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IN SILICO ANALYSIS OF ANTIDIABETIC ACTIVITY AND ADMET PREDICTION OF POTENTIAL COMPOUNDS FROM *LUFFA ACUTANGULA*

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ABSTRACT

Luffa acutangula is commonly used and considerable potential as an alternative treatment of diabetic with a molecular target action are not yet known. Preliminary study of bioinformatics to decipher the various chemical constituents of *L. acutangula* able to interact with the protein targets of an antidiabetic therapeutic. This study aims to identify the chemical constituents of *L. acutangula* are thought to interact with insulin receptor, aldose reductase, α-glucosidase, PTP-1B, GSK-3B, PPARγ, and accompanied by predictions of pharmacokinetic and toxicity. Docking molecular was conducted in AutoDock 4.2.6 with the stages initiated by preparation of macromolecule (PDB ID: 1IR3; 2PEV; 2QMJ; 4Y14; 4PTE; 4R06) and ligand, method validation, simulation to the result visualization of docking molecular. The pharmacokinetic profiles are predictable by using the SwissADME webform and toxicity estimated by Toxtree related to three best ligands on each macromolecule. The results showed that cucurbitacin B and cucurbitacin E are the most potential compounds to interact with the macromolecular with a binding energy response similar to the native ligand. Pharmacokinetic predictions show that cucurbitacin B and cucurbitacin E are deviate from one Lipinski rules (BM> 500), do not diffuse into the blood brain barrier, not as a CYP450 inhibitor and classified as Pgp substrates. The prediction of toxicity indicates that all potential compounds are classified as high toxicity compounds with risk of narcosis, except oleanolic acid and ferulic acid. But these compounds are not genotoxic or non-genotoxic carcinogens.

Keywords: *Luffa acutangula, Antidiabetic, Docking, Pharmacokinetic, Toxicity*

INTRODUCTION

Diabetes mellitus is a condition of a group of metabolic disorders characterized by elevated glucose levels and inadequate insulin production. WHO estimates the total population of people with diabetes mellitus (DM) in 2030 which places Indonesia as the 4th country with total population of 21.3 million. Riskerdas 2018 shows that herbal alternatives are the choice of DM therapy by the Indonesian people with a proportion of 35.7% of the total national DM prevalence. This fact shows that the Indonesian people have a relatively high level of trust in herbs as an option for DM therapy [(1)].

One of the plants that has potential as an alternative to DM treatment is *Luffa acutangula*. Sharmin et al., (2013) reported that the ethanolic extract of 200 mg/kgBW *L. acutangula* can reduce blood glucose levels of female Long Evans rats by 51.50% [(2)]. Fadel (2019) also reported a decrease in blood glucose levels in Wistar rats after administration of 200 mg/kgBW of *L. acutangula* seed extract by 44.10% and ethyl acetate fraction of 34.44 mg/kgBW by 45.05% [(3)]. Antidiabetic activity of *L. acutangula* is thought to be due to the presence of various chemical compounds such as Cucurbitacin and polypeptide-p glycosides (Insulin-Like Peptide) which can increase insulin sensitivity [(4)]. *L. acutangula* methanol extract has an IC_{50} of less than 150 g/mL comparable to acarbose in the inhibition of α-glucosidase enzymes [(5)].

Various chemical constituents of *L. acutangula* have been identified to be able to lower blood glucose levels so that they can be used as an antidiabetic alternative. However, the identification of the active compound against macromolecules or the target of molecular action of antidiabetic is not yet known clearly. The identification of molecular action targets is

intended to optimize targeted pharmacodynamic activity based on the interaction pattern of the drug with its target. The challenge faced in identifying the target molecular action of an active compound is a lengthy and costly assay process. These challenges can be overcome through computational experiments with in silico molecular docking techniques. The rapid development and advancement of computational techniques currently allows in silico to accelerate the process of selecting compounds to be synthesized [(6)].

Docking is one of the CADD (Computer Aided Drug Design) methods that can be used to describe the interaction of a compound with the target protein by predicting its conformation and binding energy. docking provides a scoring through molecular mechanics in the form of repulsion, hydrogen bonding, electrostatics and desolvation, as well as scoring showing the affinity of the ligand or active compound to the target protein as an indication of the mechanism of action of the compound being tested [(7)]. By utilizing molecular docking, target proteins from several chemical constituents of *L. acutangula* that have pharmacological activity as antidiabetics can be predicted and identified accurately based on scores and models of ligand and protein (macromolecule) interactions based on calculations using the AutoDock 4.0 program in AutoDock Tools. Docking result was carried out in silico to predict the pharmacokinetic and toxicity parameters of the potential compounds of *Luffa acutangula* to the macromolecules of DM therapy targets using webform SwissADME and Toxtree.

RESEARCH METHODS

Preparation of macromolecules and ligands

Insulin receptor (PDB ID: 1IR3), aldose reductase (PDB ID: 2PEV), GSK-3B (PDB ID: 4PTE), PTP-1B (PDB ID: 4Y14), α-glucosidase (PDB ID: 2QMJ) PPARγ (PDB ID: 4R06) were identified through SwissPred and SuperPred, which are webserver for target predictions of compounds. The macromolecules downloaded the structure from PDB (https://www.rcsb.org) in .pdb format. Three-dimensional structure of compounds as the ligands that have been created with VegaZZ in .mol format and then optimized with AutoDock Tools. The preparation is done through AutoDock Tools by separating the native ligand and water molecules, as well as adding hydrogen atoms.

Validation Method

Validation was carried out on the native ligand to find the right conformation. The previously prepared macromolecules were redocked with the native ligand. Conformation docking obtained is then aligned with the native ligand conformation on the crystallographic structure expressed in r*oot-mean-square deviation* (RMSD). The RMSD value states that the conformational alignment of the structure is still acceptable with a value of less than 2.5 Å, if it is smaller or closer to the value 0 then the alignment value is getting better [(8)].

Molecular Docking

The docking is carried out using the AutoDock 4.0 program with AutoDock Tools (ADT). Setting docking with rigid macromolecular format as well as GA Runs (200) and Population Size (150). Then select the Output submenu for Lamarckian GA (4.2). The results docking all the test ligands resulted in $G_{binding}$ (kcal/mol) which was then analyzed and visualized using the Discovery Studio Visualizer Biovia to see the shape or model of the anchorage formed.

Prediction of Pharmacokinetic and Toxicity Parameters

Prediction of pharmacokinetic and toxicity profiles was carried out online using webform SwissADME and Toxtree. It is enough to enter the SMILES code of ligands obtained from PubChem, then click ADMET. Furthermore, the prediction results of these compounds are

shown which consist of several parameters of absorption, distribution, metabolism and excretion. Identification of toxicity parameters through the Cramer rules, Verhaar scheme, Kroes TTC, Benigni/Bossa rules and SMART CypP50.

RESULTS AND DISCUSSION

The method of determining the structure of macromolecules used is the crystallographic method (X-ray diffraction) because it can be applied to large macromolecular structures and is more precise [(9)]. The value of conformational resolution must also be considered with the RMSD criteria <2.5Å. The organisms used are humans (*Homo sapiens*). The downloaded macromolecules in .pdb format still contain the native ligand complex and the crystallized water molecules. The native ligands and water molecules must be removed from the macromolecules through optimization so as not to interfere with the docking molecular. Native ligands are removed in order to obtain individual macromolecules that will be docked with the test ligand. The water molecule was omitted because it could mediate the interaction between the ligand and the receptor were not good due to the complexity of mathematical calculations in docking, causing docking to be longer [(8)]. Preparation or optimization is done through the AutoDock Tools program. In addition to the removal of the native ligand molecules and water molecules, preparation was also carried out by removing non-amino acid residue molecules and adding charge to adjust the chemical environment. Residues are removed because they can interfere with the interaction between the ligands and amino acid residues on the active site of the macromolecule. Removal of residues non-amino acids needs to be reviewed with a review of selected macromolecule journals attached to PDB. Residues that are not removed such as chromoprotein Mg on insulin receptor and cofactor $NADP^+$ on aldose reductase [(9)].

Table 1. Validation of macromolecules

Macromolecule	Centre			Size (Å)			RMSD (Å)	$\Delta G_{binding}$ (kcal/mol)
	X	Y	Z	X	Y	Z		
1IR3	-	29,968	7,184	40	40	44	1,65	-7,08
2PEV	23,160	-8,180	16,812	40	40	40	0,87	-9,63
4PTE	17,514	0,527	-	40	40	40	1,86	-9,12
4R06	-4,076	30,921	35,765	40	40	40	1,64	-8,11
4Y14	18,225	-	15,008	40	40	40	0,67	-14,14
2QMJ	-	22,120	-8,056	32	30	46	0,64	-11,87
	10,597	-6,547	-5,222					
	-							
	21,764							

The validation of docking is a preliminary test and it is important to do before the docking for the potential ligands. At this stage, the native ligand is re-tethered to the target protein (macromolecule). Determining the centre of the grid box is the first step in the validation. The grid box is an analogy to the space for the native ligand or the active compound to form a conformation when tethered to the macromolecule. Determination of the grid box is done to find out the coordinates of the binding site or the active side of macromolecule. Settings that have been done are setting the coordinates and grid size [(10)]. Table 1 shows the validation results of macromolecules that have been re-docked with the native ligand as well as the settings of grid box. The validation of the redocked ligands showed good results with the RMSD value of each macromolecule of <2Å. The overlay of the crystallographic native ligands is also comparable to that of the redocked ligands (figure 1). This indicates that the conformation of the redocked ligand to the target macromolecule is similar to that of the crystallographic results.

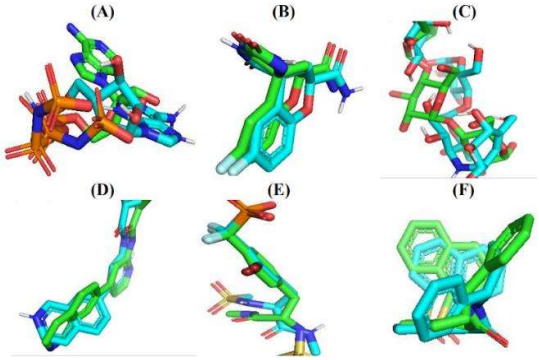

Figure 1. The overlay crystallographic ligands (green) and redocking ligands (blue); (A) IR (ANP); (B) AR (Fidarestat); (C) α-glucosidase (Acarbose); (D) GSK-3B (2WF); (E) PTP-1B (C0A); (F) PPARγ (3E7)

Insulin receptor (IR)

The insulin receptor is a protein that plays an important role in carbohydrate metabolism, especially the regulation of blood sugar levels, which is activated by the receptor tyrosine kinase. Signalling molecules *src-homology-2 domain protein* (SH2) *phosphatidylinositol-3 kinase* (PI-3K). PI-3K is an activator in the translocation of the glucose transporter protein (GLUT4), glycogen, protein synthesis, and regulates gluconeogenesis [(11)]. Hubbard described that phosphorylated insulin kinase receptors bind to a peptide substrate and an ATP analogue, namely adenylyl monophosphate (AMP-PNP) at the C-terminal site. ANP is an ATP analogue that interacts at the active site through hydrogen and non-hydrogen bonds, such as hydrophobic bonds, pi-pi interactions, and charge-attractive interactions. There are two types of charge-attractive interactions, namely Mg301 and Mg302 in the aliphatic side chain of ANP. Mg301 is an octahedral ion or an ion linked to six atoms coordinated by the aliphatic side chain of ANP. While Mg302 is connected to seven oxygen atoms in the ligand. Mg301 and Mg302 are linked together at the Asp1150 residue as a charge-attractive interaction [(12)]. Therefore, the molecule was not removed because it is a chromoprotein type (metalloprotein) or a protein that binds to Mg metal ions and is a quaternary type of insulin receptor protein.

Cucurbitacin E has the lowest bond energy value compared to all test ligands and native ligands in 1IR3 macromolecules, which is -10.26 kcal/mol. Cucurbitacin E is able to interact at *binding site* similar to ANP. Therefore, Cucurbitacin E as the best ligand is a potential compound as an insulin receptor agonist with a lower binding energy value than ATP analogues (ANP). Cucurbitacin E is known to have antidiabetic activity through the mechanism of action of the GLUT4 transporter translocation. Murtaza (2017) showed a decrease in serum glucose activity of Cucurbitacin E (0.5 mg/kgBW) in rats of <150 mg/dl or better than Orlistat [(13)]. Cucurbitacin B is the second-best test ligand with a lower bond energy value than the native ligand, which is -10.02 kcal/mol. Cucurbitacin B is known to have antidiabetic activity with insulin receptor target through AMPK regulation mechanism. Serum glucose-lowering activity of Cucurbitacin B (0.1 mg/kgBW) similar to metformin (300 mg/kgBW) of <200 mg/dl in rats within 2 hours [(14)]. Oleanolic acid also has a lower bond energy value than the native ligand, amounting to -8.88 kcal/mol. The charge-attractive interactions of Mg301 and Mg302 in ANP also occur in the aliphatic chain of oleanolic acid. The interaction of Mg301 and Mg302 charges is important because these two molecules are able to mediate the interaction between the insulin receptor caPK site with peptide and ligand substrates [(12)].

Aldose reductase (AR)

Aldose reductase is an oxidoreductase enzyme that converts glucose into the polyalcohol sorbitol by reducing the aldehyde group of glucose. Under normal conditions, sorbitol in cells remains normal, but in hyperglycemic conditions, sorbitol levels in cells become high and will be converted to fructose by the enzyme sorbitol dehydrogenase. Increased activity of this enzyme can cause the accumulation of sorbitol as a trigger for hyperglycemic. Aldose reductase inhibitors have been developed and are capable of forming structural conformations that can inhibit the activity of this enzyme, such as fidarestat and zopolrestat [(15)]. Fidarestat as the native ligand has a bond energy value of -9.63 kcal/mol with various types of interactions at the binding site. Hydrogen bonding occurs in the aliphatic fidarestat chain with residues Cys298, Leu300, Ala299, Trp111. Halogen bonding occurs in the benzene ring of fidarestat with a Val47 residue and unfavourable donors between His110 and the imidazolidine ring of fidarestat. Binding pocket inhibitor of aldose reductase called anion binding pocket consists of residues Tyr48, Lys77, His110, Trp111 and cofactor $NADP^+$ [(16)].

Cucurbitacin B has the lowest bond energy value compared to all ligands and the native ligand 2PEV, which is -10.11 kcal/mol. Cucurbitacin B is able to interact at the binding site aldose reductase similar to that of fidarestat. Cucurbitacin B has an anion binding pocket similar to Fidarestat, namely the Trp111 residue. Therefore, Cucurbitacin B is a potential compound that can be developed as an aldose reductase inhibitor. Cucurbitacin E as the second-best test ligand with a bond energy value of -9.45 kcal/mol. Cucurbitacin E does not have residues as anion binding pocket similar to fidarestat. However, there are other important interactions that are not visible in the visualization of redocked fidarestat ligands, namely Leu300 and Phe122 residues. The two residues interact hydrophobically with the benzene ring fidarestat. The interaction of oleanolic acid with the 2PEV macromolecule produces one type of hydrogen bond in the hydroxy group with the Tyr48 residue. Therefore, oleanolic acid also has anion binding pocket inhibitor aldose reductase. Similar to Cucurbitacin E, oleanolic acid has one other important residue that is not visible on visualization of the redocked fidarestat ligand, namely the Phe122 residue which interacts hydrophobically with the benzene ring of fidarestat [(16)]. It has a very potent inhibitory activity against the aldose reductase enzyme with an IC_{50} of 0.09 M [(17)].

Glycogen synthase kinease-3 (GSK-3B)

Glycogen synthase kinease-3 is a serine or tyrosine kinase that works to catalyze the phosphorylation of various cellular mediators and enzymes. Glycogen synthase phosphorylation occurs as a result of one of the protein kinases, namely glycogen synthase kinase-3, especially its beta isoform. GSK-3B becomes inactivated rapidly when insulin levels rise in the bloodstream and loses its ability to phosphorylate thereby allowing it to synthesize glycogen. GSK-3B phosphorylation can cause a decrease in insulin-dependent glycogen synthesis, so GSK-3B needs to be inhibited by exogenous inhibitors to relieve DM2 pathology [(18)]. The results docking of all ligands against 4PTE macromolecules were the best target proteins because there were three test ligands with lower bond energy values than the native ligand. 2WF as the native ligand produced a bond energy value of -8.11 kcal/mol, while cucurbitacin B, cucurbitacin E and oleanolic acid were able to produce a bond energy value lower than -10 kcal/mol. The results of the visualization of the types of interactions show that there are two types of hydrogen bonds in the Lys85 and Val135 residues. Lys85 and Val135 residues are the residues that are responsible for the interaction between the pyridine carboxamide ring in 2WF and Lys85 in the adenine pocket. Hydrophobic interactions such as pi alkyl occur between Cys199 and Val70 residues with an adenine pocket, and Ala83 and Leu188 with a pyridine carboxamide ring [(19)].

Cucurbitacin B was the first best ligand with a bond energy value of -10.43 kcal/mol. The interaction of this assay ligand with the 4PTE macromolecule did not result in the similarity of hydrogen-bonded amino acid residues to the native 2WF ligand. The similarity of the interaction is in the form of non-hydrogen bonds at residue Val70 with the cyclopentane ring of cucurbitacin B. Cucurbitacin B can inhibit GSK-3B phosphorylation through inhibition of canonical Wnt activation, translocation of galectin-3 and catenin, and expression of cyclin D1 and C-Myc in Western blot test against T47D cells (100 g/ml) and SKBR-3 cells (10 g/ml) [(20)]. Cucurbitacin E is the second-best test ligand with a bond energy value of -10.27 kcal/mol. Cucurbitacin E produces one hydrogen and non-hydrogen bonded amino acid residue in common with the native 2WF ligand. Oleanolic acid have a lower bond energy value than the native ligand, which is -10.02 kcal/mol. The interaction of this test ligand with the 4PTE macromolecule produces two important residues that are responsible for the interaction between the adenine pocket and the pyridine carboxamide ring 2WF, namely Val135 and Ala83. Oleanolic acid can inhibit glycogen phosphorylation with an IC_{50} of 14 M [(21)].

α-glucosidase

Control of postprandial hyperglycaemia is an important step for blood sugar control. It can be done by inhibiting the absorption of glucose through inhibition of carbohydrates hydrolysed by the enzyme α-glucosidase. Sim et al (2008), reported that the interaction of α -glucosidase inhibitor (acarbose) occurs through the formation of extensive hydrogen bonds with residues on the active site of the N-terminal maltase-glucoamylase subunit. The active residues such as Asp327, Asp542, His600, Asp443, Asp443, Tyr605, Asp203, Asp571 and Arg526, as well as other additional residues through hydrophobic interactions such as Tyr299, Ile328, Ile364, Trp441, Trp406, Phe450 and Met444 [(22)].

Acarbose as the native ligand has a bond energy value of -9.12 kcal/mol with the type of interaction at the binding site through hydrogen binding, HOH interactions, pi-alkyl interactions and unfavourable donors. Hydrogen bonding is the most common type of interaction found on the active site of the protein with the acarvosine ring, ranging from residues of Asp203, Asp542, Arg526, to residues of His600 and Asp327 which experienced repeated interactions. This is consistent with the research of Sim et al (2008) who stated that Asp203, Asp542, Arg526, His600 and Asp327 are active residues in the N-terminal maltase-glucoamylase subunit that interacts with the acarvosine ring. Hydrogen-water bonds (HOH) appear in the visualization results due to the use of water molecules during the docking. This is because in one ring of acarvosine there is a residue bound to a water molecule, namely Tyr605. Hydrophobic interactions such as pi alkyl interactions occur between residues Trp406, Tyr299 and Phe575 with the N-terminal side of the polypeptide between the two acarvosine rings [(22)].

Cucurbitacin B is the best ligand with the lowest bond energy value compared to all test ligands, which is -8.78 kcal/mol. This ligand has the same interaction in the form of hydrogen bonds with the native ligand that occurs in the N-terminal maltase glucoamylase subunit. Ultrasound extract of cucurbitacin B (1.584 mg/g DW) had an inhibitory activity against the α-glucosidase enzyme by 56.27% [(23)].

Table 2. Molecular Docking Results of *Luffa acutangula*

Ligands	$\Delta G_{binding}$ (kcal/mol)					
	1IR3	**2PEV**	**2QMJ**	**4PTE**	**4Y14**	**4R06**
Cucurbitacin B	-10.02	-10.11	-8.78	-10.43	-9.99	-6.40
Cucurbitacin E	-10.26	-9.45	-8.55	-10.27	-9.11	-8.31
Oleanolic acid	-8.88	-9.29	-6.95	-10.02	-6.56	-1.21
Catechin	-7.06	-8.52	-7.68	-7.19	-7.02	-7.14
Ferulic acid	-5.12	-6.63	-3.71	-5.78	-7.92	-5.62
Apigenin	-7.36	-8.11	-6.81	-7.12	-7.25	-7.08
Native ligand	-7,08	-9,63	-9,12	-8,11	-11,87	-14,14

Protein tyrosine phosphatase (PTP-1B)

Protein tyrosine phosphatase is an enzyme that plays a role in negative regulation of the insulin signal transduction pathway or activates dephosphorylation of phosphotyrosine residues, thereby inhibiting the translocation of glucose transporters. The inhibitory activity of this enzyme can increase insulin receptor phosphorylation to promote glucose transporter translocation and glucose uptake in insulin sensitive cells. The inhibitory activity of PTP-1B on the catalytic site consisted of an active nucleophile site, WPD-loop, YRD-loop and a second aryl phosphate group. The catalytic site as a binding pocket substrate or inhibitor consists of a variety of amino acids that can interact from the end of the WPD-loop to the YRD-loop [(24)].

Visualization of the C0A interaction diagram shows the interaction in the form of hydrogen bonds on the residues Ala217, Gly220, Ser216 and Ile219 with aliphatic side chains, Phe182 residues with benzene rings, and Asp48 with N atoms on the C0A pyrrole ring. Residues Arg47 and Asp48 are YRD-loop sites, catalytic active sites at residues Arg221, Gly220, Asp181, Phe182 and Ile219, and residue Cys215 as the active site of nucleophiles. Other interactions in the form of a salt bridge appear on the Arg221 residue and the interaction of the halogens on the Asp181 residue with the Br atom [(24)].

Cucurbitacin B was the first ligand with a bond energy value of -9.99 kcal/mol. This ligand has an enzyme catalytic active site, namely residues Arg221, Phe182, Ala217 and Tyr46 which are not visible on the visualization of the native C0A ligand. The active part of the YRD-loop interacts with the Asp48 residue. There have been no in vitro or in vivo studies on all cucurbitacin isolates in the inhibitory activity of the PTP-1B enzyme. Cucurbitacin E is the second-best test ligand with a bond energy value of -9.11 kcal/mol. The test ligands have protein nucleophilic active sites ranging from residues Arg221, Asp181, Ser216, Ile219 to Cys215. This ligand can form a salt bridge between the ligand and the PTP-1B catalytic site through residues of Arg221 and Asp181.

Peroxisome proliferator-activated receptor (PPARγ)

PPARγ activation can increase glucose uptake and utilization in peripheral organs, stimulate fatty acid storage in adipocytes, enhance insulin signalling, and reduce gluconeogenesis in the liver. When activated by a ligand (thiazolidinedione agonist) PPARγ binds to the 9-cis retinoic acid receptor (RXR receptor) to form a heterodimer. This binding can allow DNA to regulate genetic transcription and translation of various proteins involved in cell differentiation and glucose and lipid metabolism, in particular increasing insulin sensitivity [(25)].

The interaction of PPARγ and its agonist consists of 3 sides, namely the interaction with the AF2 residue, the interaction with the indole ring, and the interaction of the naphthalene group with the B-sheet side. The interaction of the native 3E7 ligand with stable AF2 residues forms hydrogen bonds with Tyr327 and Ser289. Others such as phenyl and ethyl groups form hydrophobic interactions with Tyr449, His449 and Leu453. There is also a pi-pi interaction

between the phenyl group and Phe282. The interaction of the naphthalene group between the B-sheet and helix-3 forms hydrophobic interactions and van der Waals bonds with residues Ile341, Met348, Cys285, Ile281, Met364 and Arg288. The interaction on the native indole ligand ring forms a hydrophobic interaction with the Cys285, Ile326 and Leu330 residues. The results docking of all test ligands did not produce a compound with a lower bond energy value than the 3E7 ligand [(26)].

Cucurbitacin E is the first ligand with a bond energy value of -8.31 kcal/mol. The Ser289 residue is similar to the native ligand residue 3E7 and is part of the AF2 residue. Ile341, Leu453, Met364, Phe282 and His449 residues are residues of the B-sheet and helix-3 sections. The Ile326 residue is part of the native indole ligand ring interaction that forms a hydrophobic interaction [(26)]. Cucurbitacin E (5 mg/kg) can increase PPARγ expression in diabetic rat muscle tissue by 12, comparable to PPARγ expression by glibenclamide 600 g/kg. This compound has a plasma insulin sensitivity of 12 mU/ml [(27).]

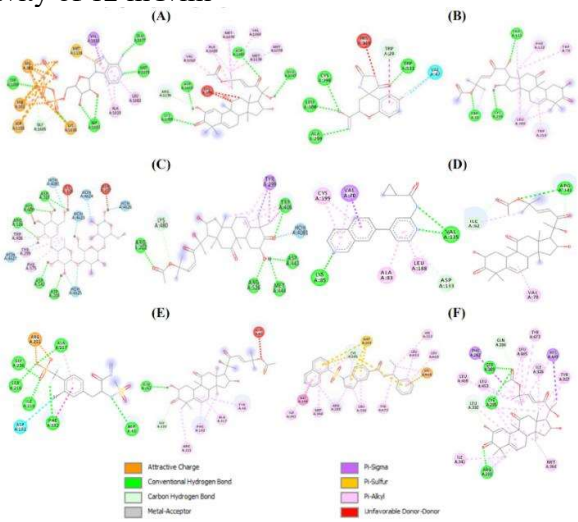

Figure 2. Visualization of macromolecule and ligand (native ligand (left) and potential ligand (right); (A) IR (ANP - Cucurbitacin E); (B) AR (Fidarestat - Cucurbitacin B); (C) α-glucosidase (Acarbose - Cucurbitacin B); (D) GSK-3B (2WF - Cucurbitacin B); (E) PTP-1B (C0A - Cucurbitacin B); (F) PPARγ (3E7 - Cucurbitacin E)

Profile of pharmacokinetic and toxicity

Prediction of physicochemical properties and pharmacokinetic profile of compounds is intended to be more effective in modifying the structure of drug compounds before synthesis after obtaining the best results from docking molecular. Prediction of pharmacokinetic profiles can be done with database to the best or potential compounds suspected of having pharmacological activity with certain target proteins. Thus, it is necessary to provide information on the pharmacokinetic profile of several compounds that can interact with the target protein of DM2 therapy. The following is a description of the pharmacokinetic profile of the best compounds conducted online through SwissADME.

Determination of the physicochemical properties of ligands when it crosses the cell membrane can be done by identifying *Lipinski's rule of five*. The conditions that must be met by potential compounds based on Lipinski's rule are molecular weight (BM) < 500 Da, log P value < 5, number of donor hydrogen bonds (HBD) < 5 and number of acceptor hydrogen bonds < 10, and molar reactivity in the range of 40 – 130. Ligands with a BM < 500 Da can easily penetrate cell membranes. The log P value indicates the polarity of the ligand in fatty or non-polar solvents when the log P value is > 5 because it can interact more easily through the

lipid bilayer and is widely distributed in the tissue. The low log P value indicates the ligand tends to dissolve in water. The number of hydrogen bonds of donors and acceptors is related to the chemical activity of drug molecules in the body [(28)].

The pharmacokinetic phase consists of the process of absorption by the gastrointestinal tract, distribution in the blood to the therapeutic target, metabolism in the liver to form active metabolites until it is excreted out of the body through certain organs. Predicted pharmacokinetic profiles showed that the three best compounds were poorly absorbed from the gastrointestinal tract, while the other three compounds showed high levels of absorption. Cucurbitacin has a fairly low solubility in water so that it shows low absorption of gastrointestinal fluids. Table 3 describes the predictions of pharmacokinetic parameters of potential compounds. Cucurbitacin as the most potent compound has a fairly large molecular weight (> 500), so it is quite difficult to penetrate cell membranes. This violates Lipinski's rule, which is that a drug candidate compound must have a molecular weight of <500. Oleanolic acid has a log P >5. This value deviates from Lipinski's rule, namely drug candidate compounds must have a log P value <5. The best test ligands have low solubility in water and do not penetrate the BBB. These compounds are also not classified as inhibitors of cytochrome P450 and OCT2 renal substrates.

Cucurbitacin B and cucurbitacin E as the potential compounds are P-glycoprotein (Pgp) *substrate* so that the penetration of the active molecules is inhibited into the blood brain barrier. Cytochrome P450 (CYP) enzymes are oxidase enzymes involved in the metabolism of various endogenous or exogenous compounds (drugs) in the liver. All of the ligands were not CYP1A2, CYP2C19, CYP2C9, CYP2D6 and CYP3A4 enzyme inhibitors. Only apigenin can catalyze or inhibit CYP1A2, CYP2D6 and CYP3A4. CYP2D6 enzyme can catalyze basic compounds with protonated atoms 4-7 A such as several types of flavonoids and alkaloids. The CYP3A4 enzyme is a type of P450 that can catalyze most of the lipophilic active molecules [(29)].
Cucurbitacin B has an oral bioavailability of 10% with a maximum plasma concentration (C_{max}) of 4.85 – 7.81 g/L. This compound has a distribution volume profile of 51.65 L/kg without inhibiting tissue in plasma. Cucurbitacin B is excreted through urine and feces[(30)]. Cucurbitacin E (1 mg/kg) has a clearance rate of 4.13 L/hour with a volume of distribution of 27.22 L. The plasma concentration reached within the initial release time of 0.45 hours is 20 ng/hour [(31)].

Table 3. Predicted Pharmacokinetic Parameters

Potential Compound	Profile of Pharmacokinetic								
	Abs. GI	BBB	Subs. Pgp	Log P	Inh. 1A2	Inh. 2C19	Inh. 2C9	Inh. 2D6	Inh. 3A4
Cucurbitacin B	Low	No	Yes	3,19	No	No	No	No	No
Cucurbitacin E	Low	No	Yes	3,47	No	No	No	No	No
Oleanolic acid	Low	No	No	6,06	No	No	No	No	No
Catechin	High	No	Yes	0,85	No	No	No	No	No
Ferulic acid	High	Yes	No	1,36	No	No	No	No	No
Apigenin	High	No	No	2,11	Yes	No	No	Yes	Yes

Cramer's rule is a general parameter used as an initial assessment of the safety properties of a potential compound. Prediction results for this parameter indicate that all the best compounds are classified in class 3 with a high risk of toxicity, except oleanolic acid and ferulic acid. Oleanolic acid as the third best compound has a low risk of toxicity because it has a common terpenoid functional group, while ferulic acid has a low risk of toxicity because it only has one aromatic ring. Cucurbitacin B and cucurbitacin E as the best ligands are classified

as compounds with a high risk of toxicity because they do not have a sulfonate group and the presence of several heteroaromatic substances as the main substance [32].

Verhaar scheme is a toxicity assessment based on a substance at risk of reactive or basic toxicity. Assessment of this parameter indicates that all of the best compounds are class 1 or groups of compounds with a risk of narcosis or basic toxicity, except that oleanolic acid cannot be classified. Cucurbitacin B and cucurbitacin E carry a risk of narcosis or basic toxicity due to the presence of the reactive substance butanone or acetophenone (aromatic ketones). Catechins and ferulic acid can be narcotic because they are influenced by physicochemical properties, such as molecular weights of more than 600 Daltons, do not have ionized groups and Log K between 0 and 6. While apigenin is at risk of narcosis or basic toxicity due to the presence of monocyclic ether reactive substances and not epoxides or peroxide [32].

Kroes TTC is a specific toxicity parameter in the analysis of dose and response data for carcinogenic compounds. The exposure threshold for a compound is not more than 0.15 g/day with a dosage size of 86 - 97%. Prediction results for Kroes TTC showed that cucurbitacin B and cucurbitacin E as the best compounds were compounds with negligible risk properties as carcinogenic compounds because they had a low probability. Other compounds are compounds with substances of which there is no particular concern for safety. Thus, all the best compounds are classified as safe from potential carcinogenic compounds [32].

Benigni/Bossa rules is a specific aspect of determining toxicity on the risk of carcinogenicity and mutagenicity by identifying the presence of genotoxic and nongenotoxic alert structures. All of the best compounds were classified as compounds that were not at risk of genotoxic carcinogenicity and non-genotoxic carcinogenicity. This is in line with the Kroes TTC parameter. However, cucurbitacin B and cucurbitacin E were identified to have a genotoxic alert structure, namely the non-saturated carbonyl-β gene or have an alkene group conjugated with a ketone [32].

Table 4. Predicted Toxicity Parameters

Potential Compound	**Profile of Toxicity**				
	Cramer rules	***Verhaar scheme***	***Kroes TTC***	***Benigni/Bossa rules***	***SMART CypP50***
Cucurbitacin B	Class 3	Class 1	Class 2	Class 1, 8, 9	Yes
Cucurbitacin E	Class 3	Class 1	Class 2	Class 1, 8, 9	Yes
Oleanolic acid	Class 1	Class 5	Class 1	Class 8, 9	Yes
Catechin	Class 3	Class 1	Class 1	Class 8, 9	Yes
Ferulic acid	Class 1	Class 1	Class 1	Class 8, 9	Yes
Apigenin	Class 3	Class 1	Class 1	Class 8, 9	Yes

Another toxicity parameter that needs to be known is the identification of certain substances or active groups that are label metabolized by the CYP3A4 enzyme using the SMARTCyp method. Prediction results showed that all the best compounds had certain active groups that were labile to metabolize by the CYP3A4 enzyme. There are several functional reactions identified in the six best compounds, ranging from aliphatic hydroxylation, aromatic hydroxylation, alcohol oxidation, O-dealkylation and epoxidation. Aliphatic hydroxylation is an oxidation reaction involving CN chain heteroatoms, while O-dealkylation is an oxidation reaction involving CO chain heteroatoms. Epoxidation is a type of non-oxidizing reaction, that is, the hydrolysis type for the conversion of epoxides to side-by-side diols. Cucurbitacin B and cucurbitacin E undergo aliphatic hydroxylation and epoxidase reactions. Oleanolic acid also undergoes a similar functional reaction but there is also an alcohol oxidase reaction. Apigenin only undergoes aromatic hydroxylation reactions, while catechins undergo various reactions

ranging from alcohol oxidase, O-dealkylation, aliphatic hydroxylation to aromatic hydroxylation. All of these functionalization reactions are biotransformation stages in phase 1 metabolism [32].

Cucurbitacin E tends to be safer with low toxicity than cucurbitacin B. Acute oral toxicity studies in rats found that cucurbitacin B can cause death in LD_{50} at a dose 14 mg/kg, while cucurbitacin E has LD_{50} value at a dose of 340 mg/kg (33). Oleanolic acid and ferulic acid are potential compounds with Cramer's rules as compounds with very low toxicity class. This is in line with research by Xiang, oleanolic acid can cause acute toxicity in rats with an LD_{50} at a dose of 2000 mg/kg [34]. LD_{50} of ferulic acid in male rats was 2445 mg/kg [35].

CONCLUSION

Cucurbitacin B, cucurbitacin E and oleanolic acid as potential compounds from *Luffa acutangula* against several antidiabetic target proteins. In silico study confirmed that the ligands shown the most effective agonism to insulin receptor and inhibitory potential against the aldose reductase and GSK-3B. The ideal bond energy value and the similarity of residues to the native ligand indicate that these compounds are potential alternative for DM2 therapy. However, the lack of a pharmacokinetic profile in the form of poor absorption of cucurbitacin and oleanolic acid indicates the need for a more intense study of the pharmacophore groups in order to increase absorption. The toxicity profile confirmed that these compounds are not genotoxic or non-genotoxic carcinogens.

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