HARIYANTI-In Silico Analysis of the Phytochemical Compounds in Carica papaya Seeds for Optimizing the Inhibitors of HMG-CoA Reductase

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In Silico Analysis of the Phytochemical Compounds in *Carica papaya* Seeds for Optimizing the Inhibitors of HMG-CoA Reductase

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Keywords: *Carica papaya* seed, HMG-CoA reductase, Molecular docking, Anti-hypercholesterolemia.

Abstract: HMG-CoA Reductase, a key enzyme in the cholesterol biosynthesis, catalyzes the conversion of 3-hydroxy-3-methyl-glutaryl coenzyme A (HMG-CoA) into mevalonate. Therefore, this enzyme is the target of the cholesterol-lowering drugs known as statins. *Carica papaya* seed extract contains phytochemical compounds that are thought to have a cholesterol-lowering effect. The present study was designed to examine the ability of the secondary metabolites of *Carica papaya* seeds as an antagonist to HMG-CoA reductase using *in silico* molecular docking. The docking analysis was carried out in PLANTS 1.2 software in which the lowest ChemPLP score, i.e., free energy, was the molecular docking parameter. Seven ligands were docked with HMG-CoA reductase receptor, three of which were benzyl glucosinolate, oleic acid, and glucotropaeolin that had the best ChemPLP scores, namely -71.5491 kcal/mol, -81.7665kcal/mol, and -85.1919 kcal/mol, respectively. Benzyl glucosinolate formed hydrogen bonds with the active site of the targeted protein. As a conclusion, this compound can inhibit the enzyme HMG-CoA reductase, and it has the potential for anti-hypercholesterolemia.

1 INTRODUCTION

Hypercholesterolemia, excessively high levels of plasma cholesterol, emerges as a strong risk factor for cardiovascular disease (CVD) (Stapleton *et al.*, 2010). Cholesterol is an important component of the cell membrane and is essential for the synthesis of various important metabolites. HMG-CoA Reductase (HMGCR), a key enzyme in the cholesterol biosynthesis, catalyzes the conversion of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) into mevalonate. Human HMGCR consists of polypeptide chains of 888 amino acids with three functional portions: residues 1-339 span the membrane of the endoplasmic reticulum eight times, while residues 340-459 connect the membrane portion to the catalytic portion (i.e., residues 460-888), which resides in the cytoplasm. This enzyme is anchored in the membrane of the endoplasmic reticulum, which has seven transmembrane domains, with the active site located in a long carboxyl-terminal domain in the cytosol (Nakanishi *et al.*, 1988). The inhibition of this enzyme results in a significant decrease in cholesterol levels (Goldstein and Brown, 1990).

Carica papaya seeds contain some compounds that are suspected to have a cholesterol-lowering effect on the mechanism of inhibiting the enzyme HMG-CoA reductase. The phytochemical substances in *Carica papaya* seeds have been reported to contain flavonoids, saponins, and tannins (Olivera *et al.*, 2007). These compounds can decrease the HMG-CoA reductase activity and, therefore, inhibit cholesterol synthesis (Siregar 2015; Afrose *et al.*, 2010). The main components of papaya seeds are fatty acids, crude protein, crude fiber, papaya oil, carpaine, benzyl isothiocyanate, benzyl glucosinolate, glucotropaeolin, benzyl thiourea, hentriacontane, β-sitosterol, caricin, and myrosin enzyme (Yogiraj *et al.*, 2014). Also, there are other compounds, such as alkaloids, steroids, essential oils, oleic acid, and palmitic acid (Satriyasa and Pangkahila, 2010). Oleic acid is part of the fatty acids found in papaya seeds. Natali *et al.* (2007) state that oleic acid has an inhibitory effect on HMG-CoA reductase enzyme.

The mechanism of interaction between HMG-CoA reductase enzyme and the compounds in papaya seed (Figure 1) can be investigated using molecular docking method. Molecular docking is used to discover compounds for potentially potent

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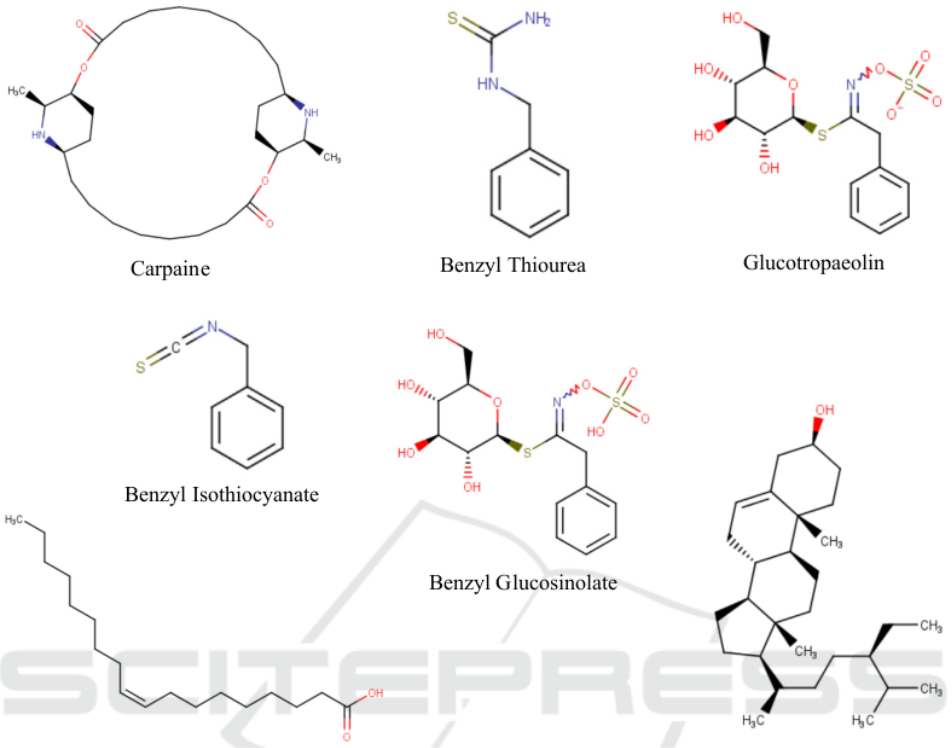


Figure 1: The ligand structures of the phytochemical compounds in *Carica papaya* seeds

drugs in relatively short periods of time (Zukrullah *et al.*, 2012). Based on this background, this research attempted to determine the mechanism of the interaction of HMG-CoA reductase enzyme and the ligands of the compounds in papaya seeds. The enzyme was rolled with each ligand in PLANTS 1.2 software and then visualized to see the interaction formed between the ligand and the receptor in Molecular Molegro Viewer (MMV) software. Using the Lipinski Rule of Five, the in silico analysis determined any compounds that had oral bioavailability.

2 MATERIALS AND METHODS

2.1 Materials

The molecular docking program was run in LINUX with UBUNTU 16 system (64 bit). The ligand design and visualization were developed using Windows 10 operating system. The software used in this research included PLANTS 1.2 (http://www.tcd.unikonstanz.de/ research/plants.php) for docking, YASARA 17.4.17 (http://www.yasara.org/viewdl.htm) for protein preparation and visualization, Marvinsketch 17.9.0 (http://www.chemaxon.com/marvin/download-user.html) for ligand preparation, and Molegro Molecular Viewer 2.5 for visualization.

The 3-dimensional crystallographic structure of HMG-CoA reductase was downloaded from the Protein Data Bank at http://www.rcsb.org/pdb in .pdb format (Purnomo, 2013). Previously, this research had consulted scientific journals to determine the receptor. The 3D structures of the ligands included simvastatin acid and simvastatin, as well as the phytochemical compounds from *Carica papaya* seeds, i.e., carpaine, benzyl isothiocyanate, benzyl glucosinolate, glucotropaeolin, benzyl

thiourea, β-sitosterol, and oleic acid. These structures were designed in Marvin Sketch in .mol2 and .mrv formats.

2.2 Methods

2.2.1 Protein Preparation

The protein macromolecule of HMG-CoA reductase with the PDB code 1HW9 for homo sapiens downloaded from the Protein Data Bank at http://www.rcsb.org/ was inserted into YASARA software for preparation. The protein macromolecules were separated from solvents and ligands or non-standard residues. The separation of macromolecules from unnecessary molecules used YASARA program (edit > delete > residue). The elimination of water molecules (edit > delete > water) and the addition of hydrogen to the structures (edit > add > hydrogen to all) were also run in this program. The results were stored with the protein name and in .mol2 format.

2.2.2 Ligand Preparation

The ligand structures were downloaded from www.pubchem.ncbi.nlm.nih.gov in 2D model. The protonation was changed at pH 7.4 using the Marvin Sketch (Calculation > Protonation > Major Microspecies), the resulted data were then stored in .mrv files. These files were opened, and a conformational search in the same software was stored in .mol2 format (Calculation > Conformation > Conformer).

2.2.3 Validation of Molecular Docking Methods

Before the virtual screening, validation was performed to determine the values of the root mean square distance (RMSD). It was run in the YASARA program (Analyze > RMSD > Molecule) by entering specific ligands and receptors in .mol2 format. A protocol was accepted if the RMSD of the heavy atom was smaller than 2.0 Å.

2.2.4 Molecular Docking with PLANTS 1.2 Software

The molecular docking was processed in PLANTS program. This software can only run in Linux operating system. All data and PLANTS applications were moved from the desktop to the root (sudo -s), and the terminal was opened in Linux afterward, The command "cp /home/desktop/PLANTS1.2 PLANTS" was typed in and followed by the command "chmod u + x PLANTS" to activate the PLANTS application. The results of the ligand and receptor preparations were stored in .mol2 and moved to the root with the command "cp /home/desktop/*.mol2."

The next step was to find the binding site using the command "./PLANTS - bind ref_ligand.mol2 5 protein.mol2". To examine whether the settings on the PLANTS were correct, this research used the command "kwrite plantsconfig", followed by "./PLANTS --mode screen plantsconfig". When the docking process was complete, the results were displayed in the terminal by entering the command "cd results /" and, then, "more bestranking.csv". From the ten docking results, the one with smaller conformation value was selected, and the result was stored using the command "cp*_entry_ (conformation number)_conf_01.mol2/home/ desktop/".

2.2.5 Molecular Docking Result Analysis And Visualization

The docking results were observed from the output in a notepad format. The complex conformation of the docking result was determined by choosing conformation based on the CHEMPLP score, i.e., the lowest free energy. The docking results were visualized using YASARA software to determine whether the hydrogen bond distance was <3.5 Å.

2.2.6 Drug Scan Analysis

The drug scan was analyzed on a website (http://chemicalize.com). The analysis involved uploading the ligand file in .mol format. Then, the results were downloaded in PDF format.

3 RESULTS AND DISCUSSION

The initial stage of the docking process was the preparation of the protein structure where the proteins were selected from the GDP site purposively. The HMG-CoA reductase enzyme in .pdb format was downloaded from the protein database organized by the Research Collaboratory for Structural Bioinformatics (RCSB) at http://www.rcsb.org/. The protein chosen for the HMG-CoA reductase was 1HW9 (PDB code). It is an enzyme complex with simvastatin acid (endogenous ligand). The protein structure

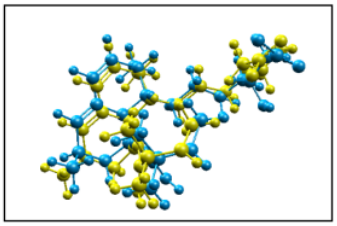


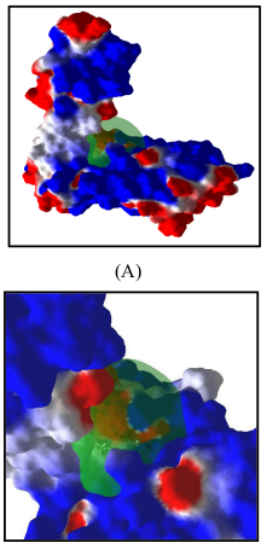
Figure 2. The Visualization of the Superposition of Endogenous Ligand and Copy Ligand in Molegro Molecular Viewer Software. The endogenous ligand is yellow; the copy ligand is blue.

downloaded from GDP generally still contains a solvent (water). In this study, the protein structures of the other residues were analyzed without the endogenous ligand structure. In other words, the protein structures depicted a protein without endogenous ligand and other molecules such as water and other single atoms; therefore, the docking process only analyzed the interaction of test compounds and proteins (Kitchen *et al.*, 200[17] These water ligands and molecules had to be removed from the protein macromolecules as they might prolong the duration of the docking simulation. The addition of the hydrogen atom in question afterward aimed to bring up the existing hydrogen atoms in the structure and create a three-dimensional form that determined the interaction with the ligand. The docking simulation of all processes involved in the preparation of the protein structure was run in the YASARA program.

The process of preparing the ligand structure aimed to achieve optimal ligand conformation. The conformation of drug molecules may depend on the acidity (pH) and ionic composition of the medium in which the drug is studied (Siswandono and Soekardjo 1998). Since the drug worked on a biological system, each ligand was subjected to protonation to obtain a structure adjusted to the blood pH, i.e., about 7.4. A total of nine ligands were tested in this study. The nine ligands consisted of one endogenous ligand (SIM) from the crystal structure of HMG-CoA reductase (for redocking process), one comparator ligand (simvastatin), and seven tes[7]gands of the compounds in papaya seeds, namely carpaine, benzyl isothiocyanate, benzyl glucosinolate, glucotropaeolin, benzyl thiourea, and oleic acid. Using a conformational search in Marvin Sketch, the optimization yielded as many as ten (10) conformations that represented the positions of all ligands against the pocket cavity. Then, the analysis proceeded with the docking process to find out which ligand conformation best represented the ligand position against the pocket cavity, as evidenced by the ChemPLP score analysis. Agistia *et al.* (2013) state that the most appropriate conformation can be identified from the output of the molecular docking run in PLANTS 1.2, which is the ChemPLP score. Therefore, the next step in this research determined the ChemPLP score of the ligand against the receptor resulted from the docking process to find out the best ligand conformation.

The identification of appropriate docking protoco[20]s a key step to a valid docking pose (Oniga *et al.*, 2017). The validation of the docking method in this study was conducted by redocking the endogen[31] ligand in the protein group downloaded from the Protein Data Bank. The [21]luation of the validation results relied on the RMSD (Root Mean Square Deviation) of the pose visualization (Moitessier *et al.*, 2008). RMSD is a measurement of two poses by comparing the positions of atoms in experimental structures with the ones in docked or predicted structures (Hawkins *et al.*, 2008) The RMSD values of successful [3]cking methods are <2.0 Å (Hevener *et al.*, 2009; Jain and Nicholls, 2008; Moitessier *et al.*, 2008). The RMSD of the validation results in this research was 1.0138 Å (<2 Å), proving that 1HW9 could be used for further analysis in this research. The closer the RMSD to zero, the more similar the poses of endogenous ligand and copy ligand. Small RMSD suggests th[6] the developed protocols are accepted, and they can be further developed for virtual screening in the discovery of new compounds (Purnomo, [6]011; 2013). Superposing the endogenous ligand and the copy ligand, the visualization validated that the atoms of the two-molecule structures had similar positions and angles (Figure 2). In other words, the conformation of the endogenous ligand structure of GDP was similar to the well-selected copy ligand in the docking process (Adelina, 2014). [6]With RMSD >2.0 Å, the visualization shows two molecules with significantly different angles and positions even though they have equal number of atoms.

Docking is a simulation method to find out the orientation between ligand and receptor. After the redocking process with endogenous ligand, the cartesian coordinates of the binding site were x= 4.0308, y= -9.4318, and z= -11.5016. Figure 3 shows the visualization of the binding site on the receptor (1HW9). The binding site is an area where protein binds to molecules and ions (i.e., ligands) that will affect the conformation and function of the



(A)

(B)

Figure 3. (A) Zoom Out, (B) Zoom in; The Visualization of the Binding Site on 1HW9 (Grid Box: Green Circle) using MMV Software Based on the Redocking Results in PLANTS 1.2 software.

protein. The binding site involves amino acid residues that play an important role in binding with ligands (Pratama *et al.*, 2016). Based on the docking scores listed in Table 1, the endogenous SIM (simvastatin acid) ligand has the lowest ChemPLP score, i.e., -101.899 kcal/mol, while the score of simvastatin (comparator ligand) is -80.3996 kcal/mol. Simvastatin acid is the active metabolite of simvastatin (Pubchem, 2017). The difference lies in the structure. Mycek *et al.* (2001) mention that the structure of simvastatin is a lactone that needs to be hydrolyzed into active drugs. This hydrolysis (simvastatin acid) adds OH-group and carboxylic group to the structure. The OH-group influences the amount of hydrogen bond interaction. Siswandono and Soekardjo (1998) explain that hydrogen bond interaction generally occurs in compounds that have clusters of, for example, OH-, and NH-. Based on the data (Table 2), the most prevalent hydrogen bond interactions in the endogenous ligand (simvastatin acid) involved the OH group, especially OH- in the carboxylic group of simvastatin acid. The difference in the number of OH-groups causes different scoring results between simvastatin and simvastatin acid.

Table 1. The Molecular Docking Results of the Comparator Ligands and Ligands in Papaya Seed against HMG-CoA Reductase Using PLANTS 1.2 Software

Ligands	CHEMPLP Scores (Kcal/mol)
Simvastatin Acid (endogenous ligand)	-101.899
Simvastatin (Comparative Ligand)	-80.3770
Carpaine	-66.6044
β-Sitosterol	-73.2094
Benzylthiourea	-55.9158
Benzyl Isothiocyanate	-52.0111
Benzyl Glucosinolate	-90.5491
Oleic acid	-81.7665
Glucotropaeolin	-85.1919

The molecular docking performed on the compounds of papaya seed against HMG-CoA reductase (Table 1) resulted in three (3) best compounds whose ChemPLP scores were lower than the comparator ligands (simvastatin). They were benzyl glucosinolate (ChemPLP score= -90.5491 kcal/mol), glukotropeolin (-85.1919 kcal/mol), and oleic acid (-81.7665 kcal/mol). The ChemPLP score of simvastatin ligand was -80.3996 kcal/mol. Schneider and Bohm (2000) mention that a smaller docking score implies a more stable bond or, in other words, a more potent compound. Serina (2013) affirms this assertion with the energy linkage to affinity, i.e., that the best ligand will have stable (free energy) performance and a better affinity. Affinity is a measure of the drug's ability to bind receptors. It is highly dependent on the molecular structure of the drug and the receptor (Siswandono and Soekardjo 1998). The ChemPLP scores of the ligands in Table 1 were compared with simvastatin to determine which ligand had the best interaction and affinity. The comparison results were validated by further analysis based on the inter-molecular bond interactions (ligand and receptor). The analysis also included the interpretation of the bond interaction between amino acid residues and ligands in Molegro Molecular Viewer (MMV).

Glucosinolate is included in the glycoside class. Glycoside is composed of two entities, namely the sugar group (glycone) and the non-sugar group (aglycone/genin). The sugar portion of a glycoside may be associated with the aglycone in various ways, and the most common one is through the oxygen (O-glycosides) atoms. However, the atoms

Table 2. The visualization Results of the hydrogen bond interaction between the ligands (endogenous ligands, test ligands, and comparator ligands) and the receptor (HMG-CoA reductase) using MMV software

Ligands	Bonded Amino Acid Residues	Bond distance (Å)	Group on Ligands
Simvastatin Acid	Asp 767	2.75	OH- group
	Gln 770	3.30	OH-group on carboxylate
	Gln 770	3.18	OH-group on carboxylate
	Glu 801	3.12	O- pada carboxylate
	Asp 690	3.14	OH-group on carboxylate
	Asp 690	3.37	OH-group on carboxylate
	Asn 771	3.03	Side chains
	Arg 702	3.26	OH-group on carboxylate
Simvastatin	Tyr 761	2.85	Lactone ring
	Gln 770	2.77	Side chains
	Gln 766	3.26	OH- group on lactone's ring
Benzyl glucosinolate	Lys 691	2.15	OH^- group on glucose
	Asp 767	2.66	OH^- group on glucose
	Gln 770	2.51	OH^- group on glucose
	Gln 770	2.41	OH^- group on glucose
	Glu 801	2.39	OH^- group on glucose
	Ser 774	2.95	Nitro group on glucose
	Tyr 761	2.32	Sulfonic group
	Tyr 761	2.77	Sulfonic group

Ligands	Bonded Amino Acid Residues	Bond distance (Å)	Group on Ligands
Glucotropaeolin	Asp 767	2.97	Nitro group
	Gln 766	3.18	Sulfonic group
	Ser 774	2.40	OH^- group on glucose
	Ser 774	3.24	OH^- group on glucose
	Gln 770	2.76	OH^- group on glucose
	Gln 770	2.60	OH^- group on glucose
	Gln 770	3.11	OH^- group on glucose
	Tyr 761	2.79	OH^- group on glucose
	Glu 801	2.62	OH^- group on glucose
	Glu 801	3.24	OH^- group on glucose
	Asn 771	3.37	OH^- group on glucose
	Ala 769	3.27	O^- on glucose ring
Oleic acid	Arg 702	3.14	OH-group
	Asp 690	3.11	OH-group
	Asp 690	2.89	OH-group
	Glu 801	2.46	OH-group
	Glu 801	2.82	OH-group

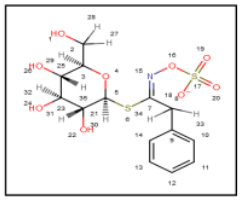


Figure 4. The Structure of Benzyl Glucosinolate (Chemicalize, 2018)

Nitrogen (N-glycosides), or sulfur atoms (S-glycosides) (Sarker and Nahar, 2009). In the glucosinolate compound, the sugar portion (glycone) is connected to a sulfur atom (S-glycoside). Glucosinolate is a secondary metabolite of almost all Brassicales families (including Brassicaceae, Capparidaceae, and Caricaceae) (James *et al.*, 1996). One example of glucosinolate found in the Caricaceae family discussed in this study is glucosinolate, derived from the benzyl glucosinolate (Figure 4) in papaya seed. Investigating the benzyl glucosinolate content in various tissues, Najamura *et al.* (2007) find the highest benzyl glucosinolate content in papaya seeds.

The receptor's interaction with ligands formed after the docking process was visualized using Molegro Molecular Viewer software. The breaking lines described the hydrogen bonds that occurred between the residues and the groups on the ligands. The observation of residual interactions (amino acids) aimed to identify any ligand-receptor interactions. The hydrogen bonding is an interaction that can stabilize the ligand bond and the receptor bond. Another ligand-receptor interaction that can improve the stability of the conformation is the electrostatic interaction and van der Walls interactions.

Table 2 shows the residual ratio of the two best ligands to simvastatin (comparator ligand) after the docking process. Simvastatin bonded to Tir 761, Gli 770, and Gli 766 (Figure 5a). The number of the hydrogen bonds formed in simvastatin tended to be lower than those of oleic acid, glucotropaeolin, and benzyl glucosinolate. The hydrogen bond distances formed in this research were greater than 3.0 Å (close to 3.5 Å), but not one of them exceeded 3.5 Å. Therefore, the hydrogen bond distance of simvastatin still qualifies for an energetically significant hydrogen bond interaction, i.e., not exceeding 3.5 Å (Marcou and Rognan, 2007). In the interaction between oleic acid ligands and HMG-CoA reductase receptors, there were five hydrogen

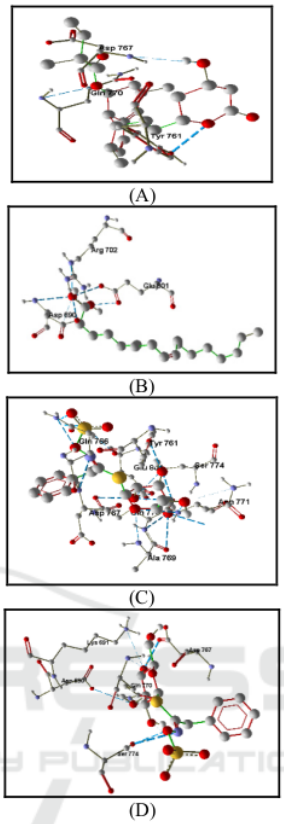


Figure 5: The Visualization of The Residual Contact of Ligand and HMG-CoA Reductase Receptor using Molegro Molecular Viewer Software: (A) Simvastatin, (B) Oleic acid, (C) Glucotropaeolin, (D) Benzyl glucosinolate.

bond interactions (Figure 5d and Table 2). The hydrogen bonds occurred in the amino acid residue Arg 702 (1 hydrogen bond interaction), Asp 690 (2 hydrogen bond interactions), and Glu 801 (2 hydrogen bond interactions). Two of the five hydrogen bonds in oleic acid had the hydrogen bond distance of greater than 3.0 Å (<3.5 Å), which meets the requirement of significant hydrogen bond (Marcou and Rognan, 2007). When compared with simvastatin as a comparator ligand in this research (Table 2), oleic acid had 34 greater number of hydrogen bond interactions. This finding is in line with Natali *et al.* (2007) who report that oleic acid exhibits an inhibition activity toward the enzyme HMG-CoA reductase.

There were twelve hydrogen bond interactions in the glucotropaeolin ligand (Figure 5c and Table 2). Most hydrogen bond interactions were formed due to a large number of electronegative atoms in the glucotropaeolin molecule; hence, the tendency to form hydrogen bonds. However, according to Lipinski (2003), the number of hydrogen bonds in the drug should not be more than ten. Otherwise, drugs will have difficulty in passing through the intestinal walls into the blood.

Pine *et al.* (1988) mention that the shorter the bond distance, the stronger the bond. The interactions in glucotropaeolin included six (6) hydrogen bonds whose distances were more than 3.0 Å, i.e., in Ala 769 (3.27 Å), Asn 771 (3.37 Å), Glu 801 (3.24 Å), Gln 770 (3.11 Å), Ser 774 (3.24 Å), and Gln 766 (3.18 Å). According to Marcou and Rognan (2007), hydrogen interactions can occur when two atoms are within 3.5 Å to each other. The hydrogen bond distance in glucotropaeolin was smaller than 3.5 Å, which satisfies the conditions for hydrogen bonding.

Glucotropaeolin was one of the three (3) best ligands in papaya seeds with a lower ChemPLP score than simvastatin (Table 1). However, after further analysis through visualization, each of its hydrogen bond distances satisfied the conditions hydrogen bonding (i.e., <3.5 Å) (Marcou and Rognan, 2007). However, it is thought to be less potent in penetrating the intestinal membrane because it does not meet the requirement proposed by Lipinski (2003), i.e., the number of hydrogen bonds should not be more than ten. Considering the number of hydrogen bond interactions, glucotropaeolin ligand could not be categorized as the best ligand.

Once proven by visualization, the number of hydrogen bond interaction between benzyl glucosinolate and amino acid residues at the receptor (Figure 6) was eight. This number was greater than the hydrogen bond interaction in simvastatin. Additionally, it conforms with the qualification set in Lipinski (2003), i.e., not exceeding 10. The amino acid residues that interacted with benzyl glucosinolate were Lys 691 (with a hydrogen bond distance of 2.15 Å), Asp 767 (2.66), Gln 770 (2.51 Å and 2.41 Å), Glu 801 (2.39 Å), Ser 774 (2.95 Å), and Tyr 761 (2.32 Å and 2.77 Å). The average length of the hydrogen bond on benzyl glucosinolate ligand was less than 3.0 Å, which is in line with the conditions for hydrogen bonding mentioned in Marcou and Rognan (2007). The average length of the hydrogen bond distance formed on benzyl glucosinolate was shorter than the comparator ligands (simvastatin) and the other two best ligands in papaya seeds (glucotropaeolin and oleic acid) (Table 2). The shorter the hydrogen bond distance, the stronger the bond (Pine *et al.*, 1988).

Drug-likeness is a qualitative concept used to describe the similarity of a compound as a drug candidate, such as the complex balance of various molecular properties and structural features that determine whether a particular molecule is similar to a known drug. These molecular properties are primarily hydrophobicity, electronic distribution, hydrogen bond characteristics, molecular size and flexibility, and other pharmacophore properties affecting the behavior of molecules in living organisms, including bioavailability, delivery properties, affinity for proteins, reactivity, toxicity, and other metabolic stability (Leeson, 2016; Mishra *et al.*, 2017). The Rule of Five (Ro5) or the Lipinski's Rule of Five is a set of in silico guidelines applied to drug discovery to prioritize compounds with a high probability of increased absorption (Doak *et al.*, 2014). This rule can be used to determine the pharmacokinetics of a compound as a drug candidate (Benet *et al.*, 2016). For drug-likeliness evaluation, it discusses four simple physicochemical parameters (namely, molecular weight ≤ 500, log P ≤ 5, hydrogen bond donor ≤ 5, hydrogen bond acceptor ≤ 10) associated with 90% of orally active drugs that have passed clinical status of phase II (Lipinski, 2004; 2016).

Based on the prediction result (Table 3) run in www.chemicalize.com using the Lipinski's Rule of Five, benzyl glucosinolate was within the threshold of the partition coefficient (log P= 2.19; <5). The log P values of benzyl glucosinolate and glucotropaeolin were lower than the endogenous compounds and ligands, but they still met the Lipinski's rule (log P <5). The log P values of benzyl glucosinolate and glucotropaeolin indicated a solubility coefficient in

Table 3. The Prediction Results Based on the Lipinski's Rule of Five (Chemicalize, 2018)

Ligands	Prediction using the Lipinski's Rule of Five			
	BM (g/mol)	Log P	H *bond Donor*	H *bond Acceptor*
Simvastatin	418.57	4.46	1	3
Benzyl Glucosinolate	408.42	2.19	4	9
Oleic acid	282.47	6.78	1	2
Glucotropaeolin	409.42	2.19	5	9
Simvastatin acid	436.59	3.9	3	6

fat or water within the range of -0.4 and 5. The molecular weights of benzyl glucosinolate, oleic acid, and glucotropaeolin were 408.42 g/mol, 282.47 g/mol, and 409.42 g/mol (<500 g/mol), and they were lower than the BM of the comparator ligand.

The hydrogen bond donors in benzyl glucosinolate and oleic acid were, respectively, 4 and 1 (<5). Meanwhile, glucotropaeolin had 5 hydrogen bond donors, which did not meet the Lipinski's rule. The number of the hydrogen bond donors in benzyl glucosinolate was higher than the comparator ligand, but it still met the standards set by Lipinski (1997). Benzyl glucosinolate and glucotropaeolin had 9 hydrogen bond acceptors (close to 10), while oleic acid had two (2). However, the Lipinski's rule states that the hydrogen bond acceptor should not exceed 10. As a conclusion, the hydrogen bond donors and acceptors in benzyl glucosinolate, oleic acid, and glucotropaeolin are compliant with the Lipinski's rule.

This rule also states that a molecular weight of more than 500 Da cannot diffuse through the cell membrane by passive diffusion. The higher the log P, the more hydrophobic the molecule. Molecules that have too hydrophobic properties tend to have high levels of toxicity because they will stay longer in the lipid bilayer and spread more widely in the body; therefore, the selectivity of the bond to the target enzyme decreases. Too hydrophilic properties (negative log P) are also not good because the molecule cannot pass through the membrane lipid bilayer. Based on the number of hydrogen bond donor and acceptor, a higher hydrogen binding capacity represents larger energy required for the absorption process to occur. In general, the Lipinski's rules describe the solubility of certain compounds to penetrate cell membranes by passive diffusion (Lipinski *et al.*, 1997). As a conclusion, benzyl glucosinolate does not violate any of the Lipinski's rules, and, thereby, it can be developed as anti-hypercholesterolemia drug candidates for oral preparations.

4 CONCLUSIONS

The results of the ligand-receptor interaction of the compounds in papaya seed (*Carica papaya* L.) against HMG-CoA reductase receptor categorized benzyl glucosinolate as the best compound because it had a ChemPLP score of -90.5491 kcal/mol and eight hydrogen bond interactions. This compound has the potential as an anti-hypercholesterolemia drug.

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