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IN SILICO TESTING OF COCOA POD HUSK (*THEOBROMA CACAO* L.) AND KERSEN LEAVES (*MUNTINGIA CALABURA* L.) EXTRACTS AS CANDIDATE DRUGS FOR TYPE 2 DIABETES

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ABSTRACT

Diabetes mellitus is a metabolic disorder caused by elevated blood glucose levels. The incidence of diabetes mellitus continues to rise globally, including in Indonesia. Type 2 diabetes mellitus is the most common type due to the inability of pancreatic beta cells to produce sufficient insulin. Kersen leaves (Muntingia calabura L.) and cocoa pod husk (Theobroma cacao L.) are natural ingredients rich in quercetin and are widely found in Indonesia. A higher quercetin content correlates with its potential as an antidiabetic candidate. The aim of this research is to develop a standardized comprehensive herbal medicine from a combination of cocoa pod husk extract and kersen leaves extract, which is expected to produce more potent antidiabetic activity with a clearly understood mechanism. This research employs an experimental laboratory method, including in silico testing, to determine the target proteins of bioactive compounds and analyze their interactions with alpha-glucosidase and GLUT4, as well as to obtain preliminary toxicity data. The in silico results show that quercetin has the best binding affinity compared to natural AGI ligands and GLUT4 target macromolecules. There is no amino acid residue similarity interaction was found between quercetin and the target macromolecules of AGIs and GLUT4, suggesting that quercetin may potentially bind to eNOS and HIF-1α receptors. The pharmacokinetic predictions indicate that quercetin has a good pharmacokinetic profile, as it meets several of Lipinski's Rule of Five criteria, making it suitable for oral use.

Keywords: ADMET, *Theobroma cacao*, *Muntingia calabura*, Diabetes Mellitus, In Silico

ABSTRAK

Diabetes mellitus adalah gangguan metabolisme yang disebabkan oleh peningkatan kadar glukosa darah. Angka kejadian diabetes melitus terus meningkat secara global, termasuk di Indonesia. Diabetes melitus tipe 2 merupakan tipe yang paling sering terjadi akibat ketidakmampuan sel beta pankreas dalam memproduksi insulin yang cukup. Daun kersen (*Muntingia calabura* L.) dan kulit buah kakao (*Theobroma cacao* L.) merupakan bahan alami yang memiliki kandungan kuercetin yang tinggi dan banyak ditemukan di Indonesia. Kadar kuercetin yang lebih tinggi berkorelasi dengan kemampuannya

sebagai kandidat antidiabetes. Tujuan dari penelitian ini adalah untuk mengembangkan obat herbal terstandar yang komprehensif dari kombinasi ekstrak kulit buah kakao dan ekstrak daun kersen yang diharapkan dapat menghasilkan aktivitas antidiabetes yang lebih poten dan mekanismenya dapat diketahui dengan jelas. Metode penelitian ini bersifat eksperimental laboratorium termasuk uji in silico yang dilakukan untuk menentukan protein target dari senyawa bioaktif dan menganalisis interaksinya dengan alfa glukosidase dan GLUT4 serta memperoleh data awal toksisitas. Hasil penelitian In Silico, senyawa kuersetin memiliki afinitas ikatan terbaik dibandingkan dengan ligan alami AGI dan makromolekul target/reseptor GLUT4. Tidak ditemukan adanya interaksi kemiripan residu asam amino antara kuersetin dengan makromolekul target AGIs dan GLUT4, sehingga senyawa kuersetin diprediksi berpotensi untuk berikatan dengan reseptor eNOS dan HIF-1α. Hasil prediksi farmakokinetik menunjukkan bahwa senyawa kuersetin memiliki profil farmakokinetik yang baik karena memenuhi beberapa persyaratan lima aturan Lipinski sehingga dapat digunakan secara oral.
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Kata kunci: ADMET, *Theobroma cacao*, *Muntingia calabura*, Diabetes Mellitus Tipe 2, In Silico

INTRODUCTION

Diabetes mellitus is currently a global health threat. The prevalence of diabetes mellitus globally is increasing, reaching 3 times in 2030. This increase has been predicted by the World Health Organization that in 2030 the prevalence will reach 21.3 million. This figure will reach 16.7 million in 2045 according to the International Diabetes Federation (Soelistijo, 2021). According to the national prevalence of diabetes mellitus data, around 20.4 million Indonesians or as many as 8.5 percent have been diagnosed with diabetes mellitus (RISKESDAS, 2019). The pathophysiology of diabetes mellitus 2 is the failure of pancreatic beta cells that cause glucose cannot be metabolized and results in insulin resistance. This results in increased glucose production in the liver (Soelistijo, 2021). The enzyme alpha-glucosidase plays an important role in the hydrolysis of carbohydrates into glucose, which when inhibited can delay glucose absorption. Acarbose as an antidiabetic agent can treat type 2 diabetes mellitus (Azizah & Novrianti, 2022).

The potential of natural ingredients as antidiabetic agents is considered relatively safer for consumption, as they tend to have fewer side effects compared to conventional medicine. The active compounds found in plants are believed to help lower blood glucose (Zahroh & Musriana, 2018). One of them is cocoa pod husk containing flavonoid compounds that have the potential to reduce blood glucose levels (Ginting et al., 2019). Likewise, kersen leaves contain flavonoids, tannins, triterpenoids, steroids and polyphenols. Flavonoids, specially quercetin as active ingredients or active compounds in plants are thought to be able to reduce blood glucose levels by increasing insulin secretion, reducing insulin resistance and encouraging glucose transporter type 4 (GLUT4) (Aligita et al., 2018). The compound content of these two plants can be developed into potential new natural medicines with effectiveness as antidiabetics.

METHOD

Research Design

Tools, Software, and Materials

This research utilizes hardware such as a laptop with the brand "ASUS" X455L, featuring specifications including an Intel Dual Core N2840 processor, up to 2.58 GHz, 2 GB DDR3 RAM, 500 GB hard disk, and Intel HD Graphics. The software used includes VegaZZ 2.4, AutoDockTools 1.5.6, BIOVIA Discovery Studio 2024 version 24.1, PyMOL 2.3.3 and web servers such as PubChem, swissADME, and proTox.

The materials used include a two-dimensional structure of the quercetin, which was drawn and optimized using VegaZZ. Two macromolecular target structures, AGIs and GLUT4, which have been

crystallographically determined, were obtained from the Protein Data Bank website. The two-dimensional structure of the quercetin test compound was meticulously crafted by utilizing the SMILES representation generated from PubChem. The SMILES results were then transcribed into the VegaZZ software under the edit → build → SMILES column and subsequently processed using the build function. The resulting compound structure was meticulously saved in .pdb format and conscientiously stored in a specific folder.

Ligand Preparation

The test compound quercetin was searched for its name on PubChem. Created in 2-dimensional form with SMILES code C1=CC(=C(C=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O) which was then copied and pasted in VegaZZ software in the edit → build → SMILES column and then click the build button. Then the compound structure will be formed which is then saved in .pdb format and placed in a specific folder.

PubChem consists of three different and interrelated main databases namely substance, BioAssay and compound. The Substance Database (SID) contains sample description information provided by individual data contributors to PubChem including chemical descriptions, chemical names (synonyms), external registration identifiers and cross-links (Fu et al., 2015).

RCSB PDB has various features that can be used for structure querying, browsing, analysis and molecular visualization. The website can also be used to perform searches including PDB ID, name, sequence and SMILES ligand. External classification and annotation systems to organize PDB data in hierarchical trees for browsing and searching (e.g. membrane protein, gene ontology and enzyme classification) (Rose et al., 2015).

Autodock (Automated Docking of Flexible Ligands to Receptors) is a program used to dock effective molecules in a fast and accurate way and can be used to predict the conformation and energy of a bond between a ligand and a receptor (Forli et al., 2016)

PyMol is one of the visualization programs used to understand a structure and produce quality three-dimensional images and display structures in several colors of a micromolecule or macromolecule such as a protein. The purpose of visualization is to better understand and explore the structure of a molecule.

VegaZZ plays a role in the molecular docking process equipped with three-dimensional graphics and is used to solve various computational chemistry problems including drug design, ligand optimization, homology modeling of proteins and molecular QSAR (Quantitative Structural Analysis Relationship) calculations (Pedretti et al., 2021).

Discovery Studio is software used to open, edit data and to analyze data generated by other software. It is designed to view and edit molecular structures, X-ray sequences and reflections, scripts and other data. Discovery Studio displays high-quality images.

The webservers used to perform small molecule toxicity prediction are pkCSM (biosig.lab.uq.edu.au/pkcsm/prediction) and proTox (http://tox.charite.de/tox) pkCSM for prediction of ADMET properties is divided into two groups: regression models to predict numerical quantification of toxicity properties and classification models into two classes.

SwissADME (swissadme.ch) is a webserver that can be used to assess pharmacokinetics and drug similarity with a compound using the boiled egg method, iLOGP and bioavailability radar (Daina & Zoete, 2016)

Research Procedure

The used ligand, which is the two-dimensional structure of the quercetin test compound, was created using SMILES by searching for the compound name on PubChem, then preparation was carried out using the VegaZZ software in .pdb format, followed by Autodock Tools to determine the number of rotatable atoms based on the number of torsions in each molecule, adding hydrogen atoms and charges, and then saved in .pdbqt format. The target macromolecules used are GLUT4 with the code 7WSN and AGIs with the code 5KZW, the structures was downloaded from the Protein Data Bank (PDB) in .pdb format. The initial preparation was carried out using Autodock Tools to remove water components and bound ligands, and the structure is saved in .pdbqt format. The ligand and target macromolecule are used for the molecular docking process using Autodock Tools. The docking process was performed, and after completion, an analysis is conducted on the affinity values (ΔG binding), the types of interactions between the test ligand and the target macromolecule, as well as its pharmacokinetic and toxicity profiles using the software Discovery Studio Visualizer, swissADME, pkCSM, and proTox.

Data Analysis

The validation of the molecular docking method is conducted to assess its appropriateness by comparing the conformation of the tethering results with the original ligand's crystallographic structure. This is evaluated through the root mean square deviation (RMSD) value, with an RMSD below 2-5 indicating the method's validity. For bond energy analysis, the binding energy (ΔG binding) values are recorded and compared across different protein-compound interactions. A lower ΔG binding value signifies a more stable binding. Interaction data is gathered by merging the docking results and macromolecular data in PyMOL, visualizing the ligand-protein complex, and using Discovery Studio Visualizer to analyze interactions between the compound and the target protein amino acid residues. The 2D interaction patterns are compared with those of the original ligand. If the patterns align, the compound is considered to potentially bind actively to the target macromolecule. As a final step, the pharmacokinetic and toxicity profile of the compound is evaluated using SwissADME and proTox 3.0, which provide information on parameters such as bioavailability, P-gp substrate activity, gastrointestinal absorption, blood-brain barrier penetration, and toxicity after running the analysis.

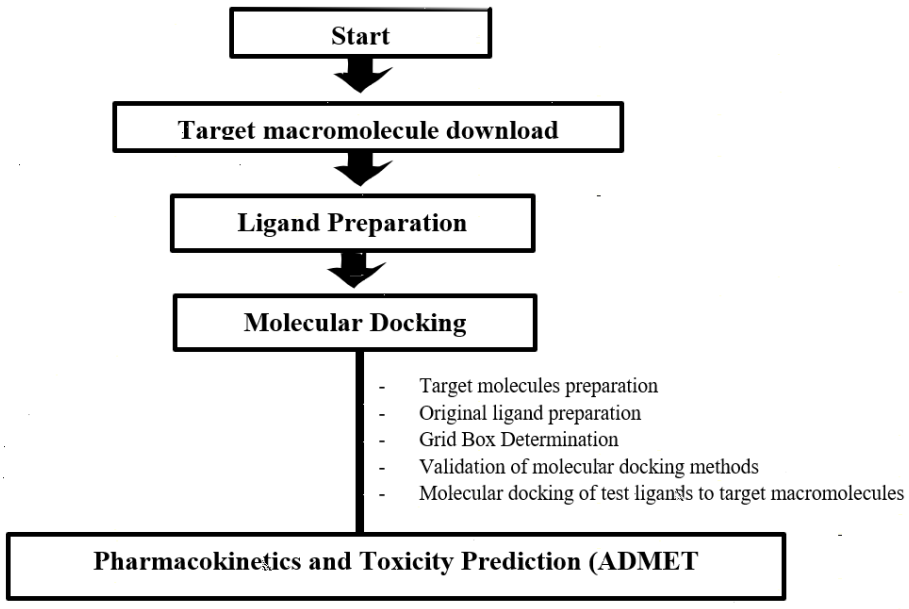


Figure 1. Schematic Procedure of In Silico Testing

RESULTS AND DISCUSSION

Quercetin is a pentahydroxyflavone having the five hydroxy groups placed at the 3-, 3'-, 4'-, 5- and 7-positions and has molecular formula $C_{15}H_{10}O_7$. It is one of the most abundant flavonoids in edible vegetables, fruit and wine. It has a role as an antibacterial agent, an antioxidant, a protein kinase inhibitor, an antineoplastic agent, an EC 1.10.99.2 [ribosyldihydronicotinamide dehydrogenase (quinone)] inhibitor, a plant metabolite, a phytoestrogen, a radical scavenger, a chelator, an Aurora kinase inhibitor and a geroprotector. It is a pentahydroxyflavone and a 7-hydroxyflavonol. It is a conjugate acid of a quercetin-7-olate.(NCBI, 2024). The two-dimensional structure of the quercetin test compound was meticulously crafted by utilizing the SMILES representation generated from PubChem.

In the real of in silico research, the molecular docking method was employed using Autodock software tools and visualized using Discovery Studio Visualizer software. The results obtained encompass the affinity value (energy binding affinity) and the interaction of ligand amino acid residues with the target macromolecule. The binding affinity data reveals the strength of the bond between the ligand and the target macromolecule. A more negative or lower affinity value indicates a more stable and stronger bond energy. The results of the affinity value in this study are presented in Table 1. Notably, in the docking with the target macromolecule AGIs, the compound quercetin surpassed the original ligand. Similarly, in the docking with the GLUT4 target macromolecule, the quercetin compound demonstrated superiority over the original ligand. It is essential to consider factors that influence the results of molecular tethering affinity values, such as the type of bond that occurs, the distance between the atoms that bind, and the geometry of the ligand and macromolecular target (NaAllah et al., 2021)

Table 1
Molecular docking results against AG1s and GLUT4

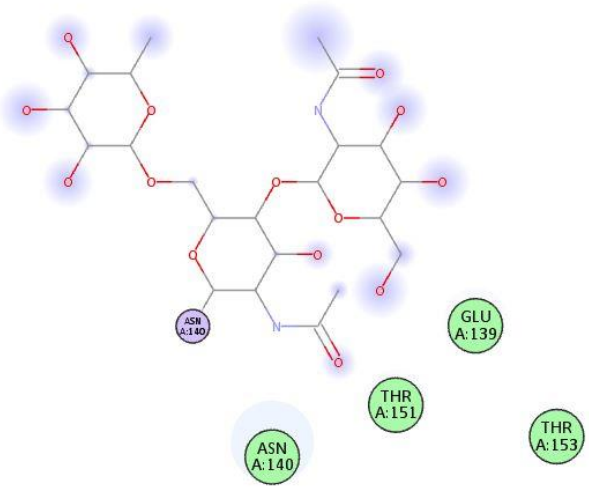
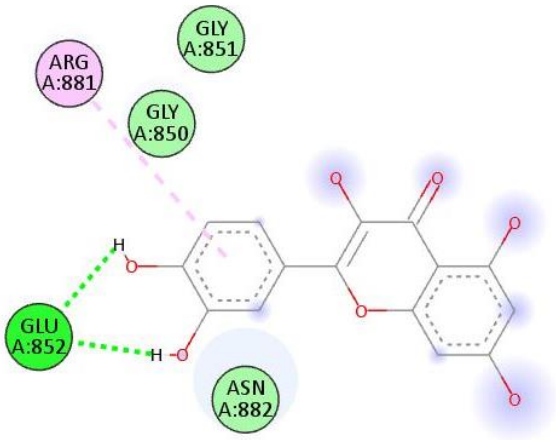
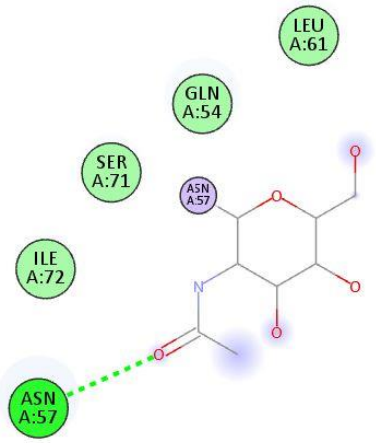
Compound	ΔG*binding affinity* (kkal/mol) ± SD	
	AG1s	GLUT4
Native Ligand	-1.25 ± 0.134	-3.05 ± 0,094
Quercetin	-2.86 ±	-4.61 ± 0.03

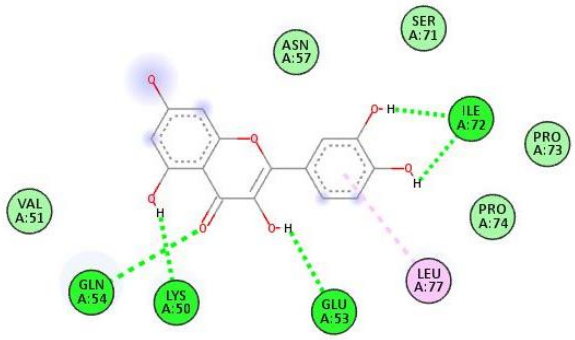
The interaction between the ligand and the macromolecule may influence the affinity value. This interaction can encompass various types, including hydrogen bonding interactions, as well as non-hydrogen bonding interactions such as Pi-sigma, alkyl, P-alkyl, and Van der Waals bonds, among others. The results of the docking and visualization of ligands with target macromolecules are provided in tables 2 and 3.

Table 2
Interaction of ligand and target macromolecule

Target macromolemule : AG1s			
Amino Acid Residue Interaction			
Compound	Non-Hydrogen	Hydrogen	Type of Interaction
Native Ligand	-	-	Tidak ada interaksi
Quercetin	Glu852	Arg881	Hydrogen bond, *Pi-Alkyl* bond
Target macromolecule : GLUT4			
Amino Acid Residue Interaction			
Compound	Non-Hydrogen	Hydrogen	Type of Interaction
Native Ligand	-	Asn57	Hydrogen bond
Quercetin	Leu77	Gln54, Lys50, Glu53, Ile72	Hydrogen bond, *Pi-Alkyl* bond

Table 3
The Visualization of the Ligan-Macromolecul target interactions

Compound	2-dimensional interaction
Target Macromolecule : AG1s	
Native Ligand	
Quercetin	
Target Macromolecule : GLUT4	
Native Ligand	

Quercetin	
Interaction Type Description : van der Waals Conventional Hydrogen Bond Alkyl Pi-Alkyl	

Based on table 2, the native ligand on the GLUT4 macromolecule binds one amino acid, namely Asn57 amino acid hydrogen bond. The result of quercetin compound docking to the macromolecule GLUT4 macromolecule has one Pi-Alkyl bond that involves alkyl groups on the amino acid residue Leu77 and four hydrogen bonds that occur between O and H atoms on amino acid Asn57. which occurs between O and H atoms on amino acids Gln54, Lys50, Glu53 and Ile72. Results quercetin docking to AGIs target macromolecules binds one amino acid with the Glu852 hydrogen bond interaction that occurs at the O atom of quercetin to the amino acid. The result of AGIs docking results in no amino acid residues because docking does not occur on the active side of the target macromolecule. The overall results of quercetin docking to macromolecules AGIs and GLUT4 each had no amino acid residues in common, neither hydrogen bonds nor other bonding interactions. hydrogen bonds and other bonding interactions. The AGIs docking results did not produce amino acid residues amino acid residues because docking does not occur on the active side of the target macromolecule.

Based on the results of molecular docking to two target macromolecules with test compounds that quercetin has a better binding energy value than the original ligand, but there is no amino acid residues with the same type of interaction as the original ligand. The process The next process was to predict the pharmacokinetics of the test compound using the webserver swissADME, pkCSM and proTox which are equipped with the parameters of each assay to assess the compound's pharmacokinetic profile.

1. Physicochemical Parameters

Identification of chemical physical properties and pharmacokinetic profiles in silico is an initial part that plays a role in supporting the development of drug compounds, namely determining the right method in a formulation, so that effective, stable and safe drug preparations are obtained. Generally, the mechanism of action of drugs is bound to the receptor site, drug molecules before reaching the receptor site interact through various membranes, biopolymers and fluid compounds outside and inside the cell so that they bind to each other to cause therapeutic effects. Chemical physical properties support the typical orientation of molecules on the receptor surface associated with biological activity, namely the absorption and distribution of drugs so that at a certain time

drug levels in large enough quantities can reach the receptor, but only drugs that have a typical structure can interact with biological receptors (Beale & Block, 2011)

The theory used to distinguish drug and non-drug compounds from compound structures and predict the likelihood of success or failure of drug compounds caused by the similarity of drugs to molecules against two or more criteria is the theory of Lipinski's rule of five. According to Lipinski's theory, drug compounds must meet several criteria, including drug compounds having a molecular weight (BM) of less than 500 g/mol, the value of the partition coefficient (logP) associated with lipophilicity or hydrophobicity, namely the ability of compounds to dissolve in fats, oils, lipids and non-polar solvents does not exceed five, the value of Hydrogen Bond Donors (HBD) or the number of hydrogen bond donors is not more than five and the value of Hydrogen Bond Acceptors (HBA) or the number of hydrogen bond acceptors is not more than ten (Chagas et al., 2018). Lipinski's rule offive is used to determine the physicochemical properties of test ligands in determining compounds belonging to the hydrophobic or hydrophilic category through cell membranes by passive diffusion. Molecular weights greater than 500 g/mol cannot diffuse through the cell membrane. Log P shows the value of the solubility coefficient in fat or water, if the log P value is more than five, the compound is more hydrophobic. Compounds that are too hydrophobic result in compounds that have a high level of toxicity because they are retained longer in the lipid bilayer and spread more widely in the body so that the selectivity of binding to the target enzyme is reduced. A negative Log P value is also unfavorable because the molecule cannot pass through the lipid bilayer membrane. The number of hydrogen bond donors and acceptors illustrates that the higher the hydrogen bonding capacity, the higher the energy required for absorption to occur. In general, Lipinski's rule explains the permeability and solubility of certain compounds through cell membranes by passive diffusion (Lipinski et al., 2001).

Based on the results of the molecular docking data carried out, there are quercetin test compounds that have values and visualization results that are in accordance with the original ligand, then predict the pharmacokinetic profile using Lipinski's rule to analyze the absorption, distribution, metabolism and excretion processes in the body.

Tabel 4
Lipinski rules of quercetin with target macromolecules

Ligand	**BM (g/mol)**	**LogP**	***Hidrogen Bond donors***	***Hidrogen Bond acceptors***	***Druglikeness***
Quercetin	302.24	1.54	5	7	*Yes*

From the data in Table 4, quercetin compounds fulfill several requirements of Lipinski's rule. The molecular weight is relatively less than 500 g/mol which makes the chemical synthesis and molecular design process not complex so that it takes a short time, the log P value is less than five in quercetin which means that the compound tends to dissolve in non-polar solvents but is relatively soluble in polar solvents so that it is good in the process of absorption and permeation. The number of hydrogen bond donor values meets the requirements of not more than ten and the number of hydrogen bond acceptor values does not meet the requirements because the test results show more than five. The greater the number of hydrogen bond donors or acceptors, the wider the polar surface area. Quercetin compounds also have a good level of similarity with oral drugs (drug likeness) indicated by the fulfillment of the lipinski criteria in their observations of most drugs that are relatively small in size and lipophilic (Kristin, 2010).

2. Absorpstion

The pharmacokinetic process includes absorption in the digestive tract, distribution through the blood, followed by metabolism into the form of active metabolite compounds and excreted out of the body (ADME). This process plays a role in the availability of drugs to reach drug target

tissues or receptors so as to cause a biological response. To provide a biological effect, the compound must pass through the absorption process to produce drug bioavailability, which is the active compound in the blood fluid (pH = 7.4) which will be distributed to tissues or organs.

Tabel 5
Lipinski rules of quercetin

Ligand	*GI abs*	*Bioavailabilitas score*	*Pgp Substrat*
Quercetin	High	0.55	No

The absorption prediction results in Table 5 show that the quercetin compound has a good gastrointestinal absorption rate with a poor bioavailability value of 0.55. The small bioavailability value is not an influential basis for the effectiveness of the compound in the target tissue. The results of Pgp substrates of all test compounds are not Pgp substrates, so it is predicted that they will be well absorbed because they are not released by cells (Finch & Pillans, 2014). Based on the bioavailability radar, quercetin compound has good pharmacokinetic parameter prediction results to become a drug that can be consumed orally. To be a drug that can be consumed orally, a compound must have good bioavailability. Figure 1 shows the bioavailability radar of quercetin in the pink area which has the optimal value of each parameter, namely lipopholity: XLOGP3 between -0.7 to +5, size: BM between 150 to 500 g/mol, polarity: TPSA between 20 to 130 Ä', solubility: Log S is not higher than 6, saturation: the fraction of carbon in sp3 hybridization is not less than 0.23 and flexibility: no more than 9 rotable bonds (Daina et al., 2017)

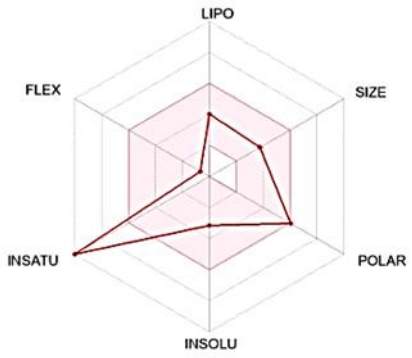


Figure 2. Compound bioavailability radar

Based on the bioavailability prediction results of the compounds presented in Table 6, it shows that quercetin in two indicators does not meet the parameters, namely TPSA polarity 131.36 Á and sp saturation value 0.00, while other parameters meet the requirements such as molecular weight 302.24 g/mol, XLOGP3 value 1.54, flexibility characterized by rotable bond 1 and solubility with a value of -3.91.

Table 6
Bioavailability Prediction

Compound	XLOGP^3	BM (g/mol)	Solubility TPSA	Polarity	Saturation	Flexibility
Quercetin	1.54	302.24	131.36	-3.91	0.00	1

3. Distribution

The drug in a sufficiently active form must be able to interact with receptors or cells target cell. The levels of active compounds in different compartments are related to distribution, metabolism and excretion of a drug. Blood brain barrier (BBB) is an essential diffusion layer that functions

as a barrier to the central nervous system). The blood brain barrier cell layer is different from the cell layer in other parts of the body due to the absence of penetration, metabolism and excretion of drugs. due to the absence of penetrators, the relationship between cells using tight junctions (TJ) and pin transport. (TJ) and transport by pinocytosis. Cell layer tight junctions restrict the flow of hydrophilic to penetrate into the BBB (Budiarsa et al., 2019). Profile prediction results distribution parameters in the table show that test compounds that can penetrate the blood-brain barrier are considered unfavorable in the distribution process. Blood-brain barrier are considered unfavorable in the distribution process because the compounds are lipophilic which affect the nervous system in the brain and tend to be difficult to excrete**.**

Table 7
Prediction results of compound distribution parameters

Ligand	BB Permeant
Quercetin	No

GI absorption and penetration of the blood brain barrier play an important role in the drug development process. Important in the drug development process, the boiled egg method can be used to calculate the polarity and lipophilicity of a compound because the boiled egg dataset has accuracy, speed and proven graphical output. In the boiled egg method, the high probability of passive absorption of the GI tract is indicated by the white region, while the yellow region represents the high probability of penetration into the blood brain barrier. In addition, the blue color indicator indicates that the molecule is actively secreted by P-glycoprotein, represented by (PGP+), while the red color indicator indicates that the compound is a nonsubstrate of P-gp, represented by (PGP-) (Daina & Zoete, 2016). Based on the results of the examination with the boileg egg method presented in Figure 2 shows that the quercetin compound predicted pharmacokinetic profile using the boiled egg method gives the result that the quercetin compound is outside of the yellow boiled egg area, which means that the compound does not penetrate the blood-brain barrier and is in the white-colored area, which means it has the ability to penetrate the blood-brain barrier. white area which means it has good digestibility. Indicator P-gp substrate indicator shows a red circle which means that the compound is not good in passing through the cell membrane because the test compound does not form a substrate with PGlycoprotein (P-gp No result).

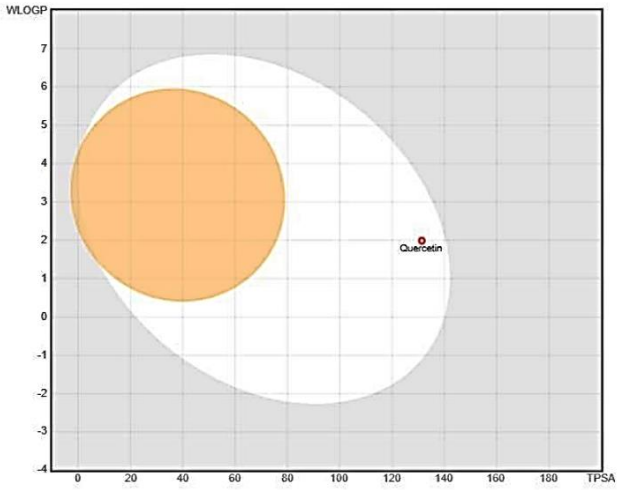


Figure 3. Boiled egg method inspection results

3. Metabolism

Drug compounds are generally metabolized in the liver (liver) with the help of Cytochrome P450 enzymes (CYP) enzymes which are isoenzymes that play an important role in the process of

metabolizing or converting drugs into active metabolites and the process of xenobiotic detoxification. Some CYP isoenzymes that are important in drug metabolism include CYP1A2, CYP2C19, CYP2C9, CYP2D6 and which often receives attention because the majority of drugs are metabolized by CYP3A4 (Kirchmair et al., 2015). Prediction of the metabolic value of the test compound using parameters of CYP1A2 inhibitors, CYP2C19 inhibitors, CYP2C9 inhibitors, CYP2D6 inhibitors and CYP3A4 inhibitors which are substrates of CYP isoenzymes including therapeutic molecules. Inhibition of isoenzymes leads to inhibition of metabolism of other drugs and the potential for drug interactions related to the pharmacokinetic profile of the pharmacokinetic profile of a compound, leading to unwanted side effects due to lower excretion (Kirchmair et al., 2015).

Tabel 8.
Prediction Result of Metabolic Parameters

Ligan	CYP1A2 Inhibitor	CYP2C19 Inhibitor	CYP2C9 Inhiibitor	CYP2D6 Inhibitor	CYP2D6 Inhibitor
Quercetin	Yes	No	No	Yes	Yes

The criteria for substrates that can be metabolized by CYP3A4 are compounds with large molecular weight and lipophilic. In general, more than 50% of drugs before further analysis are often predicted its ability as a CYP3A4 enzyme substrate first. Inhibitors of CYP3A4 enzyme cannot accelerate metabolic reactions (Kirchmair et al., 2015). CYP1A2 enzymes have a major role in the metabolism of CYP1A2 enzyme has a major role in drug metabolism in humans, based on the prediction results of CYP1A2 enzyme can accelerate the metabolic reactions of all ligands with the best molecular tethering values. Substrate criteria The substrate criteria of this enzyme are planar, aromatic, polyaromatic, heterocyclic amide and amine compounds. Enzyme CYP1A2 enzyme is found in the endoplasmic reticulum and its expression is induced by several aromatic hydrocarbons (PAHs).(PAHs). The endogenous substrate of the enzyme is unknown but it is able to metabolize some PAHs into carcinogenic intermediates (Daina & Zoete, 2016). The CYP2C19 enzyme has a pharmacokinetics in metabolizing antimalarials, antifungals and antidepressants. CYP2C19 enzyme criteria neutral or weakly basic compounds or amides with two to three hydrogen bond acceptors and generally a proton pump inhibitor compound. The CYP2C9 enzyme has a role in phase I metabolism by selectively oxidizing small lipophile molecules and limiting the bioavailability of drugs with a narrow therapeutic index of oral treatment as an enzyme in metabolism Thus, inter-individual variability in CYP2C9 protein expression and activity affects efficacy as well as drug safety with the criteria being weak acidic compounds with hydrogen bond acceptors (Elfaki et al., 2018). The enzyme CYP2D6 or often known as debrisoquin hydroxylase and is a CYP isoenzyme whose activity is inhibited by drugs such as quinidine, paroxetine, terbinafine. This enzyme functions as a catalyst for basic compounds with protonated nitrogen atoms of four to seven, such as plant compounds containing alkaloids and antidepressants, the enzyme CYP2D6 found in the liver, small intestine and kidneys can metabolize more than 50% of the drugs that are often used. Predicted metabolic profile in table 8 of quercetin compound excluding inhibitors of CYP2C19 and CYP2C9.

4. Excretion

The plasma level of the drug or compound will decrease and the duration of the drug or compound's effect depends on the rate of metabolism and excretion. Produces an effect depends on the rate of metabolism and excretion. These two factors determine the speed of drug elimination, which is expressed as half-life or T½, namely the time span during which the plasma level of the drug in the elimination phase decreases by half. Half-life depends on the speed of biotransformation and excretion of the drug. Drugs with fast metabolism, the half-life is short. Prediction process The last pharmacokinetic prediction process is excretion. The most commonly

reported basic parameters of excretion most commonly reported are T½, drug clearance and drug distribution volume, but the swiss ADME webserver cannot predict excretion parameters.

5. Toxicity

Toxicity testing is necessary for the drug development process. Based on the prediction results Toxicity prediction results using Protox webserver and pkCSM obtained toxicity prediction data in Table 9 where quercetin is not classified as hepatotoxic including toxicity class 3. Table 9 where quercetin is not classified as hepatotoxic, including toxicity class 3. The smaller the number or number, the more toxic the compound prediction is and if the larger the number, the safer the compound is the safer a compound is. The smaller the number or number, the more toxic the prediction of the compound and if the larger the number, the safer the compound.

Table 9
Toxicity Prediction Results

Compound	LD50 (mg/kg)	Toxicity Class Prediction	Hepatotoxic	Mutagens	Cytotoxicity
Quercetin	159	3	*No*	*No*	*No*

CONCLUSIONS AND SUGGESTIONS

The study suggests that quercetin compounds exhibit superior binding affinity to AG1s and GLUT4 target macromolecules compared to their native ligands, despite no observed interactions involving amino acid residue similarity. Additionally, quercetin compounds are predicted to have the potential to bind to eNOS and HIF-1α receptors. Pharmacokinetic predictions indicate that quercetin compounds possess a favorable profile, meeting several of Lipinski's rules, making them suitable for oral administration. However, these findings are based on predictive simulations, necessitating validation through in vitro and in vivo testing. The study further recommends the use of molecular docking and ADMET predictions, utilizing various software tools such as Libdock, PLANT, and ADMETlab, alongside comparative analysis with standard drugs. Expanding the research to include additional molecular targets is also suggested to enhance understanding and contribute to drug development efforts.

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ETHICAL CONSIDERATIONS

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