RESEARCH
www.commprac.com
ISSN 1462 2815

CORRELATION BETWEEN IN-SILICO TESTING AND IN-VIVO TESTING OF DERIVATIVE COMPOUNDS NAPHTHOQUINONE AND METFORMIN AND THEIR MOLECULAR MECHANISMS OF ACTION AS ANTIDIABETIC

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DOI: 10.5281/zenodo.12755529

Abstract

Diabetes mellitus often occurs in Indonesian society and people in various countries. Diabetes is known as a metabolic disorder characterized by an increase in sugar levels above normal. Metformin is an antidiabetic drug that has been declared safe and effective. Dayak onions (Eleutherine palmifolia (L.) Merr) are a typical Kalimantan plant that contains naphthoquinone derivative compounds that have anti-diabetic properties. This research aims to determine the relationship between the In-Silico Test and the In-Vivo Test for Naphthaquinone and Metformin Derivative Compounds and their Molecular Mechanism of Action as Antidiabetics. The research methods used were the PassOnline, STRING, and Molecular Docking approaches to determine the molecular mechanism and experimental testing was carried out on diabetic mice given Dayak onion extract and metformin to see changes in blood glucose levels. The results showed that the Napthaquinone derivative compound, namely 2-Anilino-1.4-Napthaquinone, when tested in silico, had a more stable docking score, namely -80.7631 compared to the native receptor ligand 1N1M -75.0595, and when 2-Anilino -1.4- Napthaquinone was compared with metformin produces a docking score of -82.6704 which is more stable than the metformin docking score of -66.7380 which works on the 3AQV protein. 2-Anilino-1.4-Napthaquinone can be a candidate for an antidiabetic drug that works on two receptors, namely 1N1M and 3AQV. The visualization results of 2-Anilino-1.4-Napthaquinone with the 3AQV receptor show many interactions bound to amino acid residues. In-vivo testing on single metformin, single Dayak onion, and a combination of both resulted in a reduction in blood glucose levels with a significance value of $p < 0.05$ when compared with the negative control.

Keywords: Eleutherine Palmifolia Ethanol Extract; Derivate Napthaquinon; Metformin; Diabetes Mellitus, Molecular Docking.

INTRODUCTION

Diabetes mellitus is a case that we often find in Indonesian society and even in people in various countries. The International Diabetes Federation (IDF) reported that in 2021 [1], Indonesia was the country with the fifth highest number of diabetes mellitus sufferers globally, reaching 19.48 million people with a diabetes prevalence of around 10.6%. Diabetes is known as a metabolic disorder characterized by an increase in sugar levels above normal. This is due to decreased insulin production in pancreatic β cells or the inability of pancreatic beta cells to work correctly [2].

Diabetes treatment can use single or combination oral synthetic drugs, which can be divided into five groups, namely insulin secretion boosters, insulin sensitivity enhancers (Peroxisome et al. (PPRAG) agonists), inhibitors of glucose absorption in

the digestive tract (α-glucosidase inhibitors), Dipeptidyl Peptidase-4 (DPP-4) inhibitor and Sodium-Glucose Co-Transporter-2 inhibitor, and controls glucose metabolism.

This is done by regulating the enzyme 5' adenosine monophosphate-activated protein kinase (AMPK) [3]. Indonesia is a country that has a variety of plants that provide abundant benefits. This encourages people to turn to traditional medicine rather than synthetic drugs [4]. WHO also recommends using medicinal plants to maintain public health and prevent and treat diseases because conventional medicine is an alternative to treatment, and the side effects caused are considered smaller [5].

One of the plants that can be used as an alternative is Dayak Onion, the Latin name *Eleutherine palmifolia* [6]. In research [5], Dayak onions at a dose of 500 mg/KgBW in diabetic rats reported a decrease in blood glucose and insulin levels in the rats also increased.

Another study also reported that dayak onion extract tested in vivo has α-glucosidase and α-amylase inhibitory activity, thus reducing carbohydrate degradation in the small intestine and the amount of glucose absorbed into the bloodstream [6]. The main compounds of *Eleutherine palmifolia* are Naphthoquinone derivatives such as Eleutherol A, B C, Eleutherine B, C, and 2-Anilino-1,4-naphthoquinone [8]. The in silico method is a computational-based drug development method that models pharmacological and physiological processes [9].

In silico testing provides predictive hypothesis results. The development of these computational methods has helped accelerate the discovery of new drugs in recent decades. In silico assays produce bond energy values, where bond energy indicates the energy required to form a bond between a ligand and a receptor. If the bond energy is lower (more negative), the bond between the ligand and the receptor is more stable. Activity will be more significant if the ligand and receptor bonds are more stable [10].

One of the research objectives using docking simulation is to select compound components that will be tested in vitro and in vivo so that the work of extraction, isolation, and elucidation, as well as testing the activity of compounds, becomes more effective and efficient. Based on this, this study is intended to analyze the mechanism of action of Naphthoquinone derivative Compounds on four different receptor/protein targets and determine the relationship between the In-Silico Test and In-Vivo Test of Naphthoquinone Derivative Compounds with Metformin and its Molecular Mechanism of Action as Antidiabetic.

MATERIALS AND METHODS

Ethical approval

Experimental studies using Wistar strain white rats were carried out based on protocols approved by the Faculty of Pharmacy, Hasanuddin University, Makassar, Indonesia (No.693/UN4.17.8/KP.06.07/2024)

Sample preparation

Dayak onion bulbs Eileiuitheirinei Palmifolia (Mill.) Uirb. Fresh (2 kg) from Samarinda City, East Kalimantan. Dayak onion samples were macerated using 96% ethanol for 3x24 hours. During soaking, stir occasionally to complete the filter and separate the filtrate and residue formed. Next, the filtrate is evaporated using a rotary evaporator until a concentrated extract is obtained.

In Vivo Testing of Diabetic Mice

Twenty-five male Wistar strain rats were bred for 3 weeks, weighing 115 -140 g. This test was divided into 5 treatment groups, each with 5 animals, namely the normal group (without intervention), the negative group with Na-Cmc intervention, the metformin intervention group (500 mg), the Dayak onion extract intervention group (0.5 gr/KgBW), the intervention group was a combination of Metformin and Dayak onion extract with a treatment period of 10 days.

The selected animals will be acclimatized for 7 days before treatment is given. After that, mice will be induced intraperitoneally with alloxan at 150 mg/kg BW dissolved in 0.9% NaCl [24]. Measurement of sugar levels was carried out 3 days after alloxan injection.

Blood glucose was measured using a Glucosameter (NESCO NO123E003716), which took blood from the tail or tail puncture. The mice used had blood glucose levels above 200 mg/dL. Then, treatment was carried out for 10 days to see changes in the blood glucose levels of the test animals.

Data Analysis

All data obtained were tested for distribution using the Shapiro-Wilk test; if it is normally distributed, analysis will continue using the One-Way ANOVA test. The difference in mean values between treatments was tested using Tukeiy's post hoc test with a significant value of $P < 0.05$.

In-Silico Testing

Compounds docked protein binding cavities using the software PLANTS, Yasara view, Marvin sketch, Pubchem, PDB, PASS Online, STRING, Pymol, and LigPlot visualization [15].

Lipinski Rule of Five Screening Tests

The work procedure in the screening test is to draw the structure, then convert the 2D structure to 3D, save it as .pdb format on the desktop, and upload the PDB file to Lipinski Drug Filters.

Lipinski's Rule of Five makes determining a molecule or compound easier based on its permeability and absorption properties [25]. 5.5.2 Protein Preparation and ref_ligand

Target protein preparation was carried out by downloading the 2JKE, 1N1M, 3AQV, and 1KNU proteins via the protein data bank website (https://www.rscb.org/) with the PDB codes 2JKE, 1N1M, 3AQV, and 1KNU—protein and ref_ligand preparation using YASARA software.

Ligand preparation

Ligand preparation is carried out using *Marvinsketch* by uploading the ref_ligand created in the ref_ligand preparation and then creating an atmosphere of pH=7.4 according to the body's pH.

The validation parameter used is an RMSD (Root Mean Square Distance) value < 2 Å; this value indicates the protocol is accepted and docking can be carried out.

Preparation of Test Compounds

The 2D test compound was obtained by copying Canonical Smile; the selected compound was the Napthaquinone derivative 2-Anilino-1,4-naphthoquinone and Eleutherol (Cardoso, 2018), which was downloaded at https://pubchem.ncbi.nlm.nih.gov/.

Then paste the compound format into the previously opened Marvin sketch, condition it at pH 7.4, and save it as ligand2D.mrv. With Marvinsketch ligand2D.mrv, 10 conformations were created and saved as ligands.mol2

Molecular Docking Simulation Process

The docking simulation was carried out using the CMD and PLANTS® (Protein-Ligand ANT System) programs based on optimizing ant colonies with the best association technique [15].

Data Analysis

The docking results were visualized using Ligplot+, a web server, to see the interaction of the test ligand with the target protein in 2D and 3D. Interaction with. Amino acid residues, inhibition constants, and binding free energies are the parameters considered. Determining the ligand conformation resulting from molecular docking is best seen and considered based on these parameters.

RESULTS

This research is divided into 2 tests, namely in-silico and in-vivo testing, so we can see the relationship between in-silico and in-vivo studies (Figure 1).

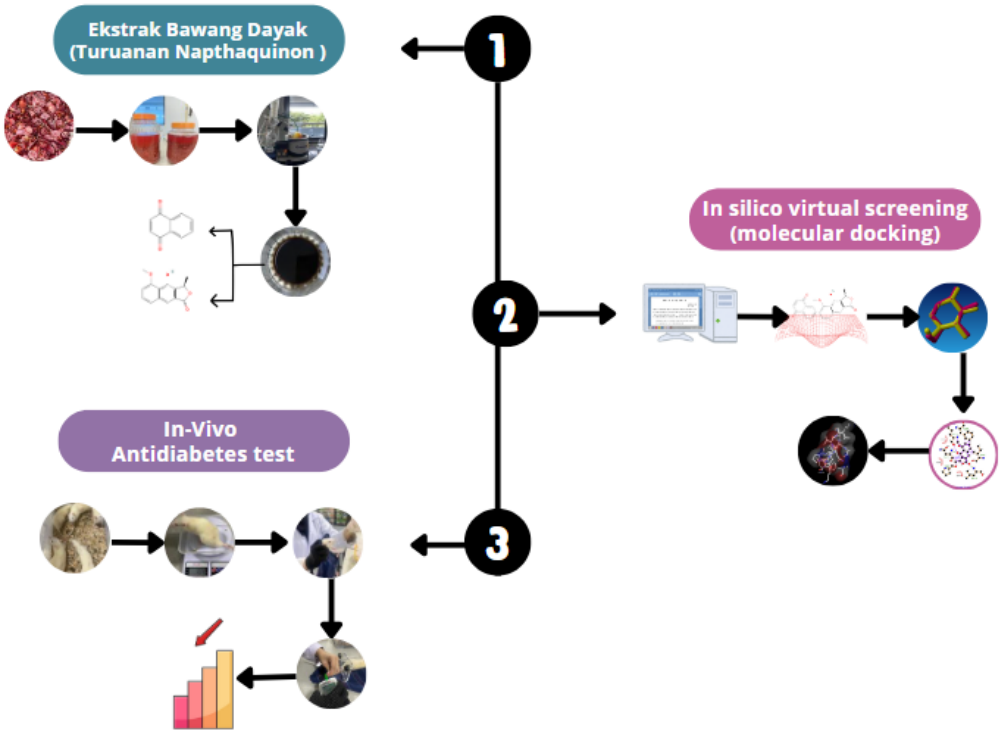


Figure 1: Combined workflow of in silico and in vivo study

Lipinski Rule of Five Screening Test

The design of an active new drug must fulfil the 'Lipinski's Rule of Five', namely: MW ≤ 500 Da, log P ≤ 5, nHBD ≤ 5, and nHBA ≤ 10 [11-2]. In this study, 2-Anilino-1,4-Napthaquinon obtained a molecular mass weight of 158.04 Da, did not have a hydrogen donor group, the obtained acceptor donor consisted of 2 groups, a log P value of 1.625 and a molar reactivity of 54.94.

As well as for Eleutherol obtained a molecular mass weight of 244.07 Da, has a hydrogen donor group of 1, the donor-acceptor obtained consists of 4 groups, and a log P value of 2.886; the prediction results show that it meets the requirements of the Lipinski rule [12].

Validasi Molecular Docking

The parameters are the *Root Mean Square Deviation* (RMSD) and visual pose. RMSD measures two poses by comparing the atomic positions between the experimental structure and the docked or predicted structure [13].

An RMSD value < 2 Å indicates a structure with the lowest energy, similar to the docked structure. RMSD results of less than 2 Armstrong indicate good formation in the quality of the docking method, which can be seen from the similarity of the structure of the docking results in Figure 2.2 below.

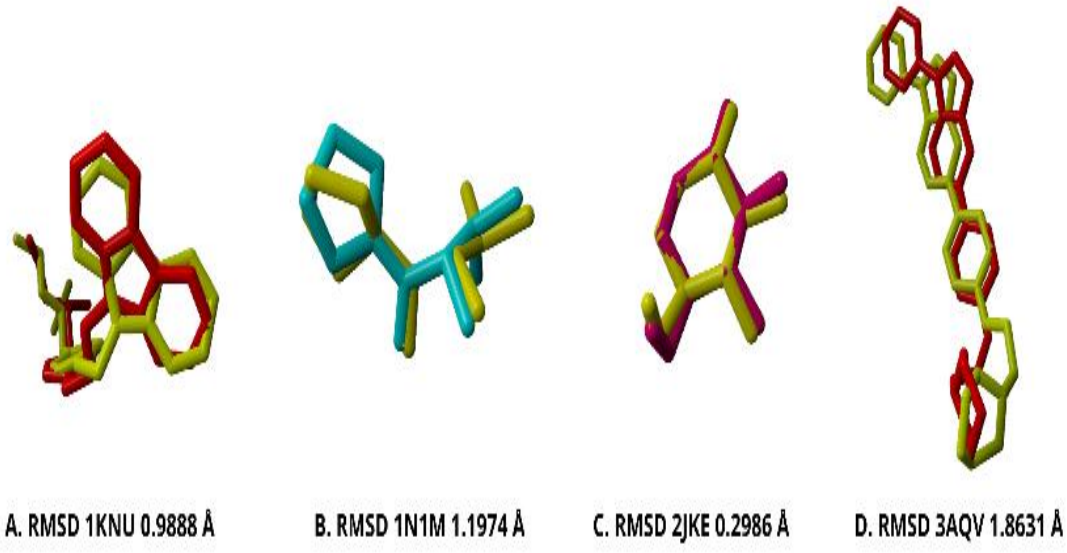


Figure 2: RMSD values of A. 1KNU (A: PPARG (Peroxisome Proliferator-Activated Receptor GAMMA) enzyme), B. 1N1M (B: Dipeptidyl Peptidase IV/CD26 enzyme), C. 2JKE (C: Alpha-glucosidase enzyme), and D. 3AQV (D: 5' adenosine monophosphate-activated protein kinase enzyme).

Molecular docking of Napthaquinone derivatives

The docking results in a score describing the strength of a stable bond between the ligand and the protein. The lower the score, the more stable the bond between the ligand and protein. The results of the molecular docking test scores for naphthoquinone derivative compounds against 2JKE, 1N1M, and 1KNU are in (table 1).

Table 1: Molecular docking test results of Napthaquinone derivatives against 2JKE, 1N1M, and 1KNU

No.	Ligand Native (1KNU)	Eleutherol	2-Anilino-1,4-naphthoquinone	Ligand Native (1N1M)	Eleutherol	2-Anilino-1,4-naphthoquinone	Ligand Native (2JKE)	Eleutherol	2-Anilino-1,4-naphthoquinone
1	-116.518	-79.609	-89.136	-74.832	-60.534	-81.127	-76.958	-65.102	-68.706
2	-117.212	-79.053	-91.602	-74.908	-60.692	-78.541	-77.294	-64.556	-66.968
3	-116.213	-78.997	-91.684	-76.408	-59.434	-79.360	-76.911	-64.659	-68.013
4	-116.241	-79.151	-86.923	-74.967	-61.554	-80.228	-77.057	-64.984	-68.177
5	-114.415	-77.752	-89.200	-75.433	-57.525	-82.618	-77.253	-64.426	-69.333
6	-115.529	-77.632	-89.088	-75.017	-58.058	-81.411	-77.284	-62.616	-69.449
7	-115.599	-77.813	-91.154	-75.240	-58.314	-80.695	-77.020	-63.374	-69.237
8	-118.065	-77.565	-89.088	-74.786	-56.883	-81.298	-77.196	-62.390	-69.802
9	-113.758	-77.469	-89.720	-74.170	-56.973	-81.670	-77.080	-64.211	-69.876
10	-114.213	-77.952	-92.218	-74.835	-56.846	-80.683	-77.196	-61.814	-68.226
Average	-115.776	-78.299	-89.981	-75.060	-58.681	-80.763	-77.125	-63.813	-68.779
SD	1.363	0.804	1.641	0.577	1.753	1.169	0.138	1.178	0.928

Then, the calculated T value was calculated, and it was obtained that the 1N1M protein had a greater result, namely 13,835, compared to the T table (α= 0.005) of 3.012 with a confidence level of 99.5%. This shows that there is a significant difference between the docking score of 2-Anilino-1.4-Napthaquinone (-80.7631) (more stable) compared to the native ligand docking score (-75.0595), so it is predicted that 2-Anilino-1.4-Napthaquinone is more potent as an antidiabetic drug candidate (Table 2).

Table 2: Test Results for Ligand 1 1M and 2-Anilino-1.4-Napthaquinone1

Ligand Native (1N1M) X 2-Anilino-1,4-naphthoquinone				
Absolute Price	α = 0.005	α = 0.01	α = 0.025	α = 0.05
T Calculate	Ttable	Ttable	Ttab Ttable el	Ttable
13.835	3.012	2.650	2.160	1.771

Then, we continued testing the Napthaquinone and Metformin derivative compounds to see their molecular mechanism of action as antidiabetics. The following are the results of the molecular docking test scores for napthaquinone and metformin derivative compounds against 3AQV (Table 3).

Table 3: Molecular docking test results of Napthaquinone derivatives against 3AQV

NO	Ligand Native (3AQV)	Eleutherol	2-Anilino-1,4-naphthoquinone	Metformin
1	-104.359	-67.655	-82.701	-67.278
2	-105.107	-69.722	-81.396	-66.815
3	-105.619	-70.278	-82.545	-67.241
4	-104.800	-68.846	-81.300	-67.106
5	-104.819	-68.431	-83.779	-67.011
6	-104.289	-71.616	-83.449	-66.649
7	-105.141	-67.185	-81.594	-66.900
8	-104.719	-71.622	-83.502	-66.101
9	-105.568	-67.853	-83.174	-65.850
10	-106.045	-70.773	-83.263	-66.429
Mean	-105.047	-69.398	-82.670	-66.738
SD	**0.565**	**1.640**	**0.931**	**0.481**

Based on the calculated T value obtained on metformin, it has a greater result, namely 48.054, compared to the T table (α= 0.005) of 3,012 with a confidence level of 99.5%. This shows that there is a significant difference between the docking score of 2-Anilino-1.4-Napthaquinone (-82.6704) (more stable) compared to the docking score of metformin (-66.7380), so it is predicted that 2-Anilino-1.4-Napthaquinone can work on proteins 3AQV, as a candidate antidiabetic drug (Table 4),

Table 4: Test results T Test Results for Metformin and 2-Anilino-1.4-Napthaquinone

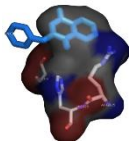
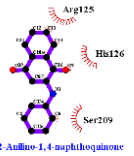
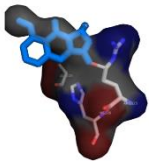
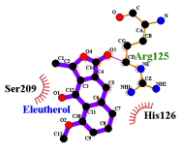
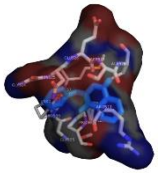
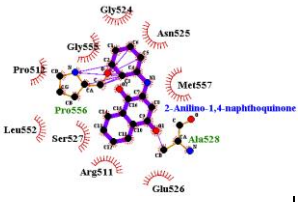
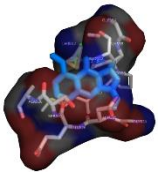
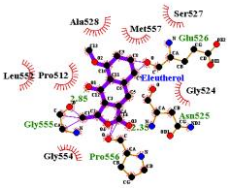
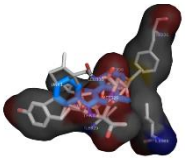
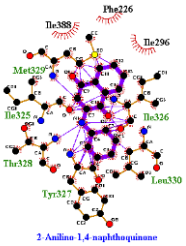
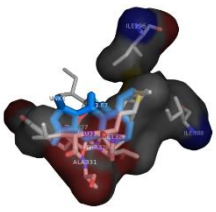
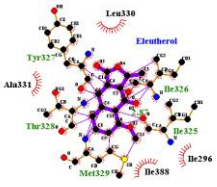
Metformin X 2-Anilino-1,4-naphthoquinone				
Absolute Price	α = 0.005	α = 0.01	α = 0.025	α = 0.05
T Calculate	Ttable	Ttable	Ttable	Ttable
48.054	3.012	2.650	2.160	1.771

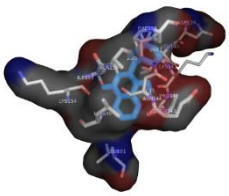
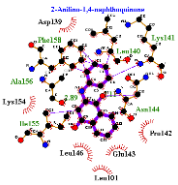
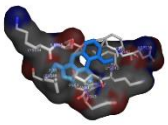
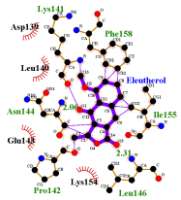
Visualization and Data Analysis Results Using Ligplot Plus.

Results of 2D visualization of hydrogen and hydrophobic compound bond interactions that appear between proteins and ligands using LigPlot+ 1.4.5 software. The number of hydrogen bonds formed can affect the amount of binding affinity or free energy [14].

The docking interactions were visualized and analyzed to see the docking results between the reference and test ligands (Table 5).

Tabel 5: Visualisasi ligplot

No.	Receptor	Compound	Visualization Results	
			3D	2D
1.	**1N1M**	2-Anilino-1,4-naphthoquinon.		
		Eleutherol		
2	**2JKE**	2-Anilino-1,4-naphthoquinon.		
		Eleutherol		
3	**1KNU**	2-Anilino-1,4-naphthoquinon.		
		Eleutherol		

4	**3AQV**	2-Anilino-1,4-naphthoquinon.		
		Eleutherol		

The interaction of amino acids allows for interactions between proteins and naphthoquinone derivative compounds, which have potential as new drug candidates. The binding area is where the protein binds to the ligand, which affects its conformation and function. Then, visualization and analysis of docking interactions on the test molecules and metformin are carried out in the image below:

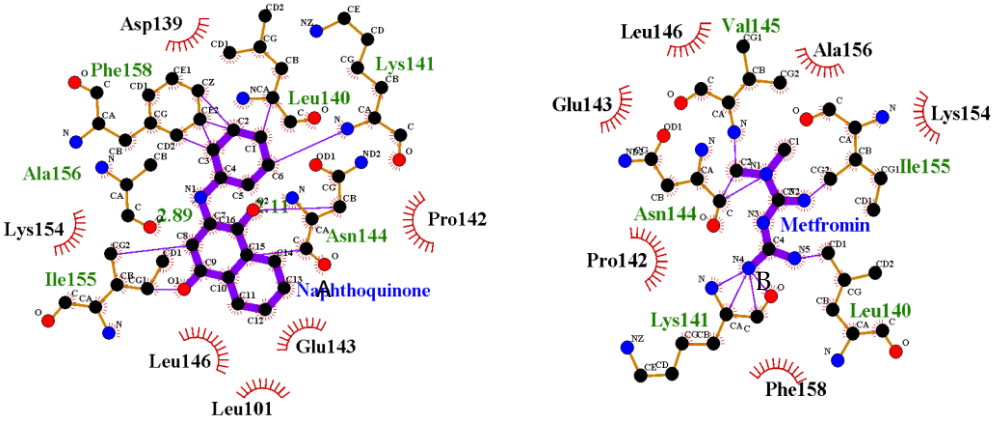


Figure 3: A: Test compound 2-Anilino-1,4-naphthoquinone, and B: Metformin

In-Vivo Test of Dayak Onion Extract Compounds

Blood glucose levels were tested on diabetic mice which were induced using Dayak onion extract at a dose of 500mg/kgbb. These results can be seen from the average glucose levels of single metformin, single Dayak onion, and the combination of both resulted in a significant reduction in blood glucose levels. This may be due to the effect of natural compounds present in Dayak onions, and when combined with metformin it has a pharmacological synergistic effect to reduce blood glucose levels. This data can be seen in **(Figure 4).**

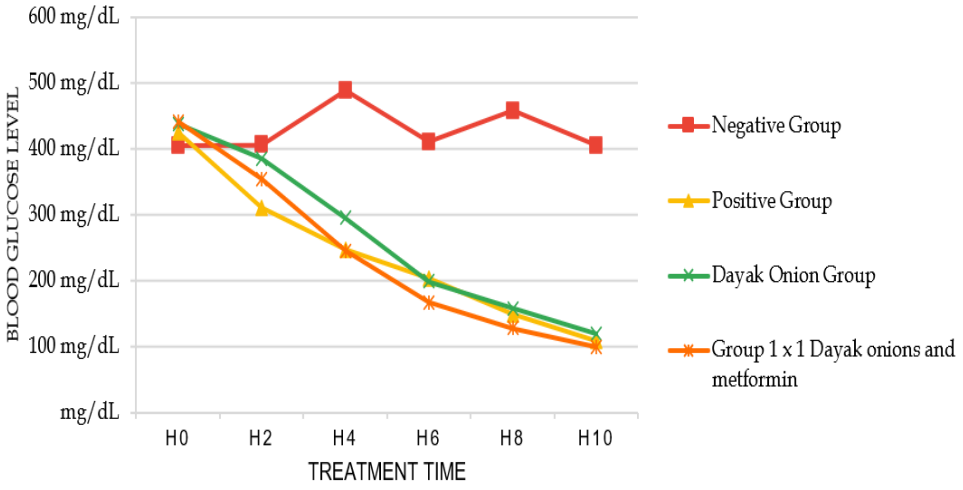


Figure 4: Data on Changes in Blood Glucose Levels

DISCUSSION

Molecular docking is an effort to search for new drug candidates derived from secondary plant metabolite compounds in-silico or computationally. Molecular docking can provide information on the interaction of metabolite compounds that act as ligands with receptors in the human body [15]. In this in-silico study, molecular docking simulations were carried out to analyze the mechanism of action of Napthaquinone Compounds derived from Dayak onions on 4 different receptor/protein targets and determine the relationship between the In-Silico Test and the In-Vivo Test of napthaquinone-derived Compounds and Metformin as Antidiabetics. Using docking simulation aims to select compound components to be tested in-vitro and in-vivo so that the extraction, isolation, and elucidation work and compound activity testing become more effective and efficient. Dipeptidyl peptidase-4 receptor (DPP4), Peroxisome proliferator-activated receptors (PPARs), and alpha-glucosidase as well as the enzyme 5' adenosine monophosphate-activated protein kinase (AMPK), were chosen because they are proteins related to glucose metabolism in the body [16]. Inhibition of the activity of this third protein can reduce blood sugar levels. Inhibition of this fourth protein is also often used to search for antidiabetic drug candidates. These receptors can be used to determine interactions between ligands and receptors in the molecular docking simulation process. In addition, this receptor is found in the human organism (Homo sapiens). The parameters are the Root Mean Square Deviation (RMSD) and visual pose. RMSD is a two-pose measurement that compares atomic positions with the experimental structure and the docked or predicted structure, where the receptor crystal structure is declared stable if it has an RMSD value < 2 Å, indicating the structure has the lowest energy, which is very similar to the docked structure. Or the constituent atoms are close to their actual state. This also shows that the software applications used (Marvin Sketch, YASARA, and PLANTS) have valid prediction capabilities under ideal conditions, without interference from other variables for the target active compounds derived from naphthoquinone with PDB codes 2JKE, 1N1M, 1KNU, and 3AQV [15]. The docking validation process obtained a value of RMSD 2JKE 0.2986 Å, RMSD 1N1M 1.1974 Å, RMSD 1KNU 0.9888 Å and RMSD 3AQV 1.8631 Å, which can be seen in Figure 3.2, the RMSD value is calculated, where the smaller it is. Based on the RMSD value, the greater the small calculation error, the

more accurate it can be said to be. The ligand used in this research is one of the compounds in Dayak onion extract (*Eleutherin palmifolia* (L.) Merr. Naphthoquinone compounds, namely 2-Anilino-1,4 naphthoquinone and Eleutherol [17]. Several studies have reported that Dayak onion extracts, tested in vitro, have α-glucosidase and α-amylase inhibitor activity, thereby reducing carbohydrate degradation in the small intestine and the amount of glucose absorbed into the bloodstream [7]. Another study was carried out in vivo using Dayak Onions at a dose of 500 mg/KgBW, which was proven to reduce blood glucose levels and increase plasma insulin levels in mice [5].

In molecular docking simulations, the results of molecular docking can be seen from the parameters of the Gibbs free energy or bond energy (ΔG) and the inhibition constant (KI) [18]. The more negative the bond energy value and the smaller the inhibition constant value indicates, the higher the ligand affinity because non-covalent interactions are stable and robust between the compound and the receptor. For docking results, data in the form of a score describes the strength of a stable bond between the ligand and protein, as seen in Table 1. Based on this table, the native ligands obtained from each protein have different results on naphthoquinone derivative compounds, namely the compounds 2-Anilino-1,4-naphthoquinone and Eleutherol. The test ligand from the compound 2-Anilino-1,4-naphthoquinone received a docking score of -80.763, more stable than the native ligand -75.05. The more stable the ligand-protein interaction is reflected by the lower (more minor) docking score, resulting in strong interaction with the Dipeptidyl peptidase-4 (DPP4) protein (1N1M.PDB) and has the potential to become an antidiabetic drug candidate with a mechanism that works on the 1N1M receptor. Then, further tests were carried out on 2-Anilino-1,4-naphthoquinone with the comparison drug metformin on a different protein, namely the 5' adenosine monophosphate-activated protein kinase (AMPK) enzyme (3AQV.PDB), it was obtained that the native ligand data had a score of -105.04 and for the test compound 2-Anilino-1,4-naphthoquinone it had a docking score of -82.67, for metformin it had a docking score of -66.73. Although this shows that the test ligand cannot exceed the native ligand of the 3AQV target protein if the test compound is compared with metformin, the docking score value of the test compound is lower, so this reflects that the ligand-protein interaction of the test compound is more stable. The ligand affinity is better-receptors and their activities [15].

Analysis of the similarity of the amino acid bonds of the test compound with the ligand is essential to review the test ligand's ability to interact with the target protein's Ligand Binding Domain (LBD) [19]. The amino acids' interaction allows contact between the protein and naphthoquinone derivative compounds, which can potentially be a new drug candidate. In this test, the 2-Anilino-1,4-naphthoquinone compound interacts with the target protein 1N1M. The native ligand has SER209 (hydrogen), ARG125, and HIS125 (hydrophobic) interactions, and for the visualization results of the 2-Anilino-1 compound, 4-naphthoquinone with 1N1M has hydrophobic bonds on the amino acid residues ARG125 and HIS125 and ARG125, the more interactions obtained, the higher the potential given. The potency of a new drug candidate is said to be good or potent if it obtains visualization test results that have the same amino acid residues as the comparison [20]. Hydrogen, hydrophobic, and electrostatic bonds can provide conformational stability in the interaction between protein and ref-ligand [21].

In this study, 2-Anilino-1,4-naphthoquinone, which binds to the target protein 1N1M, has a hydrophobic bond closer to the test molecule. However, the hydrogen bonds in

native ligand re-docking are more stable, but hydrophobic bonds such as HIS125 and ARG125 are more stable. Close to the test molecule, whereas the native ligand is far away. The second model was based on natural ligands, reference drugs, and test compounds. This model was carried out to determine the interaction between ligand and receptor and to show the biological activity of the ligand. The visualization results of the 2-Anilino-1,4-naphthoquinone compound and the 3AQV receptor show that there are hydrophobic interactions and hydrogen bonds, there are 6 hydrophobic interactions with the amino acids LYS154, LEU146, GLU143, LEU101, PRO142, and ASP139 and there are 6 hydrogen bonds, namely PHE158, LEU140, LYS141, ALA156, ASN144 and IIE155. The results of the visualization of metformin and the 3AQV receptor contain 6 hydrophobic bonds in the form of GLU143, LEU146, PRO142, PHE158, LYS154, and ALA156, but only have 5 hydrogen bonds, namely VAL145, IIE155, LEU140, and LYS141, so there are interactions of the most amino acid residues tested. Visualization of the 3AQV target protein is the compound 2-Anilino-1,4-naphthoquinone when compared with the comparison drug, namely metformin. This states that 2-Anilino-1,4-naphthoquinone has a mechanism of action as an antidiabetic that can work on two different receptors, namely 1N1M and 3AQV because it produces hydrogen, hydrophobic, and electrostatic bonds, which can provide conformational stability in the interaction between protein and ref-ligand. And by looking at the docking scores obtained [21].

People generally buy a lot of traditional medicines, and it is not uncommon for traditional and synthetic medicines to be combined to make them more effective than synthetic medicines. Combinations of synthetic and herbal medicines have become popular and are starting to become more popular than monotherapy alone [22]. Testing of Dayak onion extract combined with Metformin was carried out in-vivo in this study, using 4 groups which were divided into the negative group, positive group, Dayak onion group, and combination group of Dayak onion and metformin with a once-a-day administration interval. The dose of Dayak onion extract used is 500mg/Kg BW [5]. However, in-silico testing will be carried out first, before testing on test animals to support the results of in-vivo testing.

Working mechanisms that are related to each other can produce data on a significant reduction in blood glucose levels by looking at the data in Figure 4; The use of metformin alone, Dayak onions, and a combination of Dayak onions with Metformin resulted in a significant reduction in glucose levels. If seen from in-silico testing, the compound 2-Anilino-1,4-naphthoquinone produces a more stable docking score than Metformin; This data is strengthened by the results of in vivo testing which was carried out which resulted in a reduction in blood glucose levels in the Dayak onion extract group and when Dayak onion extract and metformin were combined, the reduction data was better than when using both alone. Metformin has been used in the community for more than 50 years. It has been declared safe and effective both in pregnancy as monotherapy and in combination with other antidiabetics [23]. Dayak onion extract, when extracted using three different methods, has a good effect in lowering blood glucose levels and is supported by the presence of flavonoid compounds and naphthoquinone as an antioxidant which can neutralize free radicals [6]. The in-silico and in-vivo test results obtained explain that the naphthoquinone derivative compound, namely 2-Anilino-1,4-naphthoquinone, can work on two receptors, namely 1N1M which represents Dipeptidyl peptidase-4 and 3AQV which represents the enzyme 5' adenosine monophosphate-activated protein kinase

(AMPK), so it has the potential to be a candidate for antidiabetic drugs. From these results, we can see the relationship between in-silico and in-vivo testing in explaining the molecular mechanisms and effectiveness of reducing blood glucose levels in experimental animals.

CONCLUSION

The 2-Anilino-1,4-naphthoquinone compound can work on two receptors, namely 1N1M which represents Dipeptidyl peptidase-4, and 3AQV which represents the 5' adenosine monophosphate-activated protein kinase (AMPK) enzyme, so it has the potential to be a drug candidate. antidiabetic. When compared with the comparison drug (metformin), the test compound had a more stable docking score, and the visualization results showed that many interactions were bound to amino acid residues. The more interactions obtained, the higher the potential given, so it has the potential to become a more effective new drug candidate. In-vivo testing on the Dayak onion group, metformin group and the combination group of metformin and Dayak onion resulted in a significant reduction in blood glucose levels when compared to the negative group. The relationship between molecular mechanisms carried out in-silico and experimentally in-vivo results in a significant reduction in blood glucose levels because both mechanisms are synergistic, and of course can be a consideration in combining synthetic drugs with herbal medicines

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