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MOLECULAR DOCKING STUDIES OF FLAVONOIDS FROM SECANG WOOD (*Caesalpinia sappan* L.) AGAINST GLUCOKINASE ENZYME AS ANTIDIABETIC CANDIDATES

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ABSTRACT

Diabetes mellitus (DM) is a metabolic disease caused by a deficiency of insulin secretion, insulin resistance, and increased hepatic glucose production. Secang wood (*Caesalpinia sappan* L.) is known to have antihyperglycemic activity. However, these compounds are not yet known. In silico studies are needed to determine the compounds that act as antidiabetics. This study performed molecular docking of flavonoid compounds in sappan wood against the 1V4S glucokinase receptor. The results showed that all flavonoid compounds of sappan wood were predicted to have antidiabetic activity because they had a lower docking score than metformin, the first-line therapy of type 2 diabetes mellitus. Butein is expected to have the best activity. It has the lowest docking score (-94.4836). Visualization of the docking results shows that butein interacts with the identical amino acid residues as metformin, namely ARG 63 and THR 65, through the formation of hydrogen bonds and Van der Waals interactions. SWISS-ADME web tool predicted that butein has good oral absorption and excretion. The toxicity prediction tool showed a slight contradiction in the mutagenic effect. Based on this research, molecular docking may be able to design new drugs, especially from butein in sappan wood (*Caesalpinia sappan* L.), as antidiabetic candidates.

Keywords: Diabetes mellitus; Caesalpinia sappan; Glucokinase; Molecular docking; Flavonoid

1. INTRODUCTION

Diabetes mellitus (DM) is a metabolic disease characterized by an increase in blood glucose levels (≥ 200 mg/dL) (Soelistijo et al., 2019). Diabetes mellitus can be caused by a deficiency of insulin secretion, insulin resistance, and increased hepatic glucose production, which can cause abnormalities in the carbohydrate, lipid, and protein metabolism system. Total or partial insulin deficiency during diabetes can impair carbohydrate metabolism resulting in inadequate glucose utilization and increased hepatic glucose production (Mahendran et al., 2014). According to the International Diabetes Federation (IDF), patients with DM in Indonesia are seventh-ranked worldwide. They are predicted to increase from 10.7 million in 2019 to 16.6 million in 2045 (IDF, 2019). From these data, it can be seen that DM is a problem that must be addressed immediately. One of them is developing new drugs from natural substances that are efficient in treating diabetes and have few adverse effects.

Secang (*Caesalpinia sappan* L.) is known to have activity in reducing blood sugar levels. Secang wood infusion with concentrations of 10% w/v, 15% w/v, and 20% w/v reduced blood sugar levels in male mice (*Mus musculus*) (Yusuf & Wati, 2019). The secang drink is reported to have an antihyperglycemic effect in adult women with prediabetes. Secang plant boiled with water can reduce fasting blood glucose levels by 14.36 ± 19.19 mg/dL (Sa'pang, 2015). In

addition, the isolation of secang wood extract is known to have antihyperglycemic activity by inhibiting alpha-glucosidase and alpha-amylase enzymes (Arsiningtyas, 2015).

Secang wood (*Caesalpinia sappan* L.) has flavonoid compounds with antidiabetic activity (Al-Ishaq et al., 2019). Flavonoid compounds contained in the secang wood are brazilin, brazilein, brazilide a, butein, (E)-3-(3,4-dihydroxybenzylidene)-7-hydroxychroman-4-one, 3-deoxysappanon b, protosappanin a, protosappanin b, protosappanin c, protosappanin d, protosappanin e, sappanchalcone, sappanone b, 3,8,9-trihydroxy-6H-benzo[c]chromen-6-one, and 3-deoxysappanchalcone (Nirmal et al., 2015).

Molecular docking is a computational method that can be used as the basis for drug discovery (Setiawan & Irawan, 2017). This method can predict the potential compound of the antidiabetic candidate. Molecular docking studies of flavonoid compounds from *Justica gendarussa* Burm.f. identified an antidiabetic activity by forming hydrogen bonds with glucokinase enzymes (Adelina, 2020). There is no information regarding the interaction of flavonoid compounds from the secang wood with the glucokinase enzyme (1V4S), so it is necessary to conduct an in silico study to determine the docking score and the interactions that occur.

2. METHODS

2.1. Tools and Materials

The docking molecule in this research used a laptop ASUS (X41C series) with specification Intel(R) Core(TM) i3-3217U CPU @1.8GHz processor, 2GB RAM, and Windows 10 Pro 64-bit operating system. The docking analysis used PLANTS (Protein-Ligand ANT-System) (http://www.tcd.uni-konstanz.de), YASARA (Yet Another Scientific Artificial Reality Application) (http://www.yasara.org), MarvinSketch (https://chemaxon.com), and Discovery Studio Visualizer (https://discover.3ds.com).

This research used the crystal structure of the human glucokinase enzyme (PDB CODE: 1V4S) as the target protein, which was downloaded from Protein Data Bank (PDB) (https://www.rcsb.org). Flavonoid compounds from secang wood (*Caesalpinia sappan* L.) and metformin as an antidiabetic drug were downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov). A total of fifteen compounds contained in secang wood were selected in this study, including Brazilin, Brazilein, Brazilide A, Butein, (E)-3-(3,4-dihydroxybenzylidene)-7-hydroxychroman-4-one, 3-Deoxysappanone B, Protosappanin A, Protosappanin B, Protosappanin C, Protosappanin D, Protosappanin E, Sappanchalcone, Sappanone B, 3,8,9-trihydroxy-6H-benzo[c]chromen-6-one, and 3-Deoxysappanchalcone.

2.2. Lipinski's Rule of Five Tests

The Lipinski rule of five tests was conducted on http://www.scfbio-iitd.res.in to determine the physicochemical properties such as molecular weight, Log P, Hydrogen Bond Donor, Hydrogen Bond Acceptor, and Molar Refractivity of flavonoid compounds from secang wood (*Caesalpinia sappan* L.).

2.3. Protein and ref_ligand Preparation

The glucokinase enzyme was prepared by downloading the protein structure from the Protein Data Bank (PDB CODE: 1V4S). Enzyme separation, with native ligands and other molecules, and the addition of hydrogen molecules were conducted using YASARA. The separated protein and native ligand structures were stored in the file. mol2.

2.4. Ligands Preparation

The ligand preparation was done by downloading the flavonoid compounds from the sappan wood (*Caesalpinia sappan* L.) as the test ligand and metformin as the comparison ligand from PubChem. The conformational search was performed using MarvinSketch and then saved as the file. mol2.

2.5. Docking Protocol Validation

The docking protocol was validated by redocking the native ligand of the glucokinase enzyme (1V4S) using YASARA to obtain the Root Mean Square Deviation (RMSD) value.

2.6. Molecular Docking

Molecular docking was carried out by docking the test ligand (flavonoid compounds from secang) and the comparative ligand (metformin) with the glucokinase enzyme (1V4S) using PLANTS through the Command Prompt (CMD) to obtain docking scores.

2.7. Docking Result Visualization

The docking results of each ligand are visualized using Discovery Studio Visualizer to see the interactions that occur.

2.8. ADME Prediction

ADME (Adsorption, Distribution, Metabolism, and Excretion) of the compound was predicted using the SWISS-ADME web tool predictor (http://www.swissadme.ch) by its canonical SMILES structure from PubChem (https://pubchem.ncbi.nlm.nih.gov).

2.9. Toxicity Prediction

Toxicity prediction of the compound was performed using Toxtree v3.1.0 software (https://toxtree.sourceforge.net/), OSIRIS tool (https://www.organic-chemistry.org/prog/peo/), and pkCSM web tool (https://biosig.lab.uq.edu.au/pkcsm/prediction) by inserting the canonical SMILES structure from PubChem (https://pubchem.ncbi.nlm.nih.gov).

3. RESULTS AND DISCUSSION

3.1. Lipinski Rule of Five

Lipinski's rule of five (Ro5) analyzes drug-like compounds or drug-likeness by looking at a ligand's physical and chemical properties (Chen et al., 2020). Parameters or criteria in the Lipinski rule of five (Ro5) can be the basis that a drug-like compound has good absorption and permeability. Based on **Table 1**, the physicochemical properties of the Secang flavonoid compounds, except protosappanin D and protosappanin E, correspond to the Ro5 rule. It can be concluded that the compounds have similarities to drugs and can be used as test ligands for molecular docking processes.

Table 1. Lipinski rule of five test results for flavonoid compounds

Ligand	Lipinski Rule Of Five Parameters				
	MW	Log P	HBD	HBA	MR
MRK (2-Amino-4-Fluoro-5-[(1-Methyl-1H-Imidazol-2-Yl)Sulfanyl]-N-(1,3-Thiazol-2-Yl)Benzamide)	349	3.001400	3	5	88.210594
Metformin	129	-1.243830	5	5	37.223495
Brazilin	286	1.382200	4	5	72.937180
Brazilein	284	0.958900	3	5	72.594383
Brazilide A	318	0.374400	2	7	72.789581
Butein	272	2.405099	4	5	72.907677
Protosappanin A	272	1.974399	3	5	71.078377
Protosappanin B	304	1.128700	5	6	78.106972
Protosappanin C	302	1.335300	4	6	77.085175
Protosappanin D	604	3.532302	8	12	151.678467
Protosappanin E	586	3.024500	7	11	146.634644
Sappanchalcone	286	2.708099	3	5	77.794876
Sappanone B	302	1.352300	4	6	76.357674
(e)-3-(3,4-Dihydroxy benzylidene)-7-hydroxychroman-4-one	284	2.462099	3	5	75.680878
3,8,9-Trihydroxy-6H-benzo[c]chromen-6-one	244	2.002999	3	5	61.830894
3-Deoxysappanchalcone	270	3.002499	2	4	76.130081
3-Deoxysappanon B	286	1.646700	3	5	74.692879

Molecular weight (MW) indicates the rate of diffusion of a molecule. Compounds with a molecular weight exceeding 500 daltons (Da) will cause the molecule to be unable to diffuse through the cell membrane. Most Secang flavonoid compounds have a molecular weight under 500 Da, and it can be predicted that they can diffuse through cell membranes readily. The Log P value indicates the bioavailability of compounds. All of the Secang flavonoid compounds have Log P lower than 5. It suggests that flavonoid compounds should be well absorbed orally. Most flavonoid compounds have Hydrogen Bond Donor (HBD) lower than five and Hydrogen Bond Acceptor (HBA) lower than 10, except protosappanin d and protosappanin e, which indicates there is not required much energy for the absorption process. Molar Refractivity (MR) most flavonoid compounds have a value between 40-130. It shows good absorption and adequate oral bioavailability (Kilo et al., 2019; Ibrahim et al., 2021).

Protosappanin D has a molecular weight of 604 Da, 8 hydrogen bond donors, 12 hydrogen bond acceptors, and 151.678467 molar refractivities, with 4 violations of the Lipinski rule of five. Protosappanin E has a molecular weight of 586 Da, 7 hydrogen bond donors, 11 hydrogen bond acceptors, and 146.634644 molar refractivities, with 4 violations of the Lipinski rule of five. Protosappanin D and E likely present absorption or permeability issues because they have more than 3 Lipinski's violations (Al Mogren et al., 2020). The native ligand (MRK) has a molecular weight of 349 Da, log P 3.001400, 3 hydrogen bond donors, 5 hydrogen bond acceptors, and 88.210594 molar refractivities. It means that the native ligand follows the Lipinski rule of five. Metformin has a violation in the Lipinski rule of five. The molar refractivity of metformin (37.2223495) is lower than 40. Based on the results of the Lipinski rule of five, native ligand (MRK) and butein have better physicochemical properties than metformin. Hence, metformin still has good bioavailability issues because it only has 1 violation of Lipinski's rule of five (Al Mogren et al., 2020).

3.2. Results of ligand-receptor Molecular Docking with PLANTS

3.2.1. Docking Protocol Validation

The docking protocol was validated by re-docking the native ligand on the target protein (1V4S). The smaller RMSD value indicates that the predicted has a suitable ligand pose because it is closer to the conformational shape of the native ligand. In comparison, the more considerable RMSD value (> 2 Å) results in a substantial difference in conformation between the predicted ligand pose and the native ligand, which can contribute to a high prediction error rate of ligand-protein interaction (Putri *et al*., 2019). The results of the RMSD analysis obtained an RMSD value of 0.6114 from the fifth conformation, which had the lowest score of -102.972 (**Figure 1**). Based on the RMSD value, it can be seen that the validation of the docking protocol is acceptable because it meets the criteria of < 2.0 Å and can be continued for further research processes.

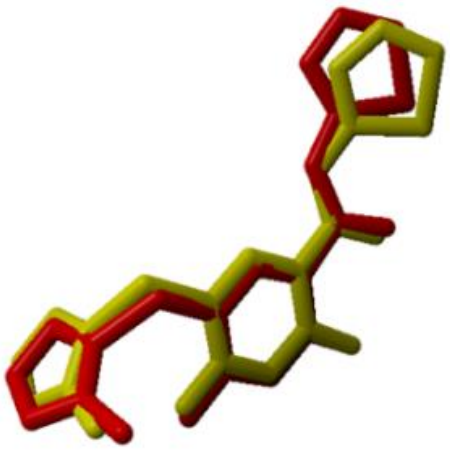

Figure 1. The pose of the native glucokinase ligand (red) and the pose of the re-docking ligand (yellow) with RMSD value = 0.6114

3.2.2. Docking Results

Molecular docking was performed using PLANTS (Protein-Ligand ANT-System) on flavonoid compounds from Secang wood (*Caesalpinia sappan* L.) and glucokinase receptors (PDB CODE: 1V4S).

The docking results between glucokinase protein (1V4S) with flavonoid ligands from Secang wood (*Caesalpinia sappan* L.) are shown in **Table 2**. Based on the docking scores, it showed that all ligands could interact with glucokinase protein. The butein compound has the lowest docking score (-94.4836) compared to the comparison ligand metformin (-59.7875). The lowest docking score indicates that butein has a good affinity and is more stable than metformin. The negative value indicates that the ligand and receptor interaction runs spontaneously (Nitami & Febriansah, 2019; Adriani, 2018). The docking score shows that butein is more stable than metformin when interacting with glucokinase receptors. It can be seen that butein compounds are potential candidates for antidiabetic drugs.

Table 2. The value of the docking ligand score from the molecular docking results

No	Ligand	Score docking	
		Average	Bestranking
1.	MRK (2-Amino-4-Fluoro-5-[(1-Methyl-1H-Imidazol-2-Yl)Sulfanyl]-N-(1,3-Thiazol-2-Yl)Benzamide)	-101.44084	-102.9720
2.	Metformin	-59.14353	-59.7875
3.	(e)-3-(3,4-Dihydroxybenzylidene)-7-hydroxychroman-4-one	-88.67528	-90.4340
4.	3-Deoxysappanchalcone	-80.73401	-81.5542
5.	3-Deoxysappanon B	-81.66752	-84.8542
6.	3,8,9-Trihydroxy-6H-benzo[c]chromen-6-one	-77.70257	-79.8999
7.	Brazilin	-77.17793	-78.2738
8.	Brazilein	-82.74491	-85.0942
9.	Brazilide A	-69.91013	-73.2438
10.	Butein	-93.65594	-94.4836
11.	Protosappanin A	-77.98330	-81.5760
12.	Protosappanin B	-67.55506	-68.4040
13.	Protosappanin C	-66.28681	-67.2410
14.	Sappanchalcone	-86.08326	-86.9112
15.	Sappanone B	-84.65582	-86.4820

3.2.3. Molecular Interactions

The molecular docking results were analyzed using the Discovery Studio Visualizer to see the interactions and the amino acid residues on the active side of the interacting protein. The interaction between the flavonoid compounds of the Secang wood and the glucokinase receptor (1V4S) resulted in hydrogen bonds, van der Waals interactions, and hydrophobic interactions (**Figure 2-Figure 4**).

The visualization analysis (**Table 3**) shows that the hydrogen bonding in butein, the best ligand with the lowest docking score, interacts with the same amino acid residues as the comparison ligand metformin, Arginine 63 (ARG 63). These hydrogen bonds provide a stable interaction between the test ligand and the glucokinase receptor (Sari et al., 2020). In addition, the hydrogen bond distance (**Table 4**) shows that MRK has more hydrogen bonds than butein and metformin. However, butein has the smallest hydrogen bond distance compared to MRK and metformin. The greater the distance of the hydrogen bonds will cause the bonds to break easily; conversely, the smaller the bond distance, the stronger the bond will be (Rachmania et al., 2015).

Butein formed one hydrogen bond with ARG 63 through the oxygen atom, one with TYR 61 through the hydroxyl group, and two with TYR 215 through the oxygen atom and carbonyl oxygen atom. Metformin only formed two hydrogen bonds with ARG 63 and LEU 451 through hydrogen atoms in amino groups. The number of hydrogen bonds formed when the protein

interacts with the ligand will contribute to the stability of the complex structure (Rachmania et al., 2015). It could explain the lower butein binding score compared to metformin.

Table 3. Interaction of native ligand (MRK), test ligand (butein), and comparison ligand (metformin) against glucokinase receptors (1V4S)

Ligand	Amino Acid Residues Involved		
	Hydrogen Bond	**van der Waals interactions**	**Hydrophobic Interaction**
MRK (Native ligand)	ARG 63, SER 64, THR 65	GLN 98, MET 210, GLU 221, HIS 218, ARG 250	TYR 214, VAL 62, ILE 159, VAL 455, PRO 66, ALA 456, ILE 211, MET 235
Metformin (Comparison ligand)	ARG 63, LEU 451	THR 65, ILE 211, GLN 98, PRO 66, VAL 62, VAL 455, ALA 456, ILE 159	-
Butein (Test ligand)	TYR 61, ARG 63, TYR 215	THR 65, MET 210, SER 64, LEU 451, ALA 201	TYR 214, PRO 66, VAL 452, ILE 159, ALA 456, VAL 62, VAL 455, ILE 211

Table 4. Hydrogen bonding distance of MRK, butein, and metformin

Bonding type	Ligand	Amino Acid Residue	Bonding Distance (Å)
Hydrogen Bond	MRK	ARG 63	4.92; 5.97; 7.40
		SER 64	3.52
		THR 65	3.75
	Metformin	ARG 63	5.35; 6.61
		LEU 451	4.46
	Butein	ARG 63	3.87
		TYR 61	5.85
		TYR 215	5.18; 6.63

Electrostatic interactions play a role in the stability of the ligand-receptor complex. Electrostatic interactions are interactions between atoms due to the differences in polarity. These interactions include weak and non-covalent interactions so that they are easily separated. However, many electrostatic interactions can significantly contribute to the conformational formation of protein. One of the electrostatic interactions is the van der Waals interaction which is a relatively weak electric attraction because of the permanent or induced polarity of the molecule. This interaction can occur in charged and uncharged residues (Arwansyah et al., 2014). The interaction between the comparison ligand metformin and the test ligand butein on the glucokinase receptor forms a van der Waals interaction with the same amino acid residue, namely Threonine 65 (THR 65).

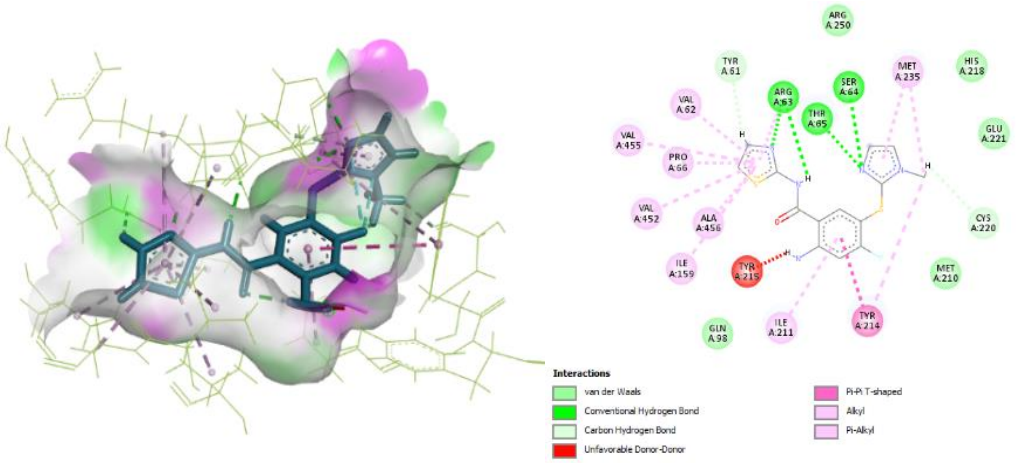

Figure 2. Pose and interaction results of MRK ligands with glucokinase receptors (1V4S)

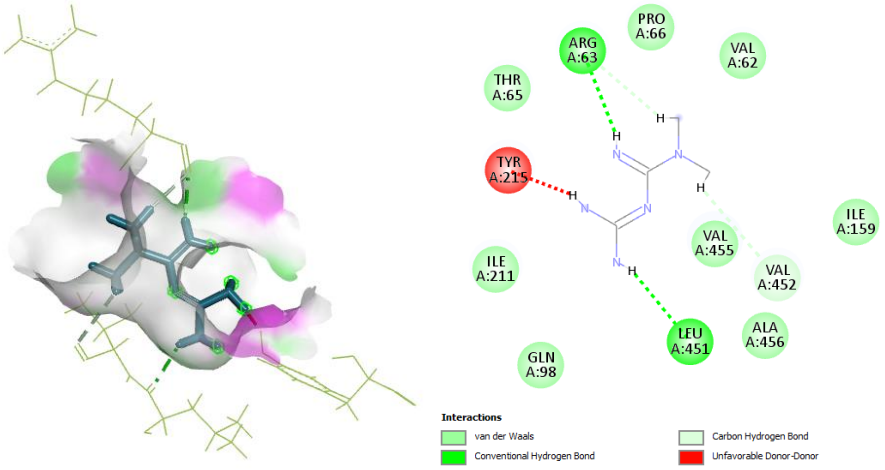

Figure 3. Pose and interaction results of metformin ligands with glucokinase receptors (1V4S)

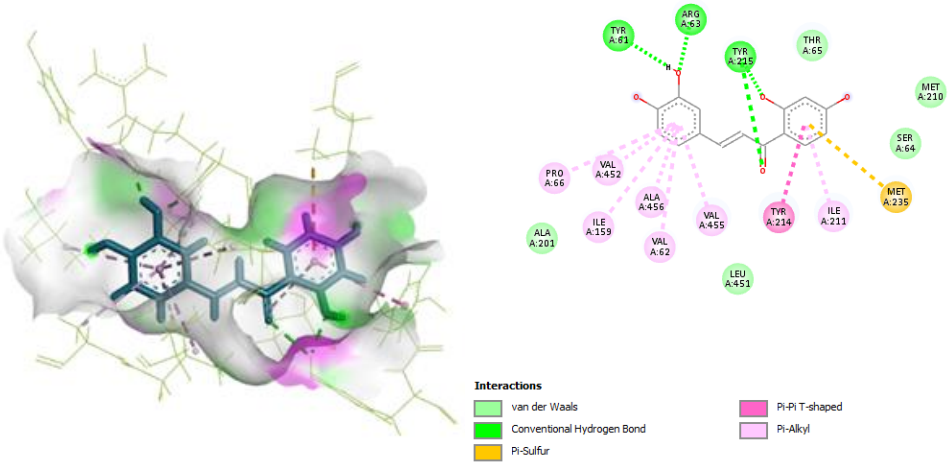

Figure 4. Pose and interaction results of butein ligands with glucokinase receptors (1V4S)

Hydrophobic interactions also play a role in the stability of the complex. These interactions avoid a liquid environment (Arwansyah et al., 2014). The hydrophobic interaction of butein with glucokinase receptors occurs with amino acid residues of Tyrosine 214 (TYR 214), Proline 66 (PRO 66), Valine 452 (VAL 452), Isoleucine 159 (ILE 159), Alanine 456 (ALA 456), Valine 62 (VAL 62), Valine 455 (VAL 455), and Isoleucine 211 (ILE 211). Metformin, as a comparison ligand, did not form a hydrophobic interaction because it does not have Pi-orbitals of the aromatic ring system (Khan et al., 2022).

Butein also forms interactions with the same amino acid residues as the native ligand (MRK) on the amino acid residue of Arginine 63 (ARG 63) via hydrogen bond; Methionine 210 (MET 210) via van der Waals interaction; Tyrosine 214 (TYR 214) (Pi-Pi T-Shaped); Valine 62 (VAL 62), Valine 452 (VAL 452), Valine 455 (VAL 455), Isoleucine 159 (ILE 159), Proline 66 (PRO 66), Alanine 456 (ALA 456), and Isoleucine 211 (ILE 211) via Pi-Alkyl interactions. It is similar to previous studies where MRK forms hydrogen bonds with Arginine 63 (ARG 63) amino acid residue. In addition, van der Waals interactions occur with Leucine 451 (LEU 451) and Serine 64 (SER 64) residue; and Pi-Alkyl interactions occur with Proline 66 (PRO 66), Valine 452 (VAL 452), and Valine 455 (VAL 455) amino acid residue (Astuty & Komari, 2022). The number of similarities between the amino acid residues that interact with MRK and butein allows the docking scores to be similar.

This research showed that butein has a potential antidiabetic drug by inhibiting glucokinase enzyme (1V4S). It is based on the docking score of butein, which is lower than the other test ligands and metformin. In addition, the interaction between butein and glucokinase through

hydrogen bonds and van der Waals interactions with the same amino acid residues as metformin binds, Arginine 63 (ARG 63) and Threonine 65 (THR 65).

3.3. ADME Properties

The results of pharmacokinetics properties for butein, metformin, and native ligand are compared in **Table 5**.

Table 5. Physicochemical and Pharmacokinetic properties of MRK (native ligand), butein, and metformin using SWISS-ADME

Parameters	Compounds		
	MRK (native ligand)	Butein	Metformin
TPSA	139.37	97.99	91.49
log Kp (cm/s)	-6.59	-5.96	-7.99
Consensus Log P	2.29	1.96	-0.89
BBB permeant	No	No	No
GI absorption	Low	High	High
CYP1A2 inhibitor	Yes	Yes	No
CYP2C19 inhibitor	Yes	No	No
CYP2C9 inhibitor	Yes	Yes	No
CYP2D6 inhibitor	Yes	No	No
CYP3A4 inhibitor	Yes	Yes	No
Pgp substrate	No	No	No

MRK's topological surface is (TPSA), butein, and metformin are less than 150 Å, indicating strong polarity. As a consequence, all compounds have good oral absorption and membrane permeation. Log Kp value (< –2.5 cm/s) shows all compounds have high skin permeability (Rajalakshmi et al., 2021). The consensus Log P value is the arithmetic mean of the five Log P prediction values (iLOGP, XLOGP3, WLOGP, MLOGP, and SILICOS-IT) (Daina et al., 2017). Based on the consensus Log P value, MRK and butein are more lipophilic than metformin (Al Mogren et al., 2020).

Pharmacokinetic properties are evaluated by brain penetration (BBB permeant) and passive gastrointestinal absorption (GI absorption). These correlations can be seen in the boiled egg model (**Figure 5**). The position of each compound is a function of WLOGP versus TPSA. Butein and metformin are in a white region, indicating a high gastrointestinal absorption probability and no BBB permeability (Ram et al., 2022). MRK is outside of the egg, predicted as not absorbed in the gastrointestinal and not brain penetrant. All compounds are displayed in a red dot, indicating non-Pgp substrate (Daina et al., 2017). It means there is no issue with the excretion process (Shweta & Rashmi, 2019).

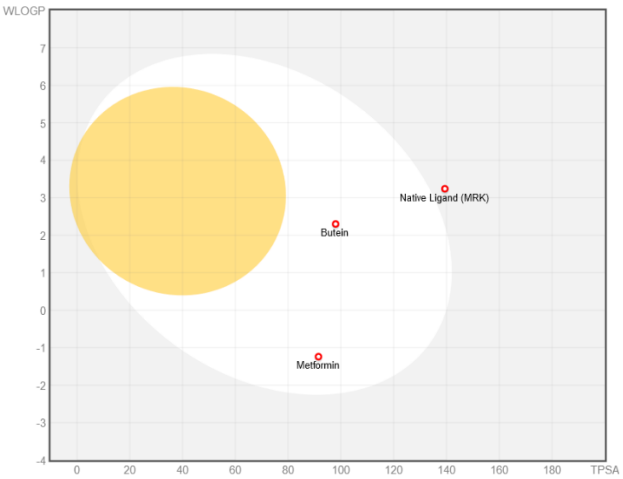

Figure 5. Boiled egg model of MRK, butein, and metformin

The interaction between compounds and cytochromes (CYP) 450 enzyme plays a vital role in liver metabolism. The CYP isoenzymes in SWISS-ADME prediction are CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4. Metformin did not inhibit any CYP isoenzymes, indicating that it is well metabolized in the liver and readily eliminated from the body. However, MRK showed as an inhibitor of all CYP isoenzymes, which means that MRK has poor elimination. Butein is predicted as a CYP1A2 inhibitor, CYP2C9 inhibitor, and CYP3A4 inhibitor. It might accumulate in the body and cause toxicity (Ononamadu & Ibrahim, 2021).

3.4. Toxicity Prediction

Toxicity prediction is critical to detect whether a compound is toxic or non-toxic. In this study, Toxtree, OSIRIS, and pkCSM were used to predict the toxicity of compounds. Toxtree prediction test showed that MRK, butein, and metformin are classified as high-class toxicity compounds based on Crammer's rule. MRK and butein have no risk based on Kroes's TTC decision tree. The structure of MRK and butein has a potential genotoxic carcinogenicity. The test compound's structures showed no significant alert for nongenotoxic carcinogenicity and potential carcinogen based on QSAR (**Table 6**).

Table 6. Toxicity prediction of MRK (native ligand), butein, and metformin using Toxtree, OSIRIS, and pkCMS

Parameters	Compounds		
	MRK (native ligand)	**Butein**	**Metformin**
Toxtree			
Crammer's rule	High Class	High Class	High Class
Kroes TTC decision tree	Negligible risk	Negligible risk	Substance would not be expected to be a safety concern
Negative for genotoxic carcinogenicity	No	No	Yes
Negative for nongenotoxic carcinogenicity	Yes	Yes	Yes
Skin irritation	Unknown	Yes	Unknown
Eye irritation and corrosion	Unknown	Unknown	Unknown
Potential carcinogen based on QSAR	No	No	No
OSIRIS			
Mutagenic	Low risk	High risk	Low risk
Tumorigenic	Low risk	Low risk	Low risk
Reproductive effect	Low risk	Low risk	Low risk
Irritant	Low risk	Medium risk	Low risk
pkCSM			
AMES toxicity	Yes	No	Yes
Hepatotoxicity	Yes	No	No
Pgp substrate	No	No	No

MRK and metformin were classified as having a low risk of mutagenic, carcinogenic, teratogenic, and irritant based on the OSIRIS tool's toxicological prediction. In contrast, butein has a high mutagenic risk and a moderate irritant risk. The pkCSM web application predicted that MRK and metformin are mutagenic. Butein, in contrast, is neither mutagenic nor hepatotoxic. Before butein can be used as a drug, it is necessary to conduct in vivo testing to ascertain its toxicity based on the three toxicity prediction tools.

4. CONCLUSION

All flavonoid compounds from the secang wood (*Caesalpinia sappan* L.) were predicted to have activity against the glucokinase enzyme as indicated by a lower docking score (-67.2410 to -94.4836) than the comparison compound metformin (-59.7875). Butein was predicted to have

the best antidiabetic activity, with a docking score of -94.4836. The interaction occurs between the flavonoid compounds of the secang wood and the glucokinase (1V4S) enzyme through the formation of hydrogen bonds, van der Waals interactions, and hydrophobic interactions. Butein showed good oral absorption and excretion. The toxicity of butein requires further study.

5. ACKNOWLEDGMENT

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6. AUTHOR DECLARATION

Authors' Contributions and Responsibilities

The authors made substantial contributions to the conception and design of the study. The authors took responsibility for data analysis, interpretation, and discussion of results. The authors read and approved the final manuscript.

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All data are available from the authors.

Competing Interests

The authors declare no competing interest.

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No additional information from the authors.

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