

IN SILICO STUDY OF *Hibiscus Sabdariffa* Linn. ACTIVE COMPOUNDS IN GLP-1R: POTENTIAL AS ANTIDIABETIC DRUG

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ABSTRAK

Diabetes melitus merupakan gangguan metabolisme yang ditandai dengan kondisi hiperglikemia akibat produksi insulin yang berkurang dan atau kondisi resistensi insulin. GLP-1 dapat meningkatkan sekresi, sensitivitas insulin, dan menurunkan kadar glukosa darah dengan cara mengikat reseptor GLP-1 (GLP-1R). Beberapa tanaman telah digunakan sebagai obat antidiabetes. Penelitian ini bertujuan untuk melihat bagaimana interaksi senyawa aktif dalam *Hibiscus sabdariffa* Linn. terhadap GLP-1R secara *in silico*. Penelitian dimulai dengan melakukan validasi internal reseptor GLP-1 (6ORV) kemudian dilakukan proses *docking* terhadap *native ligand*. Hasil validasi reseptor 6ORV mempunyai nilai RMSD 2.14Å. Hasil *score docking* 3 senyawa aktif *Hibiscus sabdariffa* Linn. terbaik adalah myricetin-3-arabinogalactoside (-10,64 kcal/mol), tetra-O-methyljeediflavanone (-9,95 kcal/mol), dan ethyl chlorogenate acid (-7,73 kcal/mol). Asam amino yang berperan terhadap afinitas pada sisi aktif ikatan ligan dengan reseptor adalah Trp 297, Trp 203, Met 204, Phe 230, Try 220, Lys 197. Temuan ini menunjukkan bahwa senyawa aktif *Hibiscus sabdariffa* Linn. dapat berikatan langsung dengan GLP-1R.

Kata kunci: Diabetes melitus, *Hibiscus sabdariffa* Linn., GLP-1R

ABSTRACT

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia due to reduced insulin production and/or insulin resistance. GLP-1 can increase insulin secretion, improve insulin sensitivity, and lower the blood glucose levels by binding to the GLP-1 receptor (GLP-1R). Several plants have been used as antidiabetic drugs. This study aims to see the interactions of the active compounds in *Hibiscus sabdariffa* Linn. and GLP-1R *in silico*. The research started with internal validation of the receptor GLP-1 (6ORV), and then the native ligand was docked. The 6ORV receptor validation results have an RMSD value of 2.14Å. The results of docking scores of the 3 best active compounds *Hibiscus sabdariffa* Linn. are myricetin-3-arabinogalactoside (-10.64 kcal/mol), tetra-O-methyljeediflavanone (-9.95 kcal/mol), and ethyl chlorogenate acid (-7.73 kcal/mol). The amino acids that contribute to the affinity at the active site of the ligand bond with the receptor are Trp 297, Trp 203, Met 204, Phe 230, Try 220, Lys 197. These findings indicate that the active compound *Hibiscus sabdariffa* Linn. can bind directly to GLP-1R.

Keywords: Diabetes mellitus, *Hibiscus sabdariffa* Linn., GLP-1R

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycemia due to reduced insulin production, insulin resistance, or both. (Biessels & Reagan, 2015) In 2019, the International Diabetes Federation (IDF) estimated that there were 463 million

individuals with DM, and this number is projected to increase to 578 million in 2030 and 700 million in 2045. (Daniels & Mietlicki-Baase, 2019) Indonesia is included in the IDF Asia-Pacific region, with 19.5 million DM cases found in Indonesia in 2021. (International Diabetes Federation, 2021)

DM causes various complications, often associated with long-term damage, dysfunction, and failure of various organs. (Kodl & Seaquist, 2008) The most common complications included persistent kidney failure (nephropathy), nerve injury (neuropathy), which increases the likelihood of ulcers and is the main cause of amputation of the lower extremities, eye disorders (retinopathy) which causes blindness, coronary heart failure, diseases involving peripheral arteries, cognitive impairment, and cerebrovascular disorders. (Saeedi et al., 2019) Some efforts are needed to treat patients appropriately in order to reduce the morbidity and mortality due to its complications.

Management of DM requires multidisciplinary action, which includes pharmacological and non-pharmacological therapy. The high incidence and prevalence of DM encourage researchers to improve and develop better therapy for DM patients that can reduce the risk of complications and increase the quality of life. Incretin-based therapy for DM is currently being developed. (Soelistijo, 2021) The effect of incretin is a response to release more insulin due to high postprandial blood glucose levels, which is responsible for 50-70% of total insulin secretion after oral glucose administration. The incretin effect is played by two hormones, namely GLP-1 and GIP. (Baggio & Drucker, 2007)

Glucagon Like Peptide-1 (GLP-1) is one of the incretin hormones produced from the proglucagon (PPG) gene and secreted by enteroendocrine L cells in response to the presence of glucose in the intestine. (Müller et al., 2019) GLP-1 shows various positive effects, such as increasing insulin secretion and sensitivity and lowering blood glucose levels. (Cho et al., 2014) Other beneficial effects of DM conditions include delayed gastric emptying, maintenance of pancreatic β cells (proliferation, neogenesis, antiapoptosis), inhibition of food intake and energy consumption, and function in increasing glucose uptake by muscle cells. (Fujiwara et al., 2019) Interestingly, GLP-1 also has anti-inflammation and antioxidant activity, and also has a positive impact on other organs such as cardiovascular and brain, and organs affected by DM complications. GLP-1 mediates its effects on various organs by binding to the GLP-1 receptor (GLP-1R) which is expressed in its related organs. GLP-1 and GLP-1R bonds

will activate this receptor and produce certain cellular signaling and corresponding physiological responses. (Yildirim Simsir et al., 2018)

Glucagon Like Peptide-1 receptor agonist is a new class of therapy for DM. This type of drug is represented by Exenatide, Liraglutide, Exenatide LAR, Albiglutide, and Taspoglutide. Despite its superiority that can naturally mimic human GLP-1, of course there are side effects that patients can experience due to its consumption, including causing nausea, vomiting, constipation, diarrhea, bloating, and other digestive disorders. (Trujillo et al., 2021) (Wharton et al., 2022) Besides that, these drugs are still rare and sold at relatively high prices. (Reid, 2012)

Currently, many studies have discussed the potential of new active compounds from natural ingredients, including those derived from plants to be developed as new drug. Indonesia is known as a country with high biodiversity, marked by the many plants that can be used in medicine, one of which is *Hibiscus sabdariffa* Linn. or Rosella which is known to have the potential to prevent DM and its complications. (Guardiola & Mach, 2014) *Hibiscus sabdariffa* Linn. is rich in bioactive compounds such as anthocyanins, quercetin, other flavonoids, organic acids, and polysaccharides, which have several beneficial physiological effects, such as antioxidant, antibacterial, anti-inflammatory, anti-cholesterol and anti-diabetic effects. (Jabeur et al., 2017)

The active compounds of herbal medicines are believed to have lower side effects, high bioavailability, and low cytotoxic effects. (Umadevi et al., 2022) Candidates for a new drug in diabetes mellitus in the botanical sector must start with an *in silico* study. *In silico* studies have been widely used to search for and design new potential drugs from natural materials and are also expected to optimize the research process and prevent losses *in vitro* and *in vivo* research steps in a precise, accurate, fast, and effective manner. The mechanism of antidiabetic effect of *Hibiscus sabdariffa* Linn. could be through GLP-1 receptor activation. Therefore, this study aims to see how the interactions of the active compounds in *Hibiscus sabdariffa* Linn. against GLP-1R *in silico* are.

MATERIALS AND METHOD

Materials and Tools

The materials used consisted of the three-dimensional structure of GLP-1R obtained through the Protein Data Bank website (PDB ID = 6ORV) and 39 compounds derived from the *Hibiscus sabdariffa* Linn. plant. This research used MarvinSketch, AutoDockTools, Ligplot+, and PyMOL software. The Software was operated on Lenovo ThinkPad with i7-4600U processor, 8GB RAM, Windows 13, and 64-bit operating system.

Ligands Preparation.

Ligands are the phytochemical constituents of *Hibiscus sabdariffa* Linn. from a study by (Herranz-López et al., 2017) as many as 39 compounds were added with N2V as the control positive ligand. Two and three-dimensional ligand structures were obtained from the Pubchem® database in .sdf format. Structures that were not found in the Pubchem® database were drawn manually using MarvinSketch. All structures were optimized in three dimensions and changed the format to .pdb using MarvinSketch. All these ligands were then given charge, set torsion and written as pdbqt format using AutoDockTools-1.5.6.

Protein Preparation

The three-dimensional structure of the GLP-1R was downloaded from the Protein Data Bank (PDB ID: 6ORV). Furthermore, the unused part including water molecules was removed, hydrogen nonpolar was added, gasteiger charged was given, and Grid box size and grid center coordinate were arranged. A grid box of 40 x 50 x 40 Å was generated around the center of the active site residues of receptor. This structure was then converted to pdbqt format. All these steps were conducted using AutoDockTools-1.5.6.

Validation of Molecular Docking Protocol

The main objective of molecular docking is to identify energetically favorable binding modes of ligands into the target receptor's binding site.(Ece, 2018) Validity is determined by redocking the native ligand to a prepared target protein. The method is said to be valid if the Root Mean Square Deviation (RMSD) value obtained is $\leq 2\text{Å}$.(Puratchikody et al., 2016) To ensure that the tested ligand binds in the ideal position for each ligand, the

binding site orientation is carried out using the blind docking method, and the results of all tested ligands show cavity with the highest affinity equal to the comparative ligands. Docking search parameter is used by the Lamarckian genetic algorithm with the number of genetic algorithms being 10 runs, population size is 150, the maximum number of energy evaluating is medium which is 2.500.000, the maximum number of generations is 27.000, spacing default is 0.375 Å, and the default docking parameter is used for running options.

The primary parameters used in the docking process are the free energy of binding (ΔG), the inhibition constant (K_i), amino acid residues, and the number of hydrogen bonds.(Kim et al., 2016) ΔG and K_i scores determine ligand affinity to the receptor. The more negative the ΔG and the lower the K_i scores indicate higher ligand affinity toward the active site of the receptor. All tested ligands are then compared with the result of control positive ligand to determine the potency of both tested ligands.(Natesan et al., 2012) The amino acid residues of ligands are then compared with amino acid residues of control positive ligand to assess the similarity of interaction between ligands and control positive ligand. More similar amino acid residues indicate a higher probability that the ligand will have a similar activity to the control positive ligand.(Pena Neshich et al., 2015) Visualization of two-dimensional and three-dimensional ligand-receptor interactions was performed using Ligplot+ and PyMOL software.

RESULTS AND DISCUSSION

Molecular docking is a computational research method that aims to estimate the interaction and affinity of a ligand with a macromolecule (usually an enzyme or protein). A ligand and protein molecule are predicted by placement techniques in a specific area (an active site) to provide optimal results (Motiejunas & Wade, 2006). The molecular docking method uses the AutoDock 4.2.6 software application. Docking validation is used successively for the x center (125.194), y center (109.599), and z center (81.738) with an RMSD value obtained from the method validation results of 2.14Å. Molecular docking results show an interaction between the active compounds *Hibiscus sabdariffa* Linn. which

interact with GLP-1R and have an activating function (as an activator). The active compounds *Hibiscus sabdariffa* Linn.

interacting with GLP-1R are described in the table below.

Table 1. The Results Of The Comparison Between ΔG And K_i Values From The Docking Of The Active Compounds *Hibiscus sabdariffa* Linn. With The GLP-1R

No	Compound Name	Binding Energy (kcal/mol)	Inhibition Constant
1	1-O-caffeoylquinic acid	-5.80	55.63 uM
2	2-O-caffeoylhydroxycitric acid	-4.19	847.27 uM
3	2-O-trans-Feruloylhydroxitric acid	-7.08	6.41 uM
4	3-Methylquercetin	-5.83	52.93 uM
5	Coumaroylquinic acid	-5.65	72.28 uM
6	5-O-Caffeoylshikimic acid	-5.58	81.63 uM
7	Caffeoylglucose	-3.18	4.65 mM
8	Chlorogenic acid	-4.88	263.31 uM
9	Chlorogenoquinone	-5.99	40.85 uM
10	Cryptochlorogenic acid	-4.60	424.20 uM
11	Cyanidine 3-sambubioside	-5.42	106.68 uM
12	Delphinidin 3-O-beta-D-sambubioside	-5.57	83.26 uM
13	Delphinidin 3-sambubioside	-5.43	104.28 uM
14	Ethyl chlorogenate acid	-7.73	2.16 uM
15	Hibiscus acid	-2.40	17.48 mM
16	Hibiscus acid dimethylester	-3.60	2.29 mM
17	Hydroxycitric acid	-2.61	12.19 mM
18	Kaempferol	-5.74	61.85 uM
19	Kaempferol-3-Glucuronide	-5.91	46.21 uM
20	Kaempferol-3-O-sambubioside	-5.91	46.65 uM
21	Kaempferol-3-O-rutinoside	-6.98	7.62 uM
22	Leucoside	-5.55	86.03 uM
23	Methyl digallate-para	-7.13	5.98 uM
24	Methyl digal llate-meta	-7.30	4.48 uM
25	Methyl-epigallocatechin	-7.65	2.47 uM
26	N-Feruloyltyramine	-6.47	18.04 uM
27	Myricetin	-5.25	142.32 uM
28	Myricetin-3-arabinogalactoside	-10.64	15.82 nM
29	Myricetin-3-glucoside	-5.34	122.59 uM
30	Neochlorogenic acid	-5.38	112.93 uM
31	Prodelfinidin-B3	-5.81	55.54 uM
32	Quercetin	-5.62	75.38 uM
33	Quercetin 3-7-diglucuronide	-5.75	60.49 uM
34	Quercetin 3-O-glucuronide	-6.13	31.95 uM
35	Quercetin 3-rutinoside	-7.12	6.09 uM
36	Quercetin 3-sambubioside	-5.11	178.74 uM
37	Quercetin-3-Glucoside	-5.53	89.01 uM
38	Tetra-O-methyljeediflavanone	-9.95	50.48 nM
39	Hibiscus acid hydroxyethylester	-4.41	582.73 uM
40	Native Ligand	-12.02	1.53 M

Bond-free energy is a vital aspect for assessing the interaction between the ligand and the receptor. This energy indicates the bond strength (affinity) between the tested ligand and the target protein. The more negative the value of the bond energy obtained, the more stable the complex formed is between GLP-1R (6ORV) and the active compound derived from *Hibiscus*

sabdariffa Linn. Increasingly negative values indicate that the compound has an interaction in the form of increasing attraction between atoms. In contrast, the repulsive forces between particles become minimal so that the conformation of the compound obtained is more stable (Manna et al., 2017). The low bond energy value also indicates that less energy is

required to carry out the binding or interaction of a ligand with a receptor. Meanwhile, the inhibition constant is directly proportional to the number of doses needed to cause pharmacological activity. The smaller the inhibition constant, the smaller the required dose is (Vargas et al., 2018). Several factors affect the binding affinity between the ligand and the receptor. Electrostatic, van der Waals, and hydrophobic interactions are important factors besides other factors such as entropy, desolvation, flexibility of the receptor structure, and the structure of the water molecule at the bond site (Haider, 2010).

In this study, three active compounds were found and had the best docking results, namely ethyl chlorogenate acid with ΔG (-7.73 kcal/mol) and K_i (2.16 μM), myricetin-3-arabinogalactoside with ΔG (-10.64 kcal/mol) and K_i (15.82 nM), and tetra-O-methyljeediflavanone with ΔG (-9.95 kcal/mol) and K_i (50.48 nM). These three compounds have the closest free bond energy to the positive control ligand value and have the highest binding affinity among the other ligands. The analysis results also obtained amino acids which are thought to play a role in the affinity of the active compounds of *Hibiscus sabdariffa* Linn. to GLP-1R, as seen in Table 2.

The target binding sites of this protein are located at Trp 297, Trp 203, Met 204, Ala 200, Leu 201, Leu 217, Phe 230, Try145, Try148, Try220, Lys 197. The essential amino acid compounds contained in the positive control ligand, namely Myricetin-3-arabinogalactoside, Tetra-O-methyljeediflavanone, and Ethyl chlorogenate acid, which are the three active compounds with the best docking, and they are Trp 297, Trp 203, Met 204, Phe 230, Try 220, Lys 197. Even though it differs from the position of the positive control ligand hydrogen bond (N2V), the presence of hydrogen bonds in the active compound with the best docking value has a vital role in the affinity between the compound and the target protein because it forms electrostatic interactions (donor and acceptor hydrogen) (Muttaqin, 2019). The amino acid residue plays a role in activating GLP-1R to carry out its function. The determined distance between the bonds indicates that the active compound has bonded with amino acid residues. In general, bonds occur at a distance of about 5 Å and not below 1 Å because there will be repulsions between atoms so that it does not meet the general bonding requirements (Faridah et al., 2021).

Table 2. Comparison Between Amino Acid Residues And The Number Of Hydrogen Bonds

Amino Acid	Native Ligand	Myricetin-3-arabinogalactoside	Tetra-O-methyljeediflavanone	Ethyl chlorogenate acid
Trp 297	✓	✓	✓	✓
Trp 203	✓	✓	✓	✓
Met 204	✓	✓	✓	✓
Ala 200	✓	✓	-	✓
Leu 201	✓	✓	-	-
Leu 217	✓	-	✓	✓
Phe 230	✓	✓	✓	✓
Try 145	✓	(H) 3.29 Å	-	-
Try 148	✓	-	-	-
Try 220	(H) 3.09 Å	✓	✓	✓
Lys 197	(H) 2.65 Å	✓	(H) 3.26 Å	✓
Cys 226	-	-	✓	✓
Cys 296	-	-	✓	✓
Val 229	-	✓	✓	✓
Met 233	-	✓	-	✓
Asp 198	-	✓	-	-
Val 194	-	-	-	-

The visualization of interactions between the top three ligands and GLP-1R used Ligplot and PyMOL. The comparison between the molecular interactions of the top three

ligands and the interactions of the positive control ligand molecules towards the same receptor is shown in Figures 1 and 2.

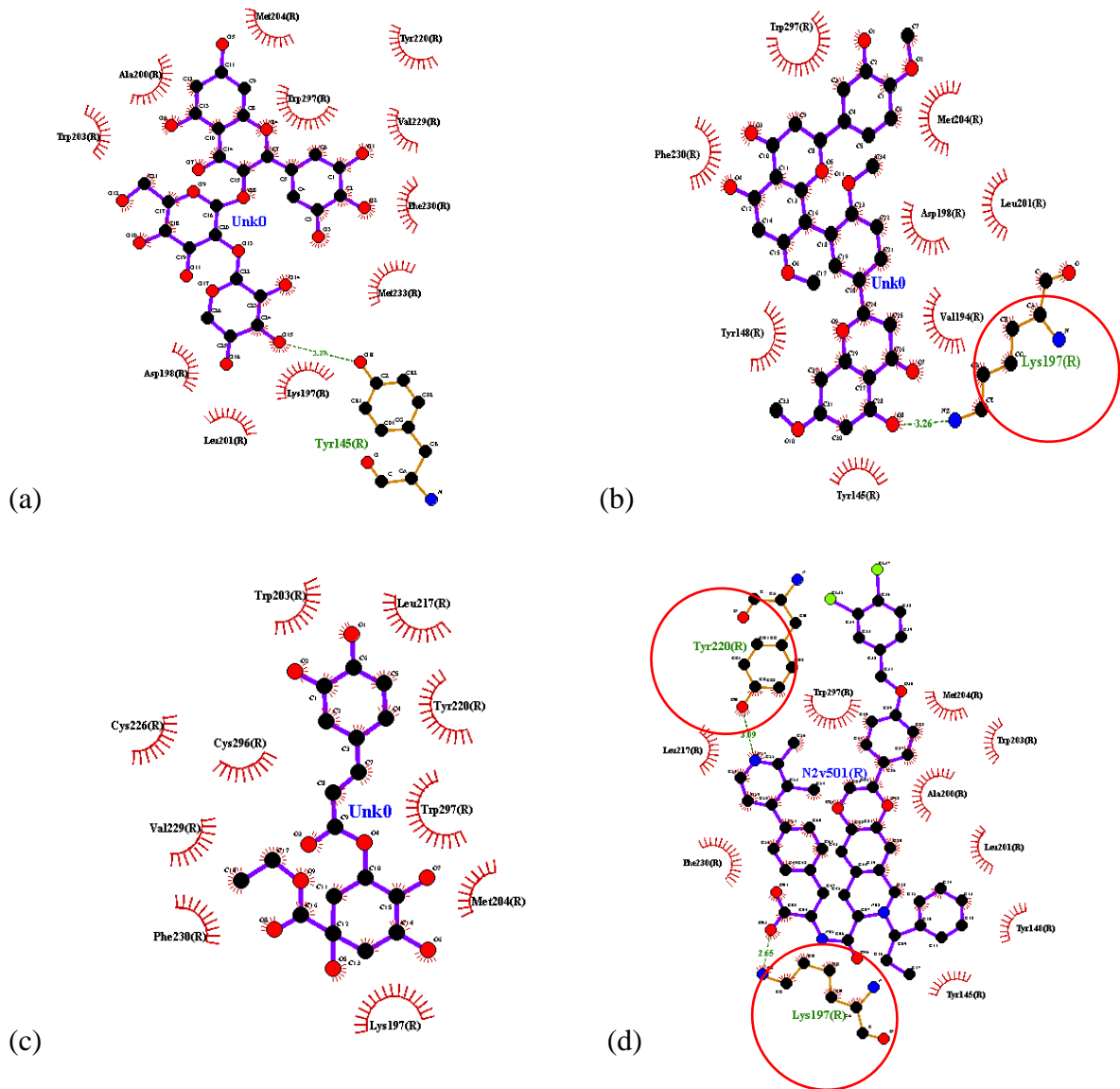


Figure 1. Results Of 2D Molecular Docking Visualization Of The Top Three Active Compounds And GLP-1R (a) Myricetin-3 arabinogalactoside And GLP-1R; (b) Tetra-O-methyljeediflavanone And GLP-1R; (c) Ethylchlorogenate acid And GLP-1R; (d) Native Ligand And GLP-1R

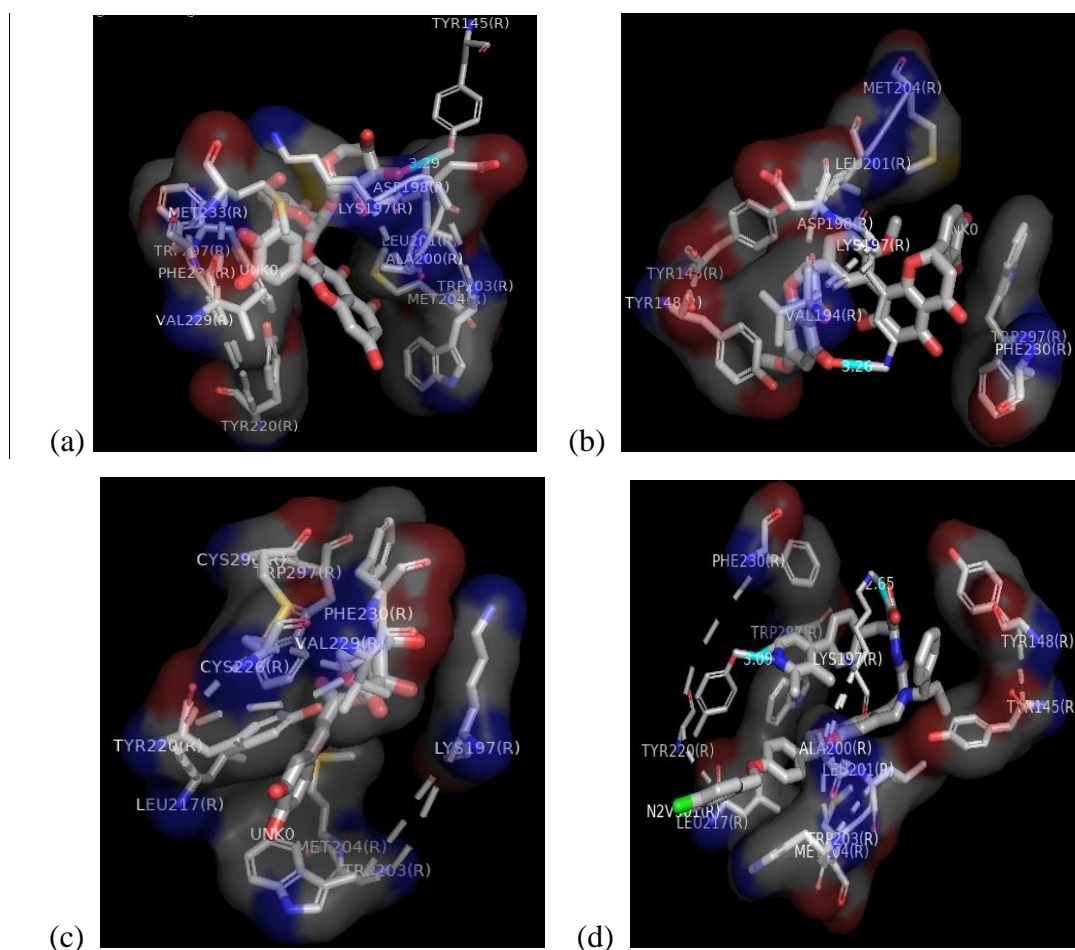


Figure 2. Results Of 3D Molecular Docking Visualization Of The Top Three Active Compounds And GLP-1R (a) Myricetin-3 arabinogalactoside And GLP-1R; (b) Tetra-O-methyljeediflavanone And GLP-1R; (c) Ethylchlorogenate acid And GLP-1R; (d) Native Ligand And GLP-1R

The compounds with the top three docking results were then examined for their physicochemical properties to see the potential of the compounds when developed into medicinal preparations. The physicochemical parameters of the tested compounds were adjusted according to the Lipinski rule. There are five rules: (1) if the test compound has a molecular weight of more than 500 Da, it will be difficult for the compound to penetrate the cell membrane; (2) a log P value greater than 5 indicates that the compound is increasingly lipophilic and binds very tightly to the membrane, making it challenging to identify targets and is also toxic; (3) However, if the log

P value is too small or negative, it is also not very good because it will be difficult for the compound to penetrate the lipid bilayer membrane; (4) Hydrogen bond donors and acceptors are the magnitudes of the hydrogen bond capacity; (5) If the hydrogen capacity is higher, the energy required in the absorption process is also higher. In general, the Lipinski rule of five (RO5) indicates the solubility of certain compounds in penetrating the membrane by passive diffusion (Brito, 2011). The test compounds are said to meet the requirements for the formation of oral preparations if there is no more than one violation of the Lipinski rules (Lipinski, 2004).

Table 3. Results Of The Lipinski Rule Of Five

Ligan	Molecular Weight (g/mol)	Hydrogen Bond Acceptor	Hydrogen Bond Donor	LogP	Description
Myricetin-3-arabinogalactoside	612.49	17	11	-1.91	Did not meet Lipinski RO5 requirements
Tetra-O-methyljeediflavanone	614.60	11	3	4.31	Did not meet Lipinski RO5 requirements
Ethyl chlorogenate acid	382.36	9	5	0.21	Met Lipinski RO5 requirements

Based on Table 3, of all the top active compounds analyzed, there was one compound that met the Lipinski RO5 requirements and met the requirements for making oral preparations. Further research is needed to determine the pharmacokinetic profile of these active

compounds. Then the active compound must have a good ADME profile and be non-toxic, and the top three test compounds will be evaluated for their toxicity through the AdmetSAR website (Cheng et al., 2012).

Table 4. Toxicity Test Results

Ligand	Toxicities		
	AMES	Carcinogenesis	AOT
Myricetin-3-arabinogalactoside	-	-	III
Tetra-O-methyljeediflavanone	-	-	III
Ethyl chlorogenate acid	-	-	III

Table 5. Ligand Pharmacokinetic Observations

Ligand	Pharmacokinetics					
	Absorption	Inhibitor CYP				
		GI	CYP1A2	CYP2C19	CYP2C9	CYP2D6
Myricetin-3-arabinogalactoside	Low	No	No	No	No	No
Tetra-O-methyljeediflavanone	Low	No	No	Yes	No	No
Ethyl chlorogenate acid	Low	No	No	No	No	No

According to the results of the analysis, the top three active compounds are not carcinogenic, non-toxic, and included in the AOT III category, which means they are slightly toxic if ingested orally. Then the three observed ligands show that these ligands have low gastrointestinal absorption. Gastrointestinal absorption can be an indicator of whether oral administration of the compound will be effective or not. Interactions of the compounds with cytochrome P450 (CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4) were identified. These iso-enzymes play a major role in drug elimination through metabolic biotransformation. If this enzyme is inhibited,

the drug or its metabolites may accumulate in the body, causing toxic effects (Yang et al., 2017). Myricetin-3-arabinogalactoside and Ethyl chlorogenate acid are predicted to be non-inhibitors for one of the isoenzymes. However, Tetra-O-methyljeediflavanone turned out to be a CYP2C9 inhibitor.

This study shows the potential of the active compound *Hibiscus sabdariffa* Linn. to interact with GLP-1R. This interaction might cause activation of the GLP-1R and initiate signal transduction and produce associated physiological responses. For example, pancreatic β cells activates several signals that have an impact on insulin secretion, increasing

proliferation, and preventing apoptosis of pancreatic β cells (Ma et al., 2014)(Kartinah et al., 2019). *Hibiscus sabdariffa* Linn. has ability to maintain circulating GLP-1 levels to reach its target in the pancreas (Domínguez Avila et al., 2017). This *in silico* study showed an additional beneficial effect of *Hibiscus sabdariffa* Linn. whose active compounds can also act as a ligand independent variable which is predicted to have antidiabetic activity by binding to GLP-1R.

CONCLUSION

Based on the docking results of 39 test compounds on *Hibiscus sabdariffa* Linn. three candidate compounds with the best binding affinity with GLP-1R were obtained, namely myricetin-3-arabinogalactoside, tetra-O-methyljeediflavanone, and ethyl chlorogenic acid. Based on visualization of the interaction of active compounds in GLP-1R, it can be concluded that amino acids play a role in the active site of the receptor, namely Trp 297, Trp 203, Met 204, Phe 230, Try 220, and Lys 197. However, the results of this study must be validated further through both *in vitro* and *in vivo* studies to elucidate the potential activity through GLP-1 and GLP-1R which produce the expected responses in diabetes mellitus management.

REFERENCE

- Baggio, L. L., & Drucker, D. J. 2007. Biology of Incretins: GLP-1 and GIP. *Gastroenterology*. 132(6): 2131–2157. <https://doi.org/10.1053/j.gastro.2007.03.054>
- Biessels, G. J., & Reagan, L. P. 2015. Hippocampal insulin resistance and cognitive dysfunction. *Nature Reviews Neuroscience*. 16(11): 660–671. <https://doi.org/10.1038/nrn4019>
- Brito, M. A. de. 2011. Pharmacokinetic study with computational tools in the medicinal chemistry course. *Brazilian Journal of Pharmaceutical Sciences*. 47(4): 797–805. <https://doi.org/10.1590/S1984-82502011000400017>
- Cheng, F., Li, W., Zhou, Y., Shen, J., Wu, Z., Liu, G., Lee, P. W., & Tang, Y. 2012. *admetSAR: A Comprehensive Source and Free Tool for Assessment of Chemical ADMET Properties*. <https://doi.org/10.1021/ci300367a>
- Cho, Y. M., Fujita, Y., & Kieffer, T. J. 2014. Glucagon-Like Peptide-1: Glucose Homeostasis and Beyond. *Annual Review of Physiology*. 76(1): 535–559. <https://doi.org/10.1146/annurev-physiol-021113-170315>
- Daniels, D., & Mietlicki-Baase, E. G. 2019. Glucagon-like peptide 1 in the brain: Where is it coming from, where is it going? *Diabetes*. 68(1): 15–17. <https://doi.org/10.2337/dbi18-0045>
- Domínguez Avila, J. A., Rodrigo García, J., González Aguilar, G. A., & de la Rosa, L. A. 2017. The Antidiabetic Mechanisms of Polyphenols Related to Increased Glucagon-Like Peptide-1 (GLP1) and Insulin Signaling. *Molecules*. 22(6): 1–16. <https://doi.org/10.3390/molecules22060903>
- Ece, A. 2018. E-Pharmacophore Mapping Combined with Virtual Screening and Molecular Docking to Identify Potent and Selective Inhibitors of P90 Ribosomal S6 Kinase (RSK). *Turkish Journal of Pharmaceutical Sciences*. 13(2): 241–248. <https://doi.org/10.4274/tjps.28290>
- Faridah, F., Sumaryono, W., Simanjuntak, P., & Triwibowo, R. R. 2021. Analysis of Pancreatic Lipase Inhibitor Activity of Chlorogenic Acid Derivatives in Green Coffee Beans as Antiobesity using In Silico. *Jurnal Ilmu Kefarmasian Indonesia*. 19(1): 125. <https://doi.org/10.35814/jifi.v19i1.946>
- Fujiwara, Y., Eguchi, S., Murayama, H., Takahashi, Y., Toda, M., Imai, K., & Tsuda, K. 2019. Relationship between diet/exercise and pharmacotherapy to enhance the GLP-1 levels in type 2 diabetes. *Endocrinology, Diabetes & Metabolism*. 2(3): 1–14. <https://doi.org/10.1002/edm2.68>
- Guardiola, S., & Mach, N. 2014. Therapeutic potential of *Hibiscus sabdariffa*: A review of the scientific evidence. *Endocrinología y Nutrición (English Edition)*. 61(5), 274–295. <https://doi.org/10.1016/j.endoen.2014.04.003>
- Haider, M. K. 2010. Computational Analysis of Protein-Ligand Interaction. In *Yuki Gosei Kagaku Kyokaiishi/Journal of Synthetic Organic Chemistry*. University of York. <https://doi.org/10.5059/yukigoseikyokaishi.54.427>

- Herranz-López, M., Olivares-Vicente, M., Encinar, J., Barrajon-Catalán, E., Segura-Carretero, A., Joven, J., & Micol, V. 2017. Multi-Targeted Molecular Effects of *Hibiscus sabdariffa* Polyphenols: An Opportunity for a Global Approach to Obesity. *Nutrients*. 9(8): 907. <https://doi.org/10.3390/nu9080907>
- International Diabetes Federation. 2021. International Diabetes Federation. In *Diabetes Research and Clinical Practice* (10th ed., Vol. 102, Issue 2). <https://doi.org/10.1016/j.diabres.2013.10.013>
- Jabeur, I., Pereira, E., Barros, L., Calhella, R. C., Soković, M., Oliveira, M. B. P. P., & Ferreira, I. C. F. R. 2017. *Hibiscus sabdariffa* L. as a source of nutrients, bioactive compounds and colouring agents. *Food Research International*. 100: 717–723. <https://doi.org/10.1016/j.foodres.2017.07.073>
- Kartinah, N. T., Fadilah, F., Ibrahim, E. I., & Suryati, Y. 2019. The Potential of *Hibiscus sabdariffa* Linn in Inducing Glucagon-Like Peptide-1 via SGLT-1 and GLPR in DM Rats. *BioMed Research International*. 2019: 1–8. <https://doi.org/10.1155/2019/8724824>
- Kim, J., Kwon, J., Kim, M., Do, J., Lee, D., & Han, H. 2016. Computational protein–ligand docking and virtual drug screening with the AutoDock suite. *Polymer Journal*. 48(7): 829–834. <https://doi.org/10.1038/pj.2016.37>
- Kodl, C. T., & Seaquist, E. R. 2008. Cognitive Dysfunction and Diabetes Mellitus. *Endocrine Reviews*. 29(4): 494–511. <https://doi.org/10.1210/er.2007-0034>
- Lipinski, C. A. 2004. Lead- and drug-like compounds: The rule-of-five revolution. *Drug Discovery Today: Technologies*. 1(4): 337–341. <https://doi.org/10.1016/j.ddtec.2004.11.007>
- Ma, X., Guan, Y., & Hua, X. 2014. Glucagon-like peptide 1-potentiated insulin secretion and proliferation of pancreatic β -cells. *Journal of Diabetes*. 6(5): 394–402. <https://doi.org/10.1111/1753-0407.12161>
- Manna, A., Laksitorini, M. D., Hudiyanti, D., & Siahaan, P. 2017. Molecular Docking of Interaction between E-Cadherin Protein and Conformational Structure of Cyclic Peptide ADTC3 (Ac-CADTPC-NH₂) Simulated on 20 ns. *Jurnal Kimia Sains Dan Aplikasi*. 20(1): 30–36. <https://doi.org/10.14710/jksa.20.1.30-36>
- Motiejunas, D., & Wade, R. C. 2006. Structural, energetic, and dynamic aspects of ligand-receptor interactions. *Comprehensive Medicinal Chemistry II*. 4: 193–212. <https://doi.org/10.1016/b0-08-045044-x/00250-9>
- Müller, T. D., Finan, B., Bloom, S. R., Alessio, D. D., Drucker, D. J., Flatt, P. R., & Fritsche, A. 2019. Glucagon-like peptide 1 (GLP-1). *Molecular Metabolism*. 30(September): 72–130. <https://doi.org/10.1016/j.molmet.2019.09.010>
- Muttaqin, F. Z. 2019. Molecular Docking and Molecular Dynamic Studies of Stilbene Derivative Compounds As Sirtuin-3 (Sirt3) Histone Deacetylase Inhibitor on Melanoma Skin Cancer and Their Toxicities Prediction. *Journal of Pharmacopolium*. 2(2): 112–121. <https://doi.org/10.36465/jop.v2i2.489>
- Natesan, S., Subramaniam, R., Bergeron, C., & Balaz, S. 2012. Binding affinity prediction for ligands and receptors forming tautomers and ionization species: Inhibition of mitogen-activated protein kinase-activated protein kinase 2 (MK2). *Journal of Medicinal Chemistry*. 55(5): 2035–2047. <https://doi.org/10.1021/jm201217q>
- Pena Neshich, I., Nishimura, L., de Moraes, F., Salim, J., Villalta-Romero, F., Borro, L., Yano, I., Mazoni, I., Tasic, L., Jardine, J., & Neshich, G. 2015. Computational Biology Tools for Identifying Specific Ligand Binding Residues for Novel Agrochemical and Drug Design. *Current Protein & Peptide Science*. 16(8): 701–717. <https://doi.org/10.2174/1389203716666150505234923>
- Puratchikody, A., Sriram, D., Umamaheswari, A., & Irfan, N. 2016. 3 - D structural interactions and quantitative structural toxicity studies of tyrosine derivatives intended for safe potent inflammation treatment. *Chemistry Central Journal*. 1–19. <https://doi.org/10.1186/s13065-016-0169-9>
- Reid, T. 2012. *Choosing GLP-1 Receptor Agonists or DPP-4 Inhibitors: Weighing*

- the Clinical Trial Evidence*. 30(1): 3–12.
<https://diabetesjournals.org/clinical/article/30/1/3/35430/Choosing-GLP-1-Receptor-Agonists-or-DPP-4>
- Saeedi, P., Petersohn, I., Salpea, P., Malanda, B., Karuranga, S., Unwin, N., Colagiuri, S., Guariguata, L., Motala, A. A., Ogurtsova, K., Shaw, J. E., Bright, D., & Williams, R. 2019. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. *Diabetes Research and Clinical Practice*. 157: 107843.
<https://doi.org/10.1016/j.diabres.2019.107843>
- Soelistijo, S. 2021. Pedoman Pengelolaan dan Pencegahan Diabetes Melitus Tipe 2 Dewasa di Indonesia 2021. *Global Initiative for Asthma*, 46. www.ginasthma.org.
- Trujillo, J. M., Nuffer, W., & Smith, B. A. 2021. *GLP-1 receptor agonists: an updated review of head-to-head clinical studies*. 1–15.
<https://doi.org/10.1177/2042018821997320>
- Umadevi, P., Manivannan, S., Fayad, A. M., & Shelvy, S. 2022. In silico analysis of phytochemicals as potential inhibitors of proteases involved in SARS-CoV-2 infection. *Journal of Biomolecular Structure and Dynamics*. 40(11): 5053–5059.
<https://doi.org/10.1080/07391102.2020.1866669>
- Vargas, J. A. R., Lopez, A. G., Piñol, M. C., & Froeyen, M. 2018. Molecular docking study on the interaction between 2-substituted-4,5-difuryl Imidazoles with different protein target for antileishmanial activity. *Journal of Applied Pharmaceutical Science*. 8(3): 14–22.
<https://doi.org/10.7324/JAPS.2018.8303>
- Wharton, S., Davies, M., Dicker, D., Lingvay, I., Mosenzon, O., Rubino, D. M., & Pedersen, S. D. 2022. Managing the gastrointestinal side effects of GLP-1 receptor agonists in obesity: recommendations for clinical practice. *Postgraduate Medicine*. 134(1): 14–19.
<https://doi.org/10.1080/00325481.2021.2002616>
- Yang, H., Chaofeng, L., Lixia, S., Jie, L., Yingchun, C., Zhuang, W., Weihua, L., Guixia, L., & Yun, T. 2017. AdmetSAR 2.0: web-service for prediction and optimization of chemical ADMET properties. *Bioinformatics*. 33(16): 1–7.
<https://doi.org/10.1093/bioinformatics/bty707/5085368>
- Yildirim Simsir, I., Soyaltin, U. E., & Cetinkalp, S. 2018. Glucagon like peptide-1 (GLP-1) likes Alzheimer's disease. *Diabetes and Metabolic Syndrome: Clinical Research and Reviews*. 12(3): 469–475.
<https://doi.org/10.1016/j.dsx.2018.03.002>