

Molecular Docking and Pharmacokinetic Prediction of Potential Compounds from *Luffa acutangula* as Antidiabetic Candidates

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ABSTRACT

Luffa acutangula is commonly used, but its considerable potential as an alternative treatment for diabetics with a molecular target action is not yet known. Preliminary study of bioinformatics to decipher the compounds of *L. acutangula* to interact with the protein targets of antidiabetic therapy. This study aims to identify the potential compounds of *L. acutangula* that are thought to interact with the insulin receptor, aldose reductase, and PTP-1B, as well as provide predictions of pharmacokinetics and toxicity. Molecular docking was conducted in AutoDock 4.2.6 with the stages initiated by the preparation of macromolecules (PDB ID: 1IR3; 2PEV; 4Y14) and ligands, molecular docking, and visualization. The pharmacokinetic profiles are predictable by using the Swiss ADME and toxicity estimates by Toxtree. The results showed that cucurbitacin B, cucurbitacin E, oleanolic acid, catechin, ferulic acid and apigenin 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 deviate from one Lipinski rule (BM > 500), do not diffuse into the blood brain barrier, are not CYP450 inhibitors, as well as classified as Pgp substrates. The prediction of toxicity indicates that all potential compounds are classified as high toxicity compounds with a risk of narcosis, except oleanolic acid and ferulic acid. These compounds are not genotoxic or non-genotoxic carcinogens.

Keywords: Antidiabetic, docking molecular, *Luffa acutangula*, pharmacokinetic prediction

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. Research of basic health of Indonesia (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 (Ministry of Health RI, 2018).

One of the plants that has potential as an alternative to DM treatment is *L. 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%. 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%. 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

(Chandramohan *et al.*, 2017). *L. acutangula* methanol extract has an IC₅₀ of less than 150 g/mL comparable to acarbose in the inhibition of α-glucosidase (Pimple *et al.*, 2011).

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 (Talele *et al.*, 2010).

Molecular 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 and electrostatics, as well as scoring showing the affinity of the ligand (Forli *et al.*, 2016). 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 interactions based on calculations using the AutoDock 4.0. 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 Method

Preparation of Macromolecules and Ligands

Insulin receptor (PDB ID: 1IR3), aldose reductase (PDB ID: 2PEV), PTP-1B (PDB ID: 4Y14), 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 .PDB format and then optimized with AutoDock Tools. There are 6 ligands anchored to each target protein, which are described in the table 2. The GC-MS of the analysis *L. acutangula* fraction resulted in the identification of 6 compounds (Suryanti *et al.*, 2017). 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 *root-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.

Table I. Validation of macromolecules

Macro-molecule	Centre			Size (Å)			RMSD (Å)	$\Delta G_{\text{binding}}$ (kcal/mol)
	X	Y	Z	X	Y	Z		
1IR3	-23,160	29,968	7,184	40	40	44	1,65	-7,08
2PEV	17,514	-8,180	16,812	40	40	40	0,87	-9,63
4Y14	-10,597	-22,120	-8,056	40	40	40	0,67	-14,14

Table I 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

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 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 macromolecule. Determining the center 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 (Rachmania *et al.*, 2016).

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.

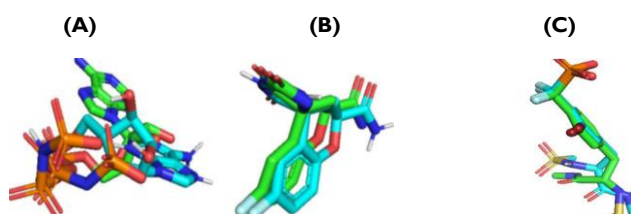


Figure 1: The overlay crystallographic ligands (green) and redocking ligands (blue); (A) IR (ANP); (B) AR (Fidarestat); (C) PTP-1B (C0A)

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. Hubbard (1997) described that phosphorylated insulin kinase receptors bind to adenylyl monophosphate (AMP-PNP). 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.

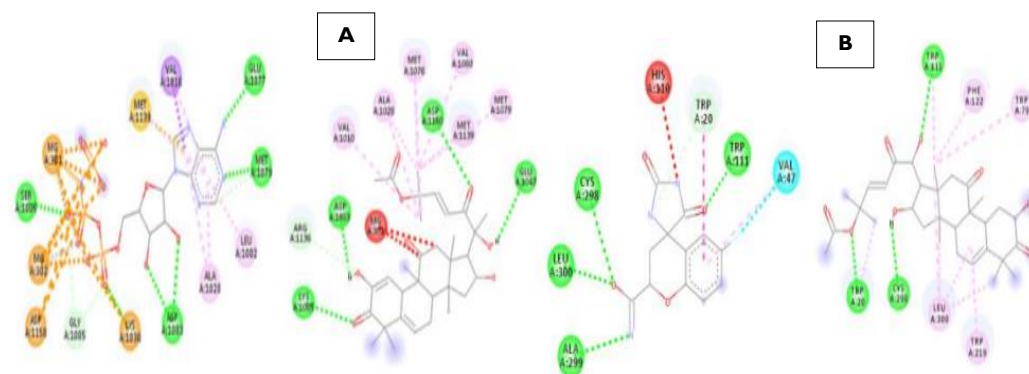
Cucurbitacin E has the lowest bond energy value compared to all test ligands and native ligands in IIR3 macromolecules, which is -10.26 kcal/mol. Cucurbitacin E is able to interact at *binding site* similar to ANP. Therefore, Cucurbitacin E as the best ligand is a potential compound as an insulin receptor 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. 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 (Kim *et al.*, 2018).

Table 2: Molecular docking results of *Luffa acutangula*

Ligands	$\Delta G_{\text{binding}}$ (kcal/mol)		
	IIR3	2PEV	4Y14
Cucurbitacin B	-10.02	-10.11	-9.99
Cucurbitacin E	-10.26	-9.45	-9.11
Oleanolic acid	-8.88	-9.29	-6.56
Catechin	-7.06	-8.52	-7.02
Ferulic acid	-5.12	-6.63	-7.92
Apigenin	-7.36	-8.11	-7.25
Native ligand	-7,08	-9,63	-11,87

Aldose reductase (AR)

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 (Steuber *et al.*, 2006). 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 unfavorable 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⁺ (Causido-siah *et al.*, 2012). Cucurbitacin B has an anion binding pocket similar to Fidarestat, namely the Trp111. 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 residues interact hydrophobically with the benzene ring fidarestat. 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. It has a very potent inhibitory activity against the aldose reductase enzyme with an IC₅₀ of 0.09 M (Takemura *et al.*, 2017)



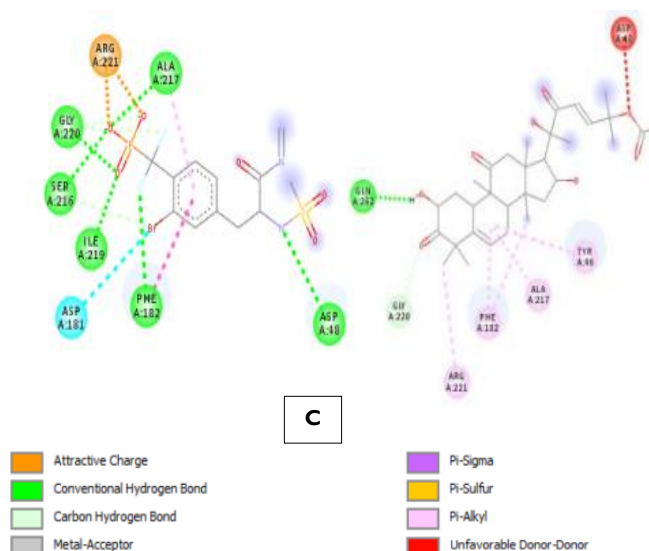


Figure 2: Visualization of macromolecule and ligand (native ligand (left) and potential ligand (right); (A) IR (ANP - Cucurbitacin E); (B) AR (Fidarestat - Cucurbitacin B); (C) PTP-IB (C0A - Cucurbitacin B)

Protein tyrosine phosphatase (PTP-IB)

Inhibitory activity of PTP-IB 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. Visualization of the C0A interaction diagram shows the 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 (Hsing *et al.*, 2020).

Prediction 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 potential ligands from docking molecular. 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 (HBA) < 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 (Lipinski *et al.*, 2001).

Predicted pharmacokinetic profiles showed that 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. Oleanolic acid has a log P >5. This value deviates from Lipinski's rule, namely drug candidate compounds must have log P <5. The potential compounds 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.

Table 3: Predicted pharmacokinetic parameters

Potential Compound	Pharmacokinetic Parameters								
	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

The compounds were not CYP1A2, CYP2C19, CYP2C9, CYP2D6 and CYP3A4 enzyme inhibitors. 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 (Zanger, 2013). Cucurbitacin B has oral bioavailability of 10% with maximum plasma

concentration of 4.85 – 7.81 g/L. This compound has a distribution volume profile of 51.65 L/kg without inhibiting tissue in plasma (Hunsakunachai *et al.*, 2019). 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 (Fiori *et al.*, 2017).

Table 4: Predicted toxicity parameters

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

Cramer's rule is general parameter used as an initial assessment of the safety properties of a potential compound. Oleanolic acid 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 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. 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 potential compounds are compounds with a risk of basic toxicity. Cucurbitacin B and cucurbitacin E carry a risk of basic toxicity due to the presence of the reactive butanone or acetophenone (Ideaconsult, 2011).

Kroes TTC is a specific toxicity parameter in the analysis of dose for carcinogenic compounds. The exposure threshold for a compound is not more than 0.15 g/day with a dosage size of 86 - 97%. Cucurbitacin B and cucurbitacin E were compounds with negligible risk properties as carcinogenic compounds because they had a low probability. Thus, all the best compounds are classified as safe from potential carcinogenic compounds. 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. 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 (Ideaconsult, 2011).

Conclusion

Cucurbitacin B, cucurbitacin E, and oleanolic acid as potential compounds from *Luffa acutangula* against several antidiabetic target proteins. 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 prediction of toxicity indicates that all potential compounds are classified as high toxicity compounds with a risk of narcosis, except oleanolic acid and ferulic acid. These compounds are not genotoxic or non-genotoxic carcinogens.

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