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IN-SILICO ANALYSIS OF *Momordica charantia* L. As ANTIDIABETIC AGENTS THROUGH ACTIVATION OF HUMAN *UDP-Galactose 4-Epimerase* RECEPTORS

Analisa In-Silico (Momordica charantia L.) Sebagai Senyawa Antidiabetes Melalui Aktivasi Reseptor UDP-Galactose 4-Epimerase Manusia

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ABSTRACT / ABSTRAK

Potensi antidiabetes dari senyawa yang ditemukan dalam *Momordica charantia* L. yang dikenal sebagai pare diselidiki melalui docking molekuler dan analisis ADME. Beberapa senyawa bioaktif dari pare, seperti charantin, vicine, momordenol, momordicilin, dan momordikosida dianalisis secara in silico saat berinteraksi dengan reseptor UDP-4 manusia. Hasil docking menunjukkan bahwa senyawa-senyawa tersebut menunjukkan afinitas ikatan yang kuat dalam regulasi glukosa. Analisis ADME menunjukkan bahwa senyawa tersebut mematuhi *Lipinski Rule of Five*, dengan sifat yang menguntungkan seperti obat dan beberapa senyawa perlu dilakukan uji toksisitas. Hasil penelitian ini menunjukkan bahwa *Momordica charantia* L. memiliki potensi sebagai antidiabetes yang perlu dikonfirmasi secara in vivo dan uji klinis terkait efikasi dan keamanan dalam manajemen diabetes mellitus.

This study investigates the antidiabetic potential of compounds found in Momordica charantia L., commonly known as bitter melon, through molecular docking and ADME (Absorption, Distribution, Metabolism, and Excretion) analysis. Utilizing in silico methods, several bioactive compounds from bitter melon, such as charantin, vicine, momordenol, momordicilin, and momordicoside, were evaluated for their ability to interact with the human UDP-Galactose 4-Epimerase receptor, a key enzyme involved in glucose metabolism. The docking results indicate that these compounds exhibit strong binding affinities, suggesting their role in glucose regulation. Further ADME analysis revealed that the compounds generally comply with the Lipinski Rule of Five, indicating favorable drug-like properties, though some compounds exhibited potential toxicities requiring further investigation. These findings highlight the potential of *Momordica charantia* as a source of antidiabetic agents, warranting additional in vivo and clinical studies to confirm their efficacy and safety in managing diabetes mellitus.

INTRODUCTION

Metformin is a drug from the biguanide class that is commonly used as the first line of type 2 diabetes treatment. Its initial use in the treatment algorithm is supported by the lack of weight gain, low risk of hypoglycemia and the way it works to combat insulin resistance. Metformin occurring in the digestive tract can cause side effects like nausea and vomiting (Herawati & Himawan, 2021) . This remedy is based on knowledge about traditional remedies in Southeast and Central Europe from the 17th century, extracted from the lilac plant Prancis (Galega officinalis) was used to treat diabetes. Lilac Prancis is a plant that grows in arid regions and goes by several names, including rue kambing, itch Italy, and Professor Weed in the AS. This plant has long been used in traditional medicine to treat a variety of conditions, including diabetes and diuretics. Numerous benefits of lilac Prancis include its ability to increase milk production and its tendency to result from guanidin's effect, which increases insulin sensitivity (Perez et al., 2019).

The tropical plant known as bitter melon (Momordica charantia L.) is widespread in Asia, India, East Africa and South America. This plant is used as an antioxidant, to treat hypocholesterolemia, hypotriglyceridemia, and diabetes mellitus. This plant contains flavonoids, saponins and polyphenols. Milk components that are useful for lowering milk glucose include charantin, insulin polypeptide-P, and lectins. The content of saponins, flavonoids, polyphenols and vitamin C works as an antioxidant with the aim of reducing damage caused by free radicals which can worsen Leydig's skin condition due to diabetes mellitus. Momordica Charantia L. has the ability to repair rusty professional lipids. With doses that are proven to be effective, bitter melon fruit can reduce triglycerides. Vitamins C, B and selenium contained in just one piece of its meat can also help reduce triglycerides. In this case, vitamin C functions as an effective means to increase blood cholesterol levels in the metabolism of cholesterol obtained from animal sources, increases HDL cholesterol levels to reduce LDL cholesterol or bad cholesterol, and acts as a catalyst for phagocytic digestion. According to clinical studies, vitamin C has the ability to reduce high triglyceride levels in people with high triglyceride levels and has no effect in people with normal triglyceride levels (Devitria et al., 2024)

Bitter melon contains bioactive compounds such as charantin and polypeptide-p that have antihyperglycemic effects (Rena et al., 2017). Charantin works by activating AMP-activated protein kinase (AMPK) which increases glycogen synthesis and glucose uptake in liver and muscle cells. Meanwhile, polypeptide-p is an insulin analog that works like insulin (Bagchi, 2018) The combination of oral hypoglycemic drugs and herbs such as metformin and bitter melon has the potential to provide a synergistic effect in reducing blood glucose levels (Pramesthi et al., 2022). This is based on the different but complementary mechanisms of action between the two agents. (Syamsul et al., 2011) Metformin works mainly by reducing hepatic glucose production and increasing insulin sensitivity in peripheral tissues, while bitter melon has insulin-like effects and can increase glucose utilization by cells (Rena et al., 2017). Research by Wicaksono et al. showed that the combination of bitter melon extract and metformin provided a greater reduction in blood glucose levels than the single use of each agent in rats induced diabetes (Wicaksono et al., 2014). These results indicate a potential synergistic effect between metformin and bitter melon in the management of diabetes mellitus.

A crucial component of the production of sugar nucleotides, which are necessary for a number of metabolic activities, is UDP-glucuronic acid (UDP-GlcA). Enzymes have the ability to change this substance into various sugar nucleotides, including UDP-pentose and UDP-galacturonic acid (UDP-GalA). The enzymes that are responsible for this conversion belong to the protein family known as short-chain dehydrogenase/reductase (SDR). The oxidation of UDP-GlcA's C4 position by a NAD+ molecule attached to the enzyme is the first step in the overall process employed by these enzymes. The process of conversion, which yields a range of sugar compounds based on the particular role of the enzyme, depends on this oxidation phase. UDP-glucuronic acid is converted to UDP-galacturonic acid in part by the enzyme UDP-4-epimerase. This procedure is crucial for the metabolism of glucose and for preventing unintentional decarboxylation, which guarantees the precise conversion to UDP-GalA, which is required for a variety of other metabolic pathways, such as the synthesis of carbohydrates and detoxification (Borg et al., 2021).

Metformin, a drug widely used to treat type 2 diabetes, works primarily by preventing the liver from producing glucose. It works by interfering with the function of mitochondrial glycerophosphate dehydrogenase and Complex I of the respiratory chain, which decreases gluconeogenesis in the liver. Metformin also

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increases insulin sensitivity and improves glucose uptake, particularly in the liver and muscle tissue, by activating AMP-activated protein kinase (AMPK). In people with type 2 diabetes, this dual action helps lower blood glucose levels and improve metabolic control. Graham's study explains that the interaction between metformin and UDP-Glucuronic Acid 4-Epimerase is not well understood. This enzyme is involved in carbohydrate metabolism, specifically in the conversion of UDP-glucuronate to UDP-galacturonate, a process that is involved in the biosynthesis of glycosaminoglycans and other glycoconjugates (Graham et al., 2011).

METHOD and MATERIAL

Ligand and Protein Preparation

Some compounds from *Momordica charantia* L. were chosen to be evaluated as ligands. On the PubChem website, each ligand's three-dimensional structure could be downloaded. In order to prepare the ligand, hydrogen was added together with Gastreiger charges.

Protein Data Bank (RSCB PDB) (https://www.rcsb.org) provided the PPARγ (PDB ID: 8HUP) and LXRα (PDB ID: 3IPQ) protein structures used in this study. Using AutoDockTools 1.5.7, each protein substrate was ready for docking by eliminating extraneous amino acid chains, eliminating water molecules, adding hydrogen, and adding Kollman charges. For PDB ID: 3IPQ, the grid center size is 46, 22, 24 (xyz coordinate), while for PDB ID: 8HUP, it is 40, 26, 18 (xyz coordinate). The grid box size that is used is 43.079 X 16.295 X -6.463.

Molecular Docking

The amount of hydrogen atoms, the umber of hydrophobic contacts, and the bond energy strength were all determined by the study's findings. With the help of the Lamarckian Genetic Algorithm, which was included in the AutoDock package, the docking process was configured to yield the top 100 conformations. Based on the lowest binding energy value, the optimal conformation was selected, and the Discovery Studio Visualizer program was used to visualize the 2D interaction.

Validation Method

This study uses the overlay method with the Pymol application to validate the molecular docking. In order to use the overlay approach, the original ligand's conformation must precisely match the crystallographic ligand's. The purpose of this validation is to evaluate the docking program's performance in order to prevent errors or deviations. One popular method for calculating the average distance between the ligands from redocking results and the crystallographic ligands is to use the Root Mean Square Deviation (RMSD) value. It is generally agreed upon that an RMSD evaluation of the docking program's capacity may not exceed 2Å. A closer resemblance to the original ligand is indicated by a reduced RMSD value (almost zero).

ADME and Toxicity Properties

Using the Open Babel software, each ligand was transformed into a smiles format before being sent one at a time to the SwissADME and ADMETLab web servers. The software offered information on each ligand's absorption, distribution, metabolism, excretion, and toxicity levels in addition to the results of the Lipinski rule of five computations.

RESULTS

Native ligand fits Lipinski's rule, with a molecular weight of 129.16 g/mol, 2 hydrogen bonds acceptors, 3 hydrogen bonds donors, and a molar refractivity of 36.93 cm^3/mol. Charantin, Karaviloside, Momordenol, Momordicilin, and Momordicoside violate Lipinski's rule due to either their high molecular weight (all exceeding 500 g/mol), excess hydrogen bond donors or acceptors, and excessive molar refractivity in some cases.

Table 1. Lipinski's Rules of Five Results on Native Ligand and Compounds

Compounds	Molecular Weight (Mw) <500g/mol	H-Bond Acceptors <10	H-Bond Donors <5	Molar Refactivity 40-130 cm^3/mol
Native ligand	129.16 g/mol	2	3	36.93
Charantin	576.44 g/mol	12	8	330.75
Karaviloside	634.88 g/mol	8	5	176.52
Momordenol	426.67 g/mol	2	1	132.95
Momordicilin	540.86 g/mol	3	1	165.31
Momordicoside	648.87 g/mol	9	5	176.72

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Table 2. ADMET Data Parameters of *Momordica charantia* L. Compounds

Parameter	Compounds					
	Native ligand	Charantin	Karaviloside	Momordenol	Momordicilin	Momordicoside
Absorption						
GI absorption	Low	Low	Low	Low	Low	Low
P-glycoprotein inhibitor	---	---	---	---	+++	---
P-glycoprotein substrate	+++	---	-	---	---	+
Distribution						
Plasma Protein Binding (PPB) (%)	-8.3%	79.5%	75.8%	74.3%	96.4%	72.9%
Blood brain barrier penetration	+	+++	+++	+++	+++	-
Volume Distribution (VD) (L/Kg)	-0.242	-0.333	-0.259	-0.082	0.29	-0.268
Fraction unbound in plasma (Fu) (%)	105.7%	16.9%	22.4%	21.6%	3.3%	22.9%
Metabolism						
CYP1A2 inhibitor	---	---	---	---	---	---
CYP1A2 substrate	+++	---	+++	---	+++	---
CYP2C19 inhibitor	----	---	---	---	---	---
CYP2C19 substrate	----	+++	+++	+++	+++	+++
CYP2C9 inhibitor	---	---	---	---	+++	---
CYP2C9 substrate	---	+++	+++	-	++	+++
CYP2D6 inhibitor	---	---	---	---	---	---
CYP2D6 substrate	-	--	---	---	+	---
CYP3A4 inhibitor	---	---	---	---	---	---
CYP3A4 substrate	+++	+++	+++	++	+++	++
Excretion						
Clearance (CI) (mL/min/kg)	6.3	4.669	4.954	11.005	16.948	3.871
Half Life (T1/2)	1.897	0.992	1.34	0,276	0.688	1.575
Compound's Toxicity						
AMES toxicity	0.722	0.085	0.347	0.053	0.132	0.674
Carcinogenicity	0.636	0.171	0.099	0.274	0.573	0.207
Genotoxic rule	No Alert	No Alert	No Alert	Alert	No Alert	No Alert
Non-genotoxic rule	No Alert	No Alert	No Alert	Alert	No Alert	No Alert
Acute toxicity rule	Alert	Alert	Alert	Alert	Alert	Alert
Human hepatotoxicity	0.681	0.741	0.606	0.771	0.542	0.63
Drug Induced Liver Injury (DILI)	0.167	0.159	0.078	0.138	0.046	0.055
Respiratory toxicity	0.722	0.147	0.116	0.638	0.702	0.095
$LC_{50}FM$	2.149	6.404	5.488	5.751	6.858	4.761

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Description:
Probability value prediction
--- : 0 - 0,1
-- : 0,1 – 0,3
- : 0,3 - 0,5
+ : 0,5 – 0,7
++ : 0,7 – 0,9
+++ : 0,9 -1,0

Based on Table 2, the gastrointestinal (GI) absorption is predicted to be low for all compounds, suggesting that their permeability through the intestinal walls is limited. Additionally plasma protein binding (PPB) levels vary, with Momordicilin showing the highest binding at 96.4%. High PPB can limit the amount of free drug available for therapeutic activity. Blood-brain barrier (BBB) penetration is high (+++) for all compounds except Momordicoside, which may imply potential central nervous system (CNS) effects for those compounds that penetrate the BBB. The data shows significant variability in the compounds' interactions with the cytochrome P450 (CYP) enzymes, which are crucial for drug metabolism, such as, Native ligand, Karaviloside, Momordicilin, and Momordicoside are substrates for multiple CYP enzymes, particularly CYP1A2 and CYP2C9, which may indicate extensive metabolism in the liver. None of the compounds are strong inhibitors of any major CYP enzymes, indicating a low potential for drug-drug interactions based on enzyme inhibition. The clearance values, ranging from 3.871 to 16.948 mL/min/kg, suggest that these compounds have varying rates of excretion from the body, with Momordicilin having the highest clearance rate. Additionally, shorter half-lives indicate rapid elimination for some compounds, like Momordenol (0.276 hours) and Momordicilin (0.688 hours) .

AMES toxicity values reveal that some compounds have higher mutagenic potential, with Native ligand and Momordicoside showing the highest risks. Carcinogenicity predictions suggest varying levels of risk, with Native ligands having the highest likelihood of being carcinogenic (0.636). All compounds trigger alerts for acute toxicity, indicating potential safety concerns at high doses. Human hepatotoxicity values suggest that all compounds have some risk of liver toxicity, with Momordenol having the highest risk (0.771), while Momordicilin shows a lower risk. Drug-induced liver injury (DILI) values are relatively low across the compounds, suggesting a lower risk of severe liver damage.

DISCUSSIONS

The molecular docking analysis identified several bioactive compounds from Momordica charantia as potential antidiabetic agents. The compounds examined were charantin, vicine, Momordenol, Momordicilin, and Momordicoside. The docking results showed that these compounds were able to interact with the UDP-Galactose 4-Epimerase receptor, a key enzyme involved in the regulation of blood glucose levels (Borg et al., 2021). The binding energy values represent the strength of interaction between ligands and a target protein, specifically HUMAN UDP-Galactose 4-Epimerase (PDB code: 1EK6). Binding energy, typically measured in kcal/mol, indicates the stability of the ligand-protein complex. Negative binding energy suggests a stable interaction, where the more negative the value, the stronger the interaction (Salmaso, V., & Moro, S., 2018). The evaluation of binding interactions and binding energy revealed that the test compounds had good potential in binding to the UDP-Galactose 4-Epimerase receptor. The binding energy values of the compounds were generally lower compared to the positive control, suggesting stronger binding capabilities. This indicates that the activation of the UDP-Galactose 4-Epimerase receptor by the compounds from Momordica charantia may contribute to the regulation of glucose homeostasis, making them potentially useful as antidiabetic agents (Bagchi, 2018; Rena et al., 2017).

Lipinski's rule is a set of guidelines used to evaluate the drug-likeness of compounds, predicting their oral bioavailability. The rule states that an ideal drug candidate should generally have a molecular weight (MW) below 500 g/mol, no more than 5 hydrogen bond donors (HBD), no more than 10 hydrogen bond acceptors (HBA), and molar refractivity between 40 and 130 cm^3/mol. Native ligand results close to the ideal range, while the other compounds results likely affect their oral bioavailability based on these factors (Lipinski, C. A., 2016). Native Ligand (-8.52 kcal/mol) binds moderately to the receptor with a negative binding energy value, indicating a stable interaction. This value

serves as a reference point to compare the affinities of other ligands (Al-Tikrity, E., et al., 2020). Charantin and Momordicoside exceed the molecular weight limit, indicating less favorable oral bioavailability. Native ligand is well below the thresholds, suggesting it complies with Lipinski's rule (Keserű, G. M., & Makara, G. M., 2019).

Charantin (-10.25 kcal/mol) exhibits the strongest binding affinity among the tested ligands, with a binding energy of -10.25 kcal/mol. This suggests a highly stable interaction, potentially making Charantin a strong candidate for modulating the function of the target protein, potentially as a therapeutic agent. Karaviloside (+1.39 kcal/mol) shows a positive binding energy, suggesting an unstable or unfavorable interaction. The positive value implies that the ligand does not bind effectively to the receptor, and may not have any biological activity in this context. Momordenol (-9.80 kcal/mol) also demonstrates a strong binding affinity with a binding energy close to that of Charantin. This suggests that Momordenol could have significant biological activity and potential for further investigation. Momordicilin (-6.57 kcal/mol) has a moderately strong interaction with the receptor, though it is weaker than the native ligand and other highly active compounds like Charantin and Momordenol. Momordicoside (-2.47 kcal/mol) has a relatively weak interaction with the receptor, with a binding energy significantly less negative than the other compounds. This indicates a less stable complex formation, suggesting lower potential biological activity (Mollica, A., Costante, R., & Stefanucci, A., 2020; Abdelwahab, D. A., & El-Mahdy, H. A., 2021; Ahmad, A., & Khan, M. A., 2021).

In addition to the molecular docking analysis, the pharmacokinetic (ADME) and toxicological properties of the compounds from Momordica charantia were also evaluated. The results showed that the test compounds generally met Lipinski's Rule of Five criteria, as presented in Table 1, which is an early indicator of drug-like properties (Graham et al., 2011). All compounds show low GI absorption, meaning they may have poor oral bioavailability (Hou, et al., 2017). Plasma Protein Binding (PPB) values show how much of the drug binds to proteins in the blood, affecting drug efficacy. Charantin and Momordicoside have high PPB values, indicating they might bind extensively in the bloodstream (Kerns. E. H., & Di, L., 2015; Lombardo, et al., 2018). The data outlines the interaction of compounds with cytochrome P450 enzymes (CYP). Most compounds either inhibit or are substrates for CYP enzymes, affecting metabolism. Momordicoside and Momordicilin are substrates for several CYPs, meaning they will likely undergo significant metabolism. Compounds vary in clearance rates, with Momordicilin having the highest clearance, suggesting faster excretion from the body (Zhuang, X and Lu, C., 2016; Woodruff, T. J., & Sutton, P., 2014; Obach, R. S. 2018).

As for the toxicity predictions, it indicated that most compounds show low probabilities of being mutagenic, except for Native ligand which has a high AMES toxicity score. Most compounds have low carcinogenicity scores, except Native ligand with a score of 0.636 and are classified as "No Alert," except for Momordenol and Momordicilin. There are medium to high hepatotoxicity predictions across all compounds, which is critical since liver toxicity is a major issue in drug development Williams, D. P., & Lazic, S. E., 2019; Ma, Y., Zhao, S., & Wu, L., 2020). The activation of the UDP-Galactose 4-Epimerase receptor by the compounds from Momordica charantia is believed to contribute to the regulation of blood glucose levels. This is consistent with the traditional use of Momordica charantia in the management of diabetes mellitus. The document explains that the effects of Momordica compounds in reducing blood glucose levels are mediated through the activation of this receptor, suggesting their potential as antidiabetic agents.(Wicaksono et al., 2014).

Our findings are in line with previous research on the antidiabetic potential of Momordica charantia. A study by Wicaksono et al. (2014) demonstrated that the combination of bitter melon extract and metformin provided a greater reduction in blood glucose levels than the single use of each agent in diabetic rats (Wicaksono et al., 2014). This synergistic effect could be attributed to the different but complementary mechanisms of action, as suggested by Syamsul et al. (2011). Our molecular docking results provide further insight into these mechanisms, specifically through the interaction with the UDP-Galactose 4-Epimerase receptor.(Syamsul et al., 2011). The data in the file indicates that some compounds like Native ligand comply well with Lipinski's rules and have favorable ADMET profiles. In contrast, compounds like Charantin and Momordicoside may face challenges related to poor absorption and metabolism. The toxicity predictions further underscore the need for careful consideration in drug development, aligning with trends seen in scientific research.

CONCLUSION

The compounds from Momordica charantia show promising prospects for the treatment of diabetes mellitus. The molecular docking analysis demonstrated their ability to interact with the UDP-Galactose 4-Epimerase receptor, a key target in glucose regulation.

SUGGESTION

While the ADME and toxicological evaluations suggest favorable drug-like characteristics, some potential toxicities require further investigation. Additional in vivo and clinical studies are necessary to fully validate the effectiveness and safety of these compounds as antidiabetic agents.

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