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Computational Study of Phytopharmaceutical Antidiabetic Potential of Gorontalo Traditional Medicine Plants

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Abstract

Diabetes Mellitus (DM) was a disease characterized by the occurrence of hyperglycemia. This study aimed to analyze the activity of compounds in traditional medicinal plants as potential antidiabetic candidates using the MM method and to analyze the interactions of compounds contained in traditional medicinal plants that could be used as antidiabetic drugs. The methods used were Molecular docking and Molecular dynamics, with the Aldose reductase 2HV5 receptor and the ligand ZST. Based on the research findings, the active compounds identified as potential antidiabetic drugs were Calebin_A and alpha_tocotrienol. Docking method validation on Protein 2HV5 using the natural ligand ZST showed an RMSD of 0.66 Å. The best results from molecular docking were obtained with Calebin_A, which had a binding affinity of -11.3 kcal/mol, and alpha_tocotrienol, which had a binding affinity of -11.2 kcal/mol. The results of the molecular dynamics method on the best complexes were evaluated by considering the changes in system energy, system temperature, pressure, RMSD, RMSF, and binding free energy (ΔG). The standard ligand ZST had a value of -30.43 kcal/mol, the test ligand calebin_A had a value of -34.48 kcal/mol, and alpha_tocotrienol had a value of -39.46 kcal/mol.

Keywords: *aldose Reductase 2HV5, antidiabetic, computational chemistry, traditional medicinal plants from Gorontalo*.

1 Introduction

Diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycemia due to abnormalities in insulin secretion, insulin action, or both. DM is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels [1]. The incidence of diabetes mellitus increased in the past few decades, primarily due to changes in lifestyle, increased prevalence of obesity, and longer life expectancy. At that time, diabetes affected more than 62 million people in India, which was over 7.1% of the adult population. Nearly 1 million people in India died each year due to diabetes [2]. DM was one of the most common non-communicable diseases and was the fourth or fifth leading cause of death in most high-income countries. According to the Diabetes Atlas published by the International Diabetes Federation, there were 382 million people living with diabetes. By 2035, this number was projected to increase to 592 million. Diabetes caused 5.1 million deaths in 2013.

In the past few decades, there has been an increased interest in alternative medicine using natural ingredients such as traditional medicinal plants to address various diseases, including diabetes. Traditional medicinal plants contained various compounds that had the potential as candidates for antidiabetic drugs. Studies have shown that traditional medicinal plants had strong antidiabetic activity, both in in vitro and in vivo research settings [3]. A review of traditional medicinal plants with antidiabetic activity indicated that these plants had multiple therapeutic dynamics in combating inflammation and diabetes that could be explored for the discovery of therapeutic preparations or co-treatment agents for both diseases [4]. An ethnobotanical survey on antidiabetic medicinal plants used by the Bodo tribe in the Kokrajhar district of Assam found that a total of 37 medicinal plants, belonging to 24 families and 33 genera, were used by traditional

healers in the Kokrajhar district to treat diabetes [5]. Furthermore, a review of traditional herbal antidiabetic drugs with a focus on scientific research conducted on antidiabetic medicinal plants, polyphenolic herbal mixtures, isolated compounds, and associated toxicity, found that natural products derived from medicinal plants played an important role as useful tools in pharmacological research for managing diabetes mellitus [6]. Therefore, traditional medicinal plants have the potential to be used as alternative treatments for diabetes, and further research is needed.

In the past few decades, there was an increased interest in alternative medicine using natural ingredients such as traditional medicinal plants to address various diseases, including diabetes. Traditional medicinal plants contained numerous secondary metabolites that had the potential as disease inhibitors. One of them was the ZST ligand, which interacted with the aldose reductase receptor 2HV5, an important target in diabetes treatment. Aldose reductase 2HV5 was a crucial target in the diabetes treatment [6–8]. In one study, a novel chromene compound isolated from Peperomia pellucida, Peperochromene A, was found to interact with aldose reductase among other proteins involved in blood glucose homeostasis. In another study, procyanidin and anthocyanidin compounds identified in grape seeds were docked with aldose reductase, and five compounds were evaluated for their ligand binding capacity. CID 9064 and 65056 showed high binding energies and strong interactions with the aldose reductase [9]. In a third study, 3-(5-arylidene-4-oxothiazolidin-3-yl)propanoic acid compounds and their analog 2-butenoic acid were synthesized and tested for their inhibitory effects on aldose reductase. Compound 4f exhibited a balanced inhibitory effect on AR at low micromolar concentrations. In a fourth study, specific flavonoid analogs that delayed the onset of microvascular complications were evaluated for their inhibitory effects on aldose reductase. Docking analysis showed that luteolin and quercetin had slightly stronger affinities for inhibiting aldose reductase compared to sulindac, and some luteolin and quercetin analogs showed stronger affinities than their original compounds [9]. In a fifth study, phosphoeleganin, a phosphorylated polyketide from the sea, was found to inhibit aldose reductase and protein tyrosine phosphatase 1B, and in silico docking, analysis was conducted to evaluate the interaction mode of phosphoeleganin with both enzymes [9]. However, the analysis of secondary metabolites (ligands) from traditional plants as DM inhibitors of the aldose reductase receptor 2HV5 had not been extensively conducted. In order to explore the antidiabetic potential of traditional medicinal plants, this study aimed to analyze the activity of 276 structures (secondary metabolites/ligands) from 22 traditional medicinal plants as candidates for antidiabetic drugs. The study began by analyzing the interactions between the natural ligand ZST and the aldose reductase receptor 2HV5 before analyzing the interactions of the two best ligands (Calebin_A and alpha-Tocotrienol) out of the 276 ligands using molecular docking and molecular dynamics methods as performed by [10–12]. Molecular docking was a computational method used to predict molecular interactions between bioactive compounds and diabetes-related targets, while molecular dynamics was used to understand the conformational changes and dynamics of these compounds [13]. This discovery can provide a strong foundation for the development of antidiabetic drugs derived from natural sources and open new opportunities in more effective and sustainable diabetes therapy.

2 Research Method

The equipment used included hardware and software. The hardware utilized was an HPL 1950 computer supported by an Intel® Xenon® CPU X5675 @ 3.07 GHz processor, equipped with a 12-core CPU. This computer ran on a 32-bit and 64-bit operating system with an x86_64-based processor. The software employed was Chem3D Professional version 15.0, Open Babel, Discovery Studio 2021 Client, AutoDock Tools, and AutoDock Vina version 1.5.7, Pymol from DeLano Scientific, Notepad++, PerlIDE, Gromacs for molecular dynamic simulations, and xmgrace. Additionally, several web servers were used to support this research, namely PubChem (https://pubchem.ncbi.nlm.nih.gov/), KNApSAcK-3D (http://knapsack3d.sakura.ne.jp/), and I-TASSER (https://zhanggroup.org/I-TASSER/) for predicting protein structures in 3D format.

The materials used consisted of secondary metabolite compounds extracted from 22 traditional medicinal plants, which can be obtained from the KNApSAcK-3D website, and the receptor used was aldose reductase. For molecular docking analysis, the data was obtained from the Protein Data Bank (PDB) at http://rcsb.org/pdb/. Aldose reductase with the PDB code 2HV5 (Figure 1) was the one bound to

the natural ligand, $C_{19}H_{12}F_3N_3O_3S$, with the IUPAC name of (1R,2S,5S)-N-{(1E,2S)-1-imino-3-[(3S)-2-oxopyrrolidin-3-yl]propan-2-yl}-6,6-dimethyl-3-[3-methyl-N-(trifluoroacetyl)-L-valyl]-3-azabicyclo[3.1.0]hexane-2-carboxamide.

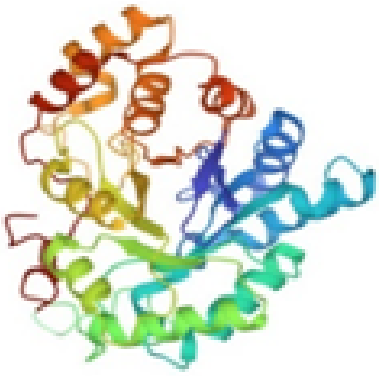

Figure 1. Antidiabetic protein template with aldose reductase variations, PDB ID 2HV5.

Water and ligands from the aldose reductase structure that were still bound were removed using the Discovery Studio software, and the results were saved in .pdb format. The structure of Aldose Reductase and the natural ligand (ZST) were shown in Figure 2.

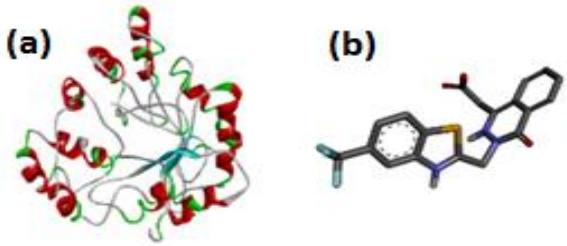

Figure 2. Prepared (a) proteins and (b) ligand

The method validation process of docking was performed using the redocking method with natural ligands from the co-crystal present in the receptor. Prior to redocking, the separated original ligand from the receptor was prepared using Autodock tools by adding hydrogen with Gasteiger charges. The observed parameter in the validation process was the RMSD of the original ligand at the selected active site. The docking method was considered valid if its RMSD was ≤ 2.5 Å. If the obtained RMSD was greater than 2.5 Å, then the used method was considered invalid.

The grid box parameter setup was performed using AutodockTools-1.5.6rc3. The coordinates of the grid box were determined based on the co-crystal ligand coordinates from the receptor file used during validation, and then the docking process was conducted using Autodock Vina.

The production simulation of Molecular Dynamics was carried out using the GROMACS 2021.3 program running on the Linux (Ubuntu) operating system. The production MD simulation was executed with the commands gmx and mdrun. The target receptor complex was simulated for 20 nanoseconds (ns). The compounds used were the complexes of aldose reductase protein with Calebin_A, alpha-Tocotrienol, and the natural ligand ZST.

The production was performed separately using different commands. The command used for protein production was pdb2gmx, while for ligand production, the command acpype in the GROMACS program was used. In the GROMACS program, hydrogen atoms were also added to the molecules. The force field used in this simulation was AMBER99SB-ILDN for protein and AMBER94 for nucleic acids [14]. The selected water model was TIP3P specified in CHARMM (MacKerell) with a 3-site rigid water molecule. In addition to the topology file, the protein coordinate file was also generated at this stage [15].

In GROMACS, there are three types of boxes: triclinic, cubic, and octahedral. The solvent box formation process used the editconf command with a triclinic box model with a diameter of 1.0 nm. The next step involved solvation and neutralization. Solvation involved dissolving the molecules in the previously formed box by editconf. In this step, the type of water model used was determined, and the required number of water molecules for solvation, typically using SPC (Simple Point Charge), was added. Neutralization involved adding Na^+ and Cl^- ions to the Aldose Reductase protein system to neutralize the previously non-neutral system [16].

The energy minimization step was performed for 50,000 steps using the steepest descent algorithm. The first equilibration, NVT, was carried out to stabilize the temperature and achieve a simulation temperature of 300 K using the modified Barendsen thermostat V-rescale. The second equilibration, NPT, was performed at a pressure of 1 bar. The temperature and pressure conditions were set to ensure that the complex had kinetic energy in the conditioned region.

During the production stage, a total of 50,000,000 steps were executed over a period of 50 ns, with a time step of 2 fs. Energy, coordinate,

and log files were saved every 10 ns. The production temperature was maintained at a constant 300 K using the modified Berendsen thermostat, and the pressure was maintained at 1 bar using the Parrinello-Rahman barostat. The production stage was run using the Gromacs program with the Grompp and mdrun commands for the specified time [17].

After the 50 ns production of Molecular Dynamics, the next step was to analyze the interactions occurring in the protein-ligand complexes. The tested ligand complexes in the Molecular Dynamics simulation were the aldose reductase-ZST, Calebin_A, and alpha-tocotrienol protein complexes. Each complex was simulated separately using the same testing parameters. The stability of the protein and ligand conformations was assessed using the Root Mean Square Deviation (RMSD), Root Mean Square Fluctuation (RMSF), and MMGBSA analysis to determine the simulated binding free energy and residue-specific free energy decomposition. The RMSD analysis was performed separately for each complex, and this simulation was carried out for 50 ns based on the Molecular Dynamics production results.

3 Results and Discussion

The validation of the molecular docking method was conducted to examine the Root Mean Square Deviation (RMSD) value. RMSD served as a parameter to describe the magnitude of changes in protein-ligand interactions between the crystal structure before and after docking, in order to determine the deviation value. The docking method was considered valid if the RMSD value was ≤ 2 Å. The RMSD value for the redocking of the natural ligand ZST in various Aldose Reductase variations was 0.66 Å. This indicated that the utilized docking method was valid, and the parameter settings met the validation criteria. The redocking of the natural ligand can be observed in the validation of the molecular docking method was conducted to examine the Root Mean Square Deviation (RMSD) value. RMSD served as a parameter to describe the magnitude of changes in protein-ligand interactions between the crystal structure before and after docking, in order to determine the deviation value. The docking method was considered valid if the RMSD value was ≤ 2 Å. The RMSD value for the redocking of the natural ligand ZST in various Aldose Reductase variations was 0.66 Å. This indicated that the utilized docking method was valid, and the parameter settings met the validation criteria. The redocking of the natural ligand can be observed in Table 1 dan Figure 3.

Table 1. Binding energy and RMSD of redocking ZST ligand (standard)

Ligand	Run	Binding energy (kcal/mole)	Cluster	RMSD (Å)
ZST	11	-11.01	1	0.66

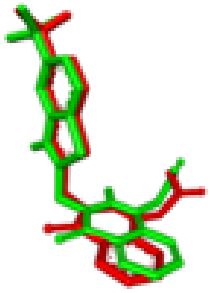

Figure 3. Overlay of the docking results for the natural ligand; before (red) and after docking (green).

The tested metabolite structures used in this study were secondary metabolite compounds derived from 22 traditional medicinal plants of the Gorontalo community, namely shallots, garlic, bitter melon, yellow wood stem, papaya, horseshoe herb, cinnamon, kaffir lime, coriander, turmeric, gendong anak (petikan kebo), castor bean, rock banana, cuplukan, cumin, horse whip, jewelweed, clove, ketapang, cocoa, soursop. There are 276 secondary metabolite structures obtained from the official KNApSAcK-3D website http://knapsack3d.sakura.ne.jp/. KNApSAcK-3D is a database website that stores information about secondary metabolites and organisms. The website also contains three-dimensional (3D) structures of all the metabolite compounds included in the original KNApSAck data.

The downloaded three-dimensional structures are generally not in optimal conditions, so they need to be prepared before being used in the docking process. The energy minimization and geometry optimization of the standard ligand structures were performed using the molecular mechanics (MM2) method 1000 times in the Chem3D Professional program. Geometry optimization and energy minimization were

performed one by one for the 276 test metabolite compounds.

The docking process was performed using the Autodock Vina program with the additional vina_windows.pl script run using the command prompt. Before docking the test ligands, redocking was performed on the natural ligands using the Autodock Vina program to determine the binding affinity values as a comparative indicator between receptor-ligand binding as an antidiabetic inhibitor agent. The docking results of several test ligands are shown in Table 2.

The docking was performed before and after docking the natural ligand, and the overlap between the ligands before and after was caused by the smaller RMSD value. The smaller the RMSD value, the tighter the ligand structure became.

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The analysis of molecular docking results in this study included the ΔG_{bind} value and Root Mean Square Deviation (RMSD), as well as the interaction between ligands and protein residues. The conformations of each docked ligand were ranked based on the ΔG_{bind} value from smallest to largest. A small ΔG_{bind} value indicated a stable conformation, while a large ΔG_{bind} value indicated the instability of the formed complex.

From the research results, the interactions between the ligands and the aldose reductase receptor yielded the best ΔG values, namely Calebin_A (-11.3) and alpha-Tocotrienol (-11.2), indicating their potential as candidates for antidiabetic agents. This was because these test ligands had ΔG values close to the natural ligand (ΔG = -11.01 kcal/mol). Subsequently, visualization was performed to observe the amino acid residues involved in binding.

Visualization and analysis of docking interactions were carried out to examine the binding results between the reference ligands and the test ligands used. The visualization results showed the interactions between amino acid residues and the ligands. The presence of interacting amino acids allowed contact between the ligand and the receptor.

The docking results of 277 test ligands on the aldose reductase protein template 2HV5 showed that the active compound Calebin_A had the lowest binding affinity, -11.3. Calebin_A was an active compound found in turmeric (*Curcuma longa* lin). The formed bond at the active site of the protein was significantly different from the natural ligand ZST. Hydrogen bonding involved one amino acid, Lys 307, and three unfavorable bump interactions with Asp 134, Thr 135, and Asn 136, as shown in Figure 4. The formation of unfavorable bonds in the ligand-protein complex could reduce its stability because these types of bonds indicated repulsive forces between two molecules or atoms. In the ligand-protein complex, unfavorable bonds could occur between charged protein regions or functional groups that repelled the ligand or other molecules bound to the complex. These bonds could disrupt the structure and conformation of the ligand-protein complex, thereby reducing its stability. The decreased stability of the ligand-protein complex due to unfavorable bonds could affect its biological function. In some cases, these repulsive interactions could lead to ligand dissociation from the protein, affect enzymatic activity, or disrupt ligand recognition by protein receptors.

Table 2. The best ligand binding affinities resulting from molecular docking

Proteins	Test ligands	*Binding affinity (kcal/mol)*	Plants
Aldosa reductase	Calebin_A	-11.3	*Kunyit*
	alpha-Tocotrienol	-11.2	*Ketumbar*
	Withaphysalin_A	-11.1	*Ceplukan*
	Curcumin	-11.0	*Kunyit*
	Proanthocyanidin_A1	-11.0	*Kakao*
	Withanolide	-11.0	*Ceplukan*
	Thalifendine	-10.8	*Batang kayu kuning*
	bis-(4- hydroxycinnamoyl)methane		
	delta-Tocotrienol	-10.8	*Kunyit*
	Apigenin_7,4'-dimethyl_ether	-10.8	*Ketumbar*
	Demethoxycurcumin	-10.6	*Sambiloto*
	Palmarumycin_CP1	-10.5	*Kunyit*
	Withangulatin_E	-10.5	*Jarak pagar*
	Anigorufone	-10.5	*Ceplukan*
	Tryptanthrin	-10.5	*Pisang batu*
	beta-Carotene_5,6-epoxide	-10.4	*Keji beling*
	Dihydrowithanolide_E	-10.2	*Pepaya*
	Geraniol	-10.2	*Ceplukan*
	Neoandrographolide	-10.2	*Bawang putih*
	Verbascoside	-10.2	*Sambiloto*
	Withangulatin_B	-10.2	*Keji beling*
	gamma-Tocopherol	-10.2	*Ceplukan*
	Hydroxyanigorufone	-10.1	*Ketumbar*
	Irenolone	-10.1	*Pisang batu*
	Lupenol	-10.1	*Pisang batu*
	Withangulatin_C	-10.1	*Keji beling*
	beta-Amyrine	-10.1	*Ceplukan*
	Chlorogenic_acid	-9.9	*Patikan kebo*
		-9.9	*Sambiloto / Pecut kuda*
	delta-Tocopherol		*Ketumbar*
	2"-O-Galloylisovitexin	-9.9	*Ketapang*
	3-O-beta-D-	-9.7	
	Glucopyranosylandrographolide.		*Sambiloto*
	alpha-Tocopherol	-9.7	*Ketumbar*
	Antheraxanthin	-9.7	*Pepaya*
	Ninandrographolide	-9.7	*Sambiloto*
	Physalin_J	-9.7	*Cuplikan*
	Andrograpanin	-9.7	*Sambiloto*
	Campesterol	-9.6	*Cengkeh*
	Mutatochrome	-9.6	*Pepayah*
	Physagulin_D	-9.6	*Ceplukan*
	Physalin_B	-9.6	*Ceplukan*
	Physalin_F	-9.6	*Ceplukan*
	Physalin_G	-9.6	*Ceplukan*
	Tetradecyl-(E)-ferulate	-9.6	*Jarak pagar*
	Withangulatin_A	-9.6	*Cepukan*
	Withangulatin_F	-9.6	*Ceplukan*
	Quercetin_3-O-rhamnosyl-(1-6)-	-9.6	
	galactoside		*Ceplukan*
	Stigmasterol-3-O-beta-D-	-9.5	
	glucopyranoside		*Cengkeh*
	Tellimagrandin_I.	-9.5	*Cengkeh*
	Wogonin_5-glucoside	-9.5	*Sambiloto*
	(+)-ar-Turmerone	-9.5	*Kunyit*
	Quercetin_3-sophoroside-7-	-9.4	
	glucuronide		*Bawang merah*

Proteins	Test ligands	*Binding affinity (kcal/mol)*	Plants
	7-O-Methylwogonin	-9.4	*Sambiloto*
	Palmarumycin_JC1	-9.3	*Jarak pagar*
	beta-Bisabolene	-9.3	*Jeruk purut*
	Bisandrographolide_B	-9.2	*Sambiloto*
	Cinaroside	-9.2	*Kakao*
	Eugeniin	-9.2	*Cengkeh*
	Taraxerol	-9.2	*Patikan kebo*
	14-Deoxy-11,14-didehydroandrographolide	-9.2	*Sambiloto*
	7R-Hydroxy-14-deoxyandrographolide	-9.1	*Sambiloto*
	Bisandrographolide_B	-9.1	*Sambiloto*
	Bisandrographolide_C	-9.1	*Sambiloto*
	Curlone	-9.1	*Kunyit*
	Dehydrocorydalmine	-9.1	*Batang kayu kuning*
	Turmeronol_B	-9.1	*Kunyit*
	Withangulatin_H	-9.1	*Ceplukan*
	Withangulatin_I	-9.1	*Ceplukan*
	alpha-Turmerone	-9.1	*Kunyit*
	alpha-Amyrine	-9.0	*Patikan kebo*
	beta-Tocopherol	-9.0	*Ketumbar*
	Chrysoeriol_7-glucoside	-9.0	*Kakao*
	Cosmosiin	-9.0	*Kakao*
	Kaempferol_4'-glucoside	-9.0	*Bawang merah*
	Progesterone	-9.0	*Bawang merah*
	Withangulatin_D	-9.0	*Ceplukan*

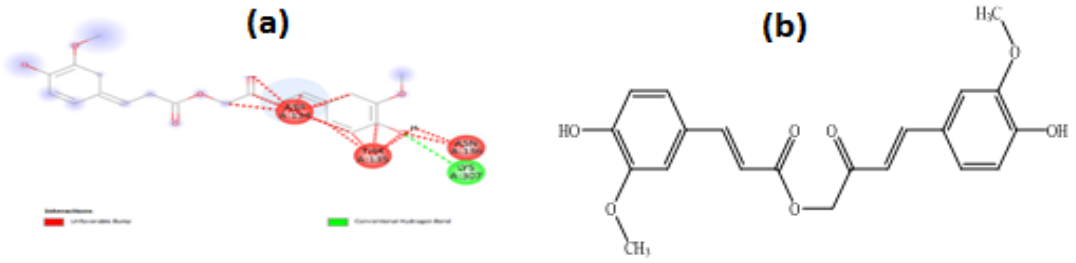

Figure 4. a) Complex Interactions of the protein 2HV5-Calebin_A complex and b) Structure of Calebin_A

In the docking results of the test ligands on the aldose reductase protein template 2HV5, it was found that the active compound alpha-tocotrienol had the lowest binding affinity, -11.2. Alpha-tocotrienol was an active compound found in coriander (*Coriandrum sativum*). The bonds formed between alpha-tocotrienol and the active site of the aldose reductase protein 2HV5 was significantly different from the bonds formed in the natural ligand condition. Several types of bonds were involved, including hydrogen bonds, pi-sigma bonds, and alkyl-alkyl bonds. The amino acids involved in these bonds were as follows: Hydrogen bonds; Cys 303 (formed a hydrogen bond with alpha-tocotrienol), pi-sigma bonds; Trp 111 (formed a pi-sigma bond with alpha-tocotrienol), Trp 20 (formed a pi-sigma bond with alpha-tocotrienol). and Alkyl-alkyl bonds; Pro 310, Phe 311, Tyr 309, Ala 299, His 110, Cys 298, Trp 79, Val 47, Tyr 48, Cys 80, Phe 115, Phe 122, and Leu 300, as shown in Figure 5. The bonds formed at the active site of the aldose reductase protein 2HV5 with alpha-tocotrienol indicated specific interactions between the ligand and specific protein residues. The differences in these bonds between the natural ligand and alpha-tocotrienol could affect the stability of the protein-ligand complex and also had an impact on its biological function.

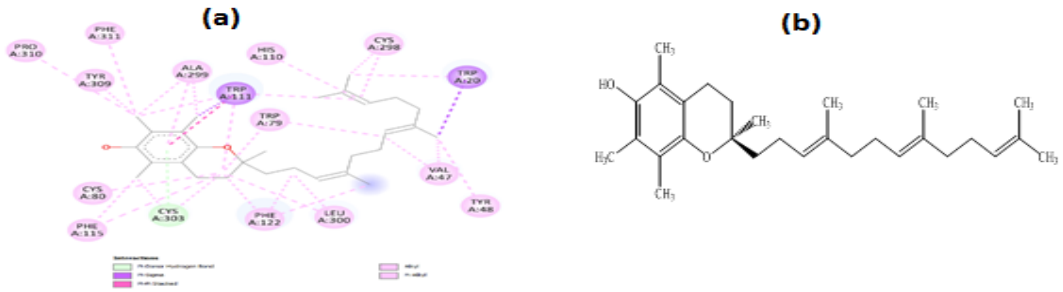

Figure 5. Interactions of the protein 2HV5-alpha-tocotrienol complex and the structure of alpha-tocotrienol.

The RMSD results that indicated the movement levels of the ZST and alpha-tocotrienol ligands were shown in Figure 6, against the Aldose Reductase protein. Fluctuations in the ZST ligand during the simulation occurred in the time range of 22.89-22.93 ns. Fluctuations in the alpha-tocotrienol ligand during the simulation occurred in the time range of 34.43-34.47 ns. And fluctuations also occurred in the Calebin_A ligand, indicating stable ligand movement. The average RMSD value for the Calebin_A ligand was 0.29 nm.

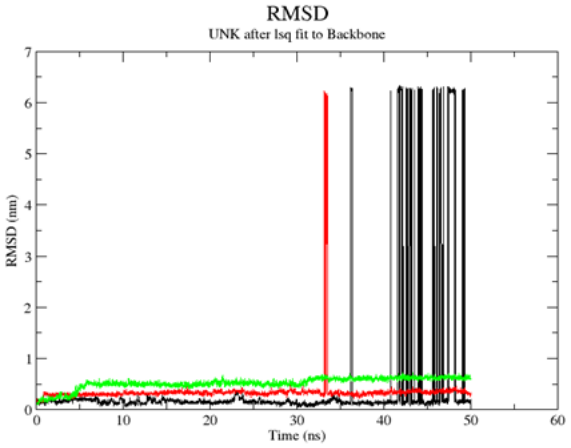

Figure 6. RMSD results of the 2HV5-ligand complex; natural ligand (black), Calebin_A (green), and alpha-tocotrienol (red).

High fluctuations in RMSD could be caused by several factors [18–21]. Firstly, the initial state of the simulation was far from the reference structure or unstable, and the complex interactions between the drug compounds and the target protein or other molecules in the biological system. Additionally, the simulation method used, such as molecular dynamics, had parameters and settings that could affect RMSD fluctuations, such as time step size, force strength, or boundary conditions. Thermal effects and simulation speed could also impact RMSD fluctuations. To address high RMSD fluctuations, approaches such as re-running the simulation, statistical analysis of the simulation results, or optimizing simulation parameters could be performed. Selecting an appropriate simulation method and paying attention to good initial conditions were also important in reducing RMSD fluctuations.

The fluctuations observed in the ligands ZST, alpha-tocotrienol, and Calebin_A with Aldose Reductase protein could have been caused by several factors, including interactions with protein residues, changes in ligand conformation, or the strength of forces between the ligand and protein. In the case of the ZST ligand, the fluctuations occurring within a specific time range might have indicated conformational changes or dynamic interactions with specific protein residues. This could have been due to environmental changes or structural alterations in the Aldose Reductase protein that affected the ZST ligand. Meanwhile, the fluctuations observed in the alpha-tocotrienol ligand within the time range of 34.43-34.47 ns could also have been caused by conformational changes or interactions with specific protein residues. These interactions were likely dynamic and could have occurred during the simulation due to changing forces between the ligand and protein. The fluctuations in the Calebin_A ligand, demonstrating stable movement, could have indicated that the ligand had more tightly bound or structured interactions with protein residues. These stable interactions could have led to more controlled and less varied fluctuations during the simulation. Overall, the fluctuations observed in these three ligands could have been attributed to complex interactions between the ligands and Aldose Reductase protein, conformational changes, or environmental alterations within the system. These factors could have influenced the movement and fluctuations of the ligands during the simulation.

High fluctuations within a specific time range could have indicated conformational changes or

dynamic interactions between the ligand and protein. However, it is important to note that high fluctuations did not necessarily indicate the invalidity of RMSD data[22–24]. Careful evaluation of other factors influencing the fluctuations, appropriate statistical analysis, and comparison with experimental data were needed to assess the validity of RMSD data in the context of high fluctuations.

High RMSD did not directly determine the validity of RMSF measurements, and both had to be evaluated separately, considering relevant factors in the study. RMSD described overall structural differences, while RMSF measured the fluctuations of individual atoms within the structure. RMSF provided insights into the flexibility and dynamics of molecules, and the validity of RMSF measurements had to be evaluated based on the context of the study, the experimental or simulation methods used, and the interpretation of relevant results.

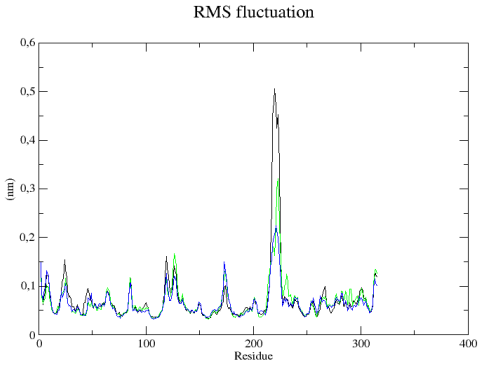

Figure 7. The RMSF results of the 2HV5-ligand complex: the natural ligand (black), Calebin_A (green), and alpha-tocotrienol (blue).

The results of the RMSF analysis of the Aldose Reductase protein and selected ligands were shown in **Error! Reference source not found.**. In the Aldose Reductase-ZST complex, the fluctuations ranged from 0.2247 nm to 0.032 nm. The highest fluctuations occurred successively at residue numbers 221, 222, 220, 219, 223, and 218. In the Aldose Reductase-alpha-tocotrienol complex, fluctuations ranged from 0.3211 nm to 0.0324 nm. The complex Aldose Reductase-alpha-tocotrienol exhibited the highest fluctuations, which could be observed at amino acid residues 223, 222, 224, 221, 225, and 219. Meanwhile, in the Aldose Reductase-Calebin_A complex, fluctuations ranged from 0.5055 nm to 0.0339 nm. This complex showed the highest fluctuations at amino acid residues 220, 219, 221, 223, 218, and 222.

Mechanics Generalized Born Surface Area (MMGBSA) is a method used to determine the total binding free energy (ΔG) of a ligand when interacting with a protein. ΔGMMPBSA consisted of the sum of the free energies in the gas phase (ΔG gas) and the solvated phase (ΔG Solv). ΔG gas represented the energy obtained from the sum of bonding and non-bonding energies. The bonding energy included bond, angle, and dihedral energies, while the non-bonding energy was contributed by van der Waals and electronic energies [25].

In the calculation of the binding free energy during a 50 ns simulation, the total binding energy (ΔG) resulted from the interaction between active protein residues and the ZST test ligand was -30.43 kcal/mol (Figure 8). The van der Waals energy ($\Delta G_{VDWAALS}$) was -43.08 kcal/mol. The electrostatic energy (ΔGEEL) formed a value of -42.34 kcal/mol, indicating the presence of interactions between charged atoms. The result of the total gas energy (ΔG_{GAS}) was -85.41 kcal/mol, while the total ΔGS_{OLV} was 54.99 kcal/mol, comprising the polar solvation energy (ΔG_{EGB}) of 60.20 kcal/mol and the non-polar solvation energy (ΔG_{ESURF}) of -5.22 kcal/mol.

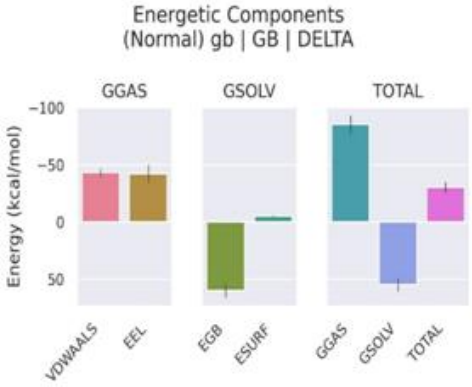

Figure 8. The value of the binding free energy for the 2HV5-ZST complex was obtained**.**

The total free energy change (ΔG) formed between the binding of active protein amino acids and the alpha-tocotrienol ligand was -39.46 kcal/mol (Figure 10). The van der Waals energy (ΔGV_{DWAALS}) and electrostatic energy (ΔG_{EEL}) were -51.31 kcal/mol and -2.26 kcal/mol, respectively. The result of the total gas energy (ΔG_{GAS}) was -53.57 kcal/mol, while the polar solvation energy (ΔG_{EGB}) was 20.35 kcal/mol and the non-polar solvation energy (ΔG_{ESURF}) was -6.23 kcal/mol.

The total free energy change (ΔG) formed in the 2HV5-Calebin_A complex was -34.48 kcal/mol (Figure 11). The Van der Waals energy

(VDWAALS) and electrostatic energy (EEL) were -46.69 kcal/mol and -16.74 kcal/mol, respectively. The total gas phase energy (GAS) was -63.43 kcal/mol, while the polar solvation energy (EGB) was 35.53 kcal/mol and the non-polar solvation energy (ESURF) was -6.58 kcal/mol.

Table 3. Active residues in the Aldosa Reduktase complex as determined by MMGBSA

Complex	ZST	Alpha-tocotrienol	Calebin_A
Active residues	TRP 20	TRP 20	TRP 20
	TRP 79	TRP 79	TRP 79
	TRP 111	TRP 111	TRP 111
	TRP 219	TRP 219	TRP 219
	VAL 47	VAL 47	
	TYR 48	TYR 48	TYR 48
	TYR 309	TYR 209	TYR 209
			TYR 309
	CYS 80	CYS 298	CYS 80
	CYS 298		CYS 298
	CYS 303		CYS 303
	HIS 110	HIS 110	HIS 110
	PRO 112	PRO 211	PRO 211
	PRO 310	PRO 215	PRO 261
		PRO 218	PRO 310
		PRO 261	
	THR 113	THR 19	THR 19
			THR 113
	PHE 115	PHE 122	PHE 115
	PHE 121		PHE 122
	PHE 122		PHE 311
	PHE 311		
	ALA 299		ALA 299
	LEU 300	LEU 212	LEU 300
		LEU 300	
	ZST 316		
		GLY 18	GLY 18
		LYS 21	LYS 21
		LYS 77	LYS 77
		LYS 262	
		ASP 43	ASP 43
		ASP 216	ASP 216
		GLN 49	GLN 183
		GLN 183	
		ASN 160	
		SER 210	SER 210
		SER 214	SER 214
		ILE 260	ILE 260
		UNK 316	UNK 316
			LYS 262

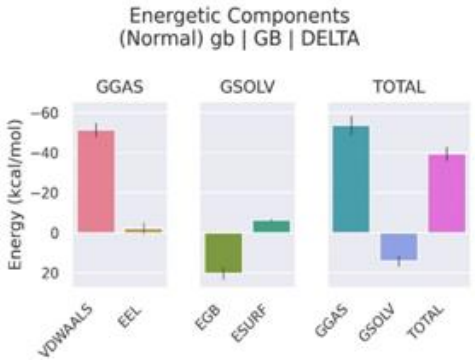

Figure 10. The value of the binding free energy for the 2HV5-alpha-tocotrienol complex

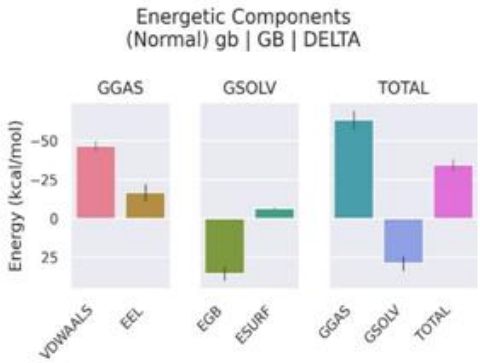

Figure 11. The value of the binding free energy for the 2HV5-Calebin_A complex.

The differences in residues involved in the binding of the Aldosa Reduktase protein and the selected ligands were observed. The highest total number of residues was found in the Aldosa Reduktase-Calebin_A complex, with a total of 32 active residues, while the alpha-tocotrienol test ligand had 30 active residues, as shown in Table 3. This number was significantly higher compared to the natural ligand ZST, which only had 21 active residues. This indicated that the test ligands had stronger binding than the natural ligand and had the potential to be candidates for antidiabetic drugs.

From the data provided in Table 4, it can be concluded that in both simulation methods, molecular docking and molecular dynamics, all complexes (Aldose reductase with ZST, alpha-tocotrienol, and Calebin_A) exhibited negative binding affinity, indicating stable interactions between the ligands and the target protein. The comparison of binding affinity between molecular docking and molecular dynamics methods showed that in all complexes, the binding affinity generated by molecular dynamics was lower (more negative) than that of molecular docking. This suggested that molecular dynamics simulations might have provided more accurate results in predicting the strength of interactions between the ligands and the target protein.

Furthermore, it could be observed that alpha-tocotrienol had the lowest binding affinity (more negative) in both simulation methods, indicating stronger interactions with Aldose reductase compared to ZST and Calebin_A. However, it is important to note that the binding affinity recorded in this table was based on simulation results and did not reflect actual conditions. These results would need to be verified through laboratory experiments to accurately determine the strength of interactions between the ligands and the target protein.

Table 4. Comparison of binding affinities of ligands from molecular docking and molecular dynamics simulations.

Complex compound		***Binding affinity* (kkal/mol)**	
		Molecular docking	Molecular dynamic
Aldosa reductase	ZST	-11.01	-30.43
	alpha-tocotrienol	-11.2	-39.46
	Calebin_A	-11.3	-34.48

4 Conclusion

Based on the results of molecular docking and molecular dynamics simulations, this study successfully identified 72 secondary metabolite compounds from the traditional medicinal plant Goronatalo with better binding affinity than the natural ligand ZST to aldose reductase protein, indicating the potential of these compounds as promising candidates for antidiabetic agents. Furthermore, the comparison between simulation methods showed that molecular dynamics simulations provided better interactions between the secondary metabolite compounds alpha-tocotrienol and Calebin_A and aldose reductase protein compared to the results of molecular docking simulations. These findings highlight the importance of using molecular dynamics simulations in predicting ligand-protein

interaction strengths. Thus, this research provides new insights into the development of antidiabetic drugs based on promising secondary metabolite compounds.

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