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RESEARCH ARTICLE

Potential of Quercetin in Bitter Melon (*Momordica charantia L*) as an Anti-diabetic: In Silico Study

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Abstract

Purpose: The purpose of this study is to predict the in silico molecular interactions of bitter melon quercetin with GLP-1 receptors using the comparable compounds of myricetin and allosteric modulators. Methods: The in silico test was used to predict the quercetin molecular interactions by docking using the Molegro Virtual Docker computer program. The receptor used was human GLP-1, code PDB: 5VEX with allosteric modulators ligand (97V_1201 [A]). As part from predicting molecular interactions, this study also carried out the prediction of its pharmacokinetic properties (ADME) and the toxicity of quercetin and myricetin using the pkCSM online tool program. The data analysis was performed by comparing the docking bond energies between quercetin, the allosteric modulator ligands and the myricetin comparators at the target receptor. The lower the bond energy of the ligands to the target receptor, the more stable the bonds are. This can be used to predict the biological activity of the compound. Results: The in silico test results showed that the bond energy of quercetin = -70.2678 kcal/mol, myricetin = -105,298 kcal/mol and allosteric modulators = -126,992 kcal/mol. Conclusions: The above test results indicate that quercetin has antidiabetic potential by activating the GLP-1 receptor although it is lower than the myricetin and allosteric modulators. The results of the in silico test using the pkCSM online tool program showed that the quercetin compound had good pharmacokinetic properties and a low toxicity level.

Keywords: *ADME, GLP-1 receptor, Quercetin, Myricetin, Allosteric modulators.*

Introduction

Diabetes Mellitus (DM) is a group of metabolic diseases with the main characteristic of hyperglycemia that occurs due to abnormalities in insulin secretion, insulin action or both [1]. Based on a report by the [2], the number of DM sufferers in the world is 463,000,000 people. It is estimated that DM sufferers in the world in 2030 will increase by 24.8% to 578,000,000 people.

Furthermore, the IDF stated that in 2019, Indonesia ranked 7th - after China, India, the USA, Pakistan, Brazil and Mexico to be among the top 10 countries with the most DM sufferers in the world. According to Made and Pathni (2018), this condition requires a continuous, comprehensive and multi-factor management strategy [3]. It is also known that pharmacological interventions to treat hyperglycemia increase the insulin sensitivity and secretion through drug administration, either by increasing the concentration of Glucagon-Like Peptide-1 (GLP-1) by inhibiting dipeptidyl peptidase-4 (DPP4) and the Glucagon-Like Peptide-1 Receptor Agonist (GLP-1 RA) or by delaying the absorption of glucose in the small intestine (alpha-glucosidase inhibitors) [4, 5]. The Indonesian Endocrinology Association (PERKENI) consensus states that GLP-1 RA-based DM treatment is a new approach.

The excretion = ADME) and interaction of the compounds with the receptors, the predicting of the mechanism of action and the selectivity and toxicity of the compounds is now modelled through computer simulation methods. This test has several advantages including security, being free from chemical waste, easy, low cost and able to shorten the research time [6]. The bitter melon plant (Momordica charantia L) is a herbal plant that has potential as an antidiabetic [7]. Bitter melon is rich in various bioactives used for managing type 2diabetes. GLP-1 RA can work on the pancreatic beta cells, thereby increasing insulin secretion [8].

GLP-1 R is activated by GLP-1 which is an incretin hormone that plays a role in controlling the secretion of insulin, glucagon and somatostatin to facilitate glucose treatment in cells while also increasing the proliferation and survival of pancreatic ß cells in experimental animals [9].GLP-1 R can also be activated by other bioactive compounds, namely flavonoids, but there are no reports of previous research on this activation based on the in silico test.

The in silico test is an approach to testing used to predict the chemical properties of molecular physics and the pharmacokinetic properties (absorption, distribution, metabolism components) of minerals, alkaloids, vitamins, steroid saponins, polypeptides and aromatic essential oils [10]. Bitter melon also contains a group of phenolic acid compounds and flavonoids.

The phenolic acid compound group consists of gallic acid while the flavonoid group consists of kaempferol and quercetin [11]. One of the phytochemicals from the flavonol group among the flavonoids is quercetin with a chemical structure of 2- (3, 4-dihydroxyphenyl) 3, 5, 7-trihydroxychromen -4-one. Quercetin is part of a group of polyphenols that are widely distributed in the secondary metabolites of plants.

Quercetin has been found to function as an anti-mutagenic, anti-oxidative, anti-inflammatory, anticancer/chemo preventive, neuroprotective, antihypertensive and with the ability to the lower blood glucose level [12]. In addition to its main function as an antioxidant, quercetin from bitter melon can also be developed as an antidiabetic drug or medicine.

The requirement for it to become a drug candidate is that it must have better activity than the previous drug and have few side effects. The process of making a drug requires long and rigid testing stages. The initial stage is to determine the chemical characteristics of the compound molecules, its pharmacokinetic properties (ADME), the description of the drug interactions with the receptors and its toxicity. After determining the chemical properties of the drug candidate compound or molecule, the molecule can be manipulated. Molecular manipulation or structural modification is in order to synthesize a number of parents and offspring, to identify the structure and to test its biological activity.

Changes in the structure of a compound will change the physicochemical properties of the compound including its lipophilic, electronic and steric properties. This can cause changes in the biological activity of the compound. In order to be more effective and efficient at making structural modifications, before the compound is synthesized, an effort is needed to predict the chemical properties of the compound molecule, its pharmacokinetic properties (ADME), the description of the drug interactions with the receptors and its toxicity [13].

Therefore this study is very important in terms of determining the potential of quercetin as an anti-diabetic in silico. To determine the activity of the bioactive compounds of bitter melon, especially quercetin, on the activation of the GLP-1 receptor, it is necessary to conduct an in silico test using the help of a computer program, both offline and online.

As a comparison, myricetin was used which has been proven in research to be a potential drug candidate for the treatment of type 2 DM as a GLP-1R agonist [14]. For the ligands or molecules that have shown good biological activity and are able to bind to the desired biological target (receptor) (docking process) in the protein data bank (PDB), the data is the Structure of the human GLP-1 receptor complex with NNC0640 (PDB ID/Code: 5VEX).

Methods

Materials

The 3D structure (PDB ID/Code: 5VEX) of the human GLP-1 receptor complex with NNC0640 which can be downloaded from http://www.rcsb.org/pdb/home.do.The 3D structure of quercetin, myricetin and the allosteric modulator can be downloaded from https://pubchem.ncbi.nlm.nih.gov/compound/.

Tools

A set of computers with Windows 8 64 bit specifications and the ChemDraw Professional 16.0, Chem3D 16.0, and Molegro Virtual Docker 5 programs were used. ChemOffice is an application that can be used in chemistry. The functions of this application include creating chemical structures, creating chemical names for chemical structures, making chemical structures from the chemical names, calculating molecular formulas and molecular weights and estimating the NMR spectrum of different chemical structures.

Chem Office consists of ChemDraw and Chem3D. Chem Draw Professional and Chem 3D are software that provide chemical structure drawing and analysis for scientists. It works as a drawing tool for biological pathways andcommon pathway elements such as membranes, DNA, enzymes, receptors etc are included [15].

Molegro Virtual Docker (MVD) is a protein-ligand docking simulation program that allowed us to carry out docking simulations in a fully integrated computational package. Molegro Virtual Docker is an integrated platform for predicting protein-ligand interactions. Molegro Virtual Docker handles all aspects of the docking process from the preparation of the molecules to the determination of the potential binding sites of the target protein, in addition to the prediction of the binding modes of the ligands. MVD has been successfully applied to hundreds of different proteins with a docking performance level similar to other docking programs such as AutoDock4 and Auto Dock Vina [16].

Procedure

Activity Prediction (molecular docking)

The compounds to be docked, namely quercetin, myricetin and the allosteric modulators, were drawn as 2D structures using ChemDraw Professional 16.0 and then converted to 3D using Chem3D 16.0 to determine the most stable conformation. After measuring the minimum energy, it was stored in the form of mol2 {SYBYL2 (*. Mol2)}. The protein structure of the GLP-1 receptor (PDB ID/code: 5VEX) was obtained from the Protein Data Bank. The results obtained were in the form of a Rerank Score (RS) which is the energy required in the ligand-receptor interaction process. From this value, the quercetin molecular interactions with GLP-1R can be predicted.

Prediction of Physicochemical, Pharmacokinetic, and Toxicity of Compounds (pkCSM)

The prediction of physicochemical properties such as molecular weight (BM), the logarithm of the octanol/water partition coefficient (Log P), the number of bonds between the atoms that can rotate (Torsion), the Hydrogen Bond Acceptors (HBA), the Hydrogen Bond Donors (HBD) and the Polar Surface Activity (PSA) were carried out using the pkCSM online tool. The prediction of the pharmacokinetic properties (ADME: absorption, distribution, metabolism and excretion) and the toxicity of quercetin, myricetin and the allosteric modulators were also carried out using the pkCSM online tool.

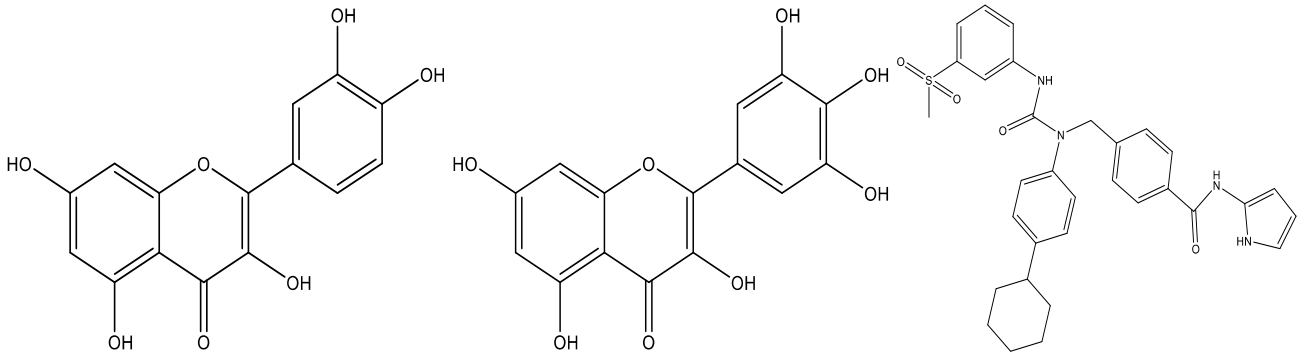
First, quercetin, myricetin and the allosteric modulators were drawn as a 2D molecular structure using the ChemDraw Professional 16.0 program and then copied into the Chem3D 16.0 program to create 3D structures saved as an *.sdf file. Second, the structure of quercetin, myricetin and the allosteric modulators was translated into the Simplified Molecular Input Line Entry System (SMILES) format using the help of the Online SMILES Translator (https://cactus.nci.Nih.gov/translate/). In the SMILES format, the compounds are processed using the pkCSM online tool (http://biosig.unimelb.edu.au/pkcsm/prediction) to predict ADME and compound toxicity.

To predict toxicity or lethal dose (LD50) orally in a globally harmonized system (GSH), the Protox online tool (http://tox.charite.de/tox/) was used [6,13].

Results

The results of making a 2D structure using ChemDraw Professional 16.0 are shown in Figure 1. The 2D structures were then used to make a 3D structure using Chem3D 16.0.

The 3D structure used in all stages of docking is shown in Figure 2.



(a) (b) (c)

Figure 1: The 2D structures of the following compounds. (a) Quercetin; (b) Myricetin; (c) Allosteric modulators

(a) (b) (c)

Figure 2: The 3D structure of the compounds stored in the form of mol2 (SYBYL2).(a) Quercetin; (b) Myricetin; (c) Allosteric modulators

Activity Prediction with Docking and Amino Acid Analysis

The protease receptors downloaded from the Protein Data Bank (PDB) code: 5VEX and imported into the Molegro Virtual Docker program are as shown in Figure 3. The detection results of the interaction site between the ligands and receptors (cavity) on Protease 5VEX receptor (A) are shown in Figure 4. The cavity used was cavity 1 (volume 1509.89) with active Ligan97V_1201 [A]. This cavity was used because it has an area where the original ligand interacts with the protease enzyme.

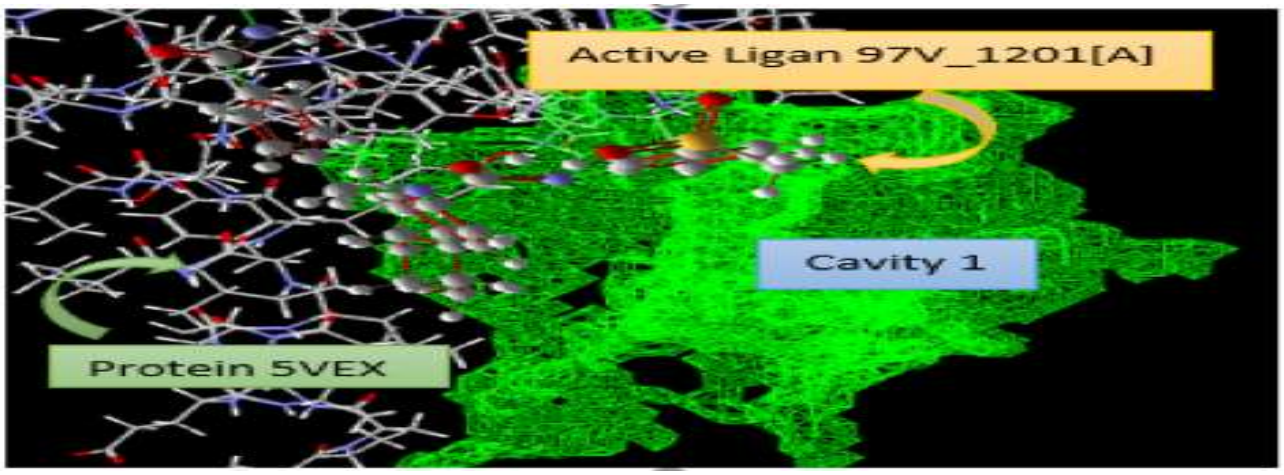
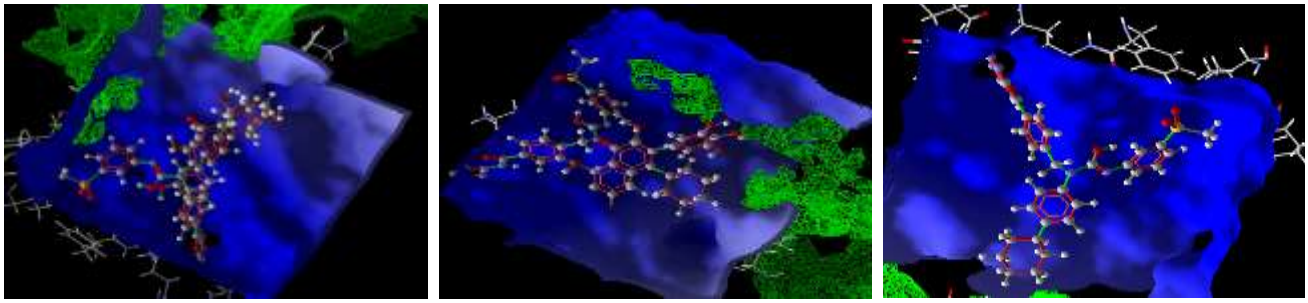


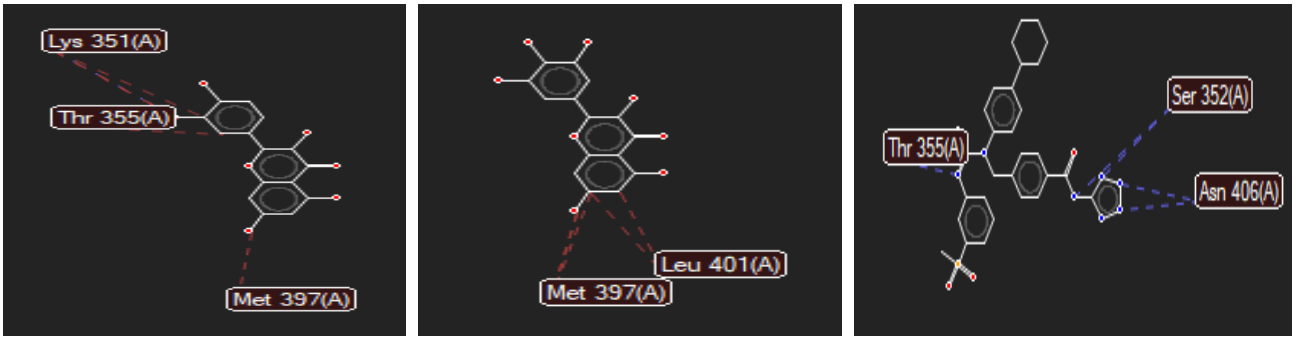
Figure 3: Protease Receptors (PDB code 5VEX) with cavity 1 (volume 1509.89) and active Ligand97V1201 [A]



(a) (b) (c)

Figure 4: Cavity 1 and Ligands (a) Quercetin; (b) Myricetin; (c) Allosteric modulators

In the interaction between the ligands and receptors, there are ligand interactions with several amino acids residues of the 5VEX protease receptor. The amino acids involved in the interaction process of quercetin, myricetin and the allosteric modulators with the 5VEX protease receptor can be seen in Figure 5 and Table 1.



(a) (b) (c)

Figure 5: The interactions between the ligands and 5VEX [A]receptors (H-Bond, electronic, steric). (a) Quercetin; (b) Myricetin; (c) Allosteric modulators

Table 1: The interactions between the ligands and 5VEX[A]receptors (H-Bond, electronic, steric)

Ligand	Hydrogen bonds and amino acid residues		Electrostatic interactions and amino acid residues		Steric interactions and amino acid residues	
Quercetin	2	Lys 351(A) Thr 355(A)	0	-	3	Thr 355(A) Lys 351(A) Met 397(A)
Myricetin	0	-	0	-	2	Leu 401(A) Met 397(A)
97V_1201[A] (Allosteric modulators)	3	Ser 352(A) Asn 406(A) Thr 355(A)	0	-	3	Ser 352(A) Asn 406(A) Thr 355(A)

The results of the re-docking of quercetin, myricetin and the allosteric modulators with the 5VEX protease receptor can be seen in Table 2.

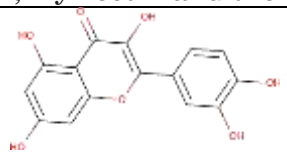
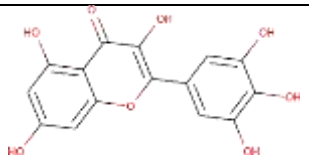
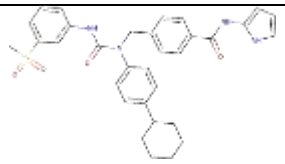
Table 2: Results of the re-docking using the Molegro Virtual Docker program

File name	Ligands	Rerank Score
Quercetin.mvdml	Quercetin	-70.2678
Myricetin.mvdml	Myricetin	-105.298
97V_1201 [A].mvdml	97V_1201 [A] (Allosteric modulators)	-126.992

Prediction of the Physicochemical and Pharmacokinetic Properties and the Toxicity of the Compounds (pkCSM)

The results of the in silico prediction of the values of the physicochemical parameters of quercetin, myricetin and the allosteric modulators can be seen in Table 3.

Table 3: Prediction of the in silico values of the parameters for the physicochemical properties of quercetin, myricetin and the allosteric modulators

SMILES Structure	 **Quercetin**	 **Myricetin**	 **Allosteric modulators**
BM	302.238	318.237	570.715
LogP	1.988	1.6936	6.9569
Torsion	1	1	8
HBA	7	8	4
HBD	5	6	3
PSA (A^2)	122.108	126.902	240.263

Description: SMILES = Simplified Molecular Input Line Entry System, BM = Molecular Weight; Log P = logarithm of the octanol/water partition coefficient; Torsion = bonds between atoms that can rotate; HBA = Hydrogen Bond Acceptors; HBD = Hydrogen Bond Donors; PSA = Polar Surface Activity.

The prediction results of the in silico pharmacokinetic properties (ADME) and the toxicity of quercetin, myricetin and the allosteric modulators can be seen in Table 4.

Table 4: Prediction of the in silico pharmacokinetic properties (ADME) and the toxicity of quercetin, myricetin and the allosteric modulators

ADMET	**Quercetin**	**Myricetin**	**Allosteric modulators**
Intestinal absorption (human) (%)	77.207	69.705	100
Skin Permeability (log Kp)	-2.735	-2.735	-2.735
VDss (human) (log L/kg)	1.559	0.243	-1.264
BBB permeability (log BB)	-1.098	-1.694	-0.966
CYP2D6 substrate (Yes / No)	No	No	No
CYP2D6 inhibitor (Yes / No)	No	No	No
Total Clearance (log ml/min/kg)	0.407	0.394	-0.154
Renal OCT2 substrate (Yes / No)	No	No	No
AMES toxicity (Yes/ No)	No	No	No
LD50(mol/kg)	2.471	2.581	2.609

VDSS = Steady State of Volume Distribution, BBB = Blood Brain Barrier, CYP2D6 = Cytochrome P2D6, Renal OCT2 = Renal Organic Cation Transporter 2. LD = Lethal Dose

Discussion

The interaction of the amino acid residues of the protease receptor with the compounds occurs through the lipophilic/hydrophobic, electronic and steric bonds. In Figure 5 and Table 1, the difference in the interactions between each compound of quercetin, myricetin and the allosteric modulators with the 5VEX protease receptor can be seen. This is because there are differences in the spatial configuration of the structures of the three compounds. The binding energy of quercetin with the 5VEX [A] receptor is higher than it is for myricetin and the allosteric modulators. Quercetin has a re-ranking score of -70.2678 kcal/mol while myricetin has a score of -105,298 kcal/mol and the allosteric modulators have a score of -126,992 kcal/mol.

The ranking score shows that quercetin has a higher energy than myricetin and the allosteric modulators, which means that it is less stable when binding to the receptor when compared to myricetin and the allosteric modulators. Lipinski et al. (1997) analyzed 2,245 drugs from the World Drugs Index baseline and concluded that the compounds studied here are difficult to absorb and have low permeability[17].

If the molecular weight is greater than 500, the partition coefficient log value of octanol / water (log P) is greater +5. It has a donor H-bond (HBD) which is expressed by the number of O-H and N-H groups greater than 5 and an acceptor H-bond (HBA) expressed by the number of O and N atoms. This is greater than 10 and with a violation of more than 2. The above analysis is known as Lipinski's law of five because all values are multiples of the number five [17].

From Table 3, it can be seen that quercetin and myricetin have a molecular weight of less than 500 while the allosteric modulators have a molecular weight of more than 500, a logP value of less than 5, acceptor hydrogen of less than 10 and a hydrogen donor of 5. It can thus be concluded that the quercetin and myricetin compounds will be easily absorbed, except for the allosteric modulators.

Compounds or molecules are said to have good absorption if the absorption value is> 80% and poor absorption if <30%. The main place for the absorption of drugs given orally is in the intestine [18]. From Table 4, it can be seen that the intestinal absorption (human) value of the quercetin and myricetin compounds is close to 80% but not less than 30%, while the allosteric modulator compound value is more than 80%.

This shows that the three compounds have good absorption abilities. The compounds are said to have relatively low skin permeability if they have a log Kp value> -2.5 (Pires, Blundell, and Ascher 2015). From Table 4, it can be seen that the skin permeability value (log Kp) of quercetin, myricetin and the allosteric modulators ranges from -2.7, which is less than -2.5.

The three compounds have good skin permeability. Furthermore, the Steady State of Volume Distribution (VDss) is the theoretical volume that the total dose of the drug needs to be distributed evenly at in order to have the same concentration as in blood plasma. The higher the VDss value, the more the drug is distributed in the tissues of the body rather than the plasma. According to Pires, Blundell, and Ascher (2015), a compound is said to have a low volume distribution if the Log VDss value is <-0.15, and high if > 0.45.

From Table 4, it can be seen that the VDss value of the compound quercetin = 1.559, myricetin = 0.243 and the allosteric modulators = -1.264. This shows that the quercetin compound has a high solubility in the network because it has high distribution capabilities. It can induce molecular signal effects in the cells compared to myricetin and the allosteric modulators [6].

The allosteric modulators have a low VDss value, so it is predicted that these compounds cannot be distributed evenly in the blood plasma. The ability of drugs to cross the Blood Brain Barrier (BBB) is an important parameter to consider helping reduce the side effects and toxicity or to increase the efficacy of drugs whose pharmacological activity is present in the brain.

Brain-blood permeability was measured in vivo in an animal model as log BB, which is the logarithmic ratio of brain-to-plasma concentrations. According to Pires, Blundell and Ascher (2015), a compound is said to be able to penetrate the blood-brain barrier well if it has a Log BB value> 0.3 and it cannot be properly distributed if the log BB <-1. From Table 4, it can be seen that the log BB value of the compound quercetin = -1.098 and myricetin = -1.694, which is less than -1.

It can therefore be predicted that the two compounds cannot penetrate the blood brain barrier well while allosteric modulators = -0.966, which is greater than -1. This means that the compound is able to penetrate the blood brain barrier moderately [6]. It is generally known that most metabolic reactions will involve oxidation processes. Cytochrome P450 is an important detoxification enzyme in the body and it is mainly found in the liver.

It works by oxidizing foreign organic compounds including drugs and facilitating the excretion of these compounds. Enzyme inhibitors such as grapefruit juice can affect drug metabolism and are therefore contraindicated against cytochrome P450 enzymes. It is therefore important to assess the ability of the compound to inhibit cytochrome P450, which in this study is represented by the cytochrome CYP2D6 isoform (CYP2D6).

From Table 4, it can be seen that the compounds quercetin, myricetin and the allosteric modulators do not affect or inhibit the CYP2D6 enzyme, so it can be predicted that the three compounds tend to be

metabolized by the P450 enzyme. To predict the process of compound excretion, the Total Clearance (CLTOT) and the Renal Organic Cation Transporter 2 (OCT2) substrate constants were measured. CLTOT is a combination of hepatic clearance (metabolism in the liver and bile) and renal clearance (excretion through the kidneys).

This is related to bioavailability and it is important to determine the dosage level needed to reach steady-state concentrations. From Table 4, it can be seen that the CLTOT value of quercetin = 0.457, myricetin = 0.394 and allosteric modulators = -0.154. From these values, the rate of the compound excretion can be predicted. Organic Cation Transporter 2 is a transporter in the kidneys which plays an important role in the disposition and clearance of drugs and endogenous compounds.

OCT2 substrates also have the potential to cause side interactions when given together with OCT2 inhibitors. From Table 4, it can be seen that the three compounds do not affect the OCT2 substrate so it can be predicted that the three compounds are not OCT2 substrates. To determine the toxicity of a compound, the Ames Toxicity test was done.

The Ames Toxicity test is a widely used method of assessing the mutagenic potential of compounds using bacteria. If the test result is positive, this indicates that the compound is mutagenic and can act as a carcinogen. From Table 4, it can be seen that the three compounds are predicted not to cause mutagenic effects because the Ames Toxicity test results are negative.

Conclusion

The quercetin bond energy is higher than that of myricetin and the allosteric modulators. The comparison of the bond energy values shows that quercetin has potential as an antidiabetic by activating GLP-1R. However, the in silico tests using the molecular docking method showed that the bond stability is still low compared to myricetin and the allosteric modulators.

The predicted pharmacokinetics (ADME) and the toxicity of quercetin compounds mean that it is well absorbed in the intestine, has good skin permeability, can be distributed evenly to have the same concentration as blood plasma and bodily tissues, is less able to penetrate the blood-brain barrier, tend to be metabolized by enzyme P450, does not cause mutagenic effects, and has low cytotoxic activity based on the pkCSM online tool test.

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