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Molecular Docking of Anthocyanin Compound as Anti-Hyperlipidemia Against PPARα, HMG-CoA Reductase and ACAT Proteins

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ABSTRACT
Hyperlipidemia can increase the risk of cardiovascular diseases, such as coronary heart disease (CHD), the number one cause of death worldwide each year. Therefore, needed efforts to reduce the prevalence of cardiovascular disease, one of which is parijoto fruit (*Medinilla speciosa*), which has a high anthocyanin content. Previous research stated that parijoto extract reduced total cholesterol, LDL, triglycerides (TG), and increased HDL levels in the blood of hyperlipidemic rats. However, the molecular mechanism of anthocyanin in reducing lipid levels is still unknown, so an in silico study using molecular docking techniques is necessary. Molecular docking provides molecular modeling of receptor proteins and their interactions with ligands. This study aims to determine the potential of anthocyanin as an anti-hyperlipidemia compound through inhibiting PPARα protein, HMG-CoA reductase, and ACAT in silico with molecular docking techniques. Several stages in this research are data collection of receptor proteins and ligand, download of receptor proteins and ligand, molecular docking, and data analysis. Molecular docking includes protein separation with accompanying components, receptor and ligand preparation, running molecular docking using PyRx and AutoDock Vina, docking result validation, and visualization of docking results using Biovia Discovery Studio 2020. The molecular docking results were analyzed by looking at the affinity energy value (kcal/mol) and the complex conformation between the receptor-ligand. The results showed that the anthocyanin compound had a docking score of -8.8 kcal/mol; -6.0 kcal/mol; and -7.6 kcal/mol for PPAR-α protein, HMG Co-A reductase, and ACAT proteins, respectively. This value is similar to the docking score produced by Simvastatin as a positive control which is -7.8 kcal/mol, -6.4 kcal/mol, and -7.7 kcal/mol. Anthocyanin compounds have the potential to inhibit PPAR-α protein, HMG Co-A reductase, and ACAT and act as anti-hyperlipidemic in silico.

Keywords: *ACAT, Anthocyanin, Anti-hyperlipidemia, HMG Co-A reductase, Molecular docking.*

1. INTRODUCTION

Hyperlipidemia can increase the risk of cardiovascular diseases, such as coronary heart disease (CHD), the number one cause of death worldwide each year. In 2018, the prevalence of CVD comprising CHD, stroke, and hypertension in adults ≥20 years of age was 49.2% overall [1]. Therefore, an effort is needed to reduce the prevalence of cardiovasculardisease, one of which is parijoto fruit (*Medinilla speciosa*) with high anthocyanin content. The

total anthocyanin content of the peel and fruit extract of parijoto (*M. speciosa*) was 208.75 mg/L and 173.7 mg/L, respectively [2]. Previous research stated that methanol extract could reduce total cholesterol, LDL, triglycerides (TG), and increase HDL levels in the blood of hyperlipidemic rats [3, 4]. However, the molecular mechanism of anthocyanin compounds in reducing lipid levels is still unknown, so an in silico study using molecular docking techniques is necessary.

Molecular docking is widely used as early research in the discovery of bioactive compounds that can be used as drug candidates [5] because it can provide molecular modeling of receptor proteins and their interactions with ligands [6, 7]. Molecular docking is one of the most frequently used methods in structure-based drug design (SBDD) because of its ability to predict, with a substantial degree of accuracy, the conformation of small-molecule ligands within the appropriate target binding site [8]. This study will simulate the docking of anthocyanin compounds to proteins involved in lipid metabolism, including Peroxisome proliferator-activated receptor alpha (PPARα) [9], 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase [10, 11] and acyl-CoA cholesterol acyltransferase (ACAT) proteins [11].

The PPARα is a physiological receptor for hypolipidemic fibrates. This transcription factor controls the expression of various genes involved in most lipid metabolic pathways, including fatty acid oxidation, triglyceride (TG) synthesis and breakdown, lipoprotein metabolism, gluconeogenesis, and various other metabolic pathways [12]. Another protein that plays a role in lipid metabolism is Hidroxymethylglutaryl Coenzyme A Reductase (HMG-CoA reductase) [11]. Flavonoid compounds can inhibit the action of the HMG-CoA reductase enzyme [13], which is an enzyme that catalyzes the initial steps of cholesterol biosynthesis. When cholesterol is transported from the intestine to the liver, HMG-CoA reductase that converts HMG-CoA to mevalonate in cholesterol synthesis will be inhibited so that endogenous cholesterol synthesis products by the liver will be reduced [10]. Another therapeutic strategy for hypercholesterolemia and atherosclerosis is ACAT inhibition [14]. ACAT catalyzes cholesterol esterification from cholesterol and fatty acyl coenzyme A to cholesterol esters [14, 15]. Inhibition of ACAT can cause a decrease in cholesterol levels, reduce the assembly and secretion of lipoproteins containing apolipoprotein B such as VLDL and LDL, and inhibit the formation of foam cells in the arterial walls [14].

This study aims to determine the potential of anthocyanin compounds in inhibiting PPARα protein, HMG-CoA reductase, and ACAT in silico with molecular docking techniques and determine potential receptor targets that play a role in the anti-hyperlipidemic mechanism of anthocyanin compounds.

2. METHODOLOGY

This research is a bioinformatics approach using the Autodock Vina molecular docking method [16]. Several steps in this study:

2.1. Data collection of receptor proteins and ligand compounds

The first step was determination of protein receptors and ligand compounds. This research used PPARα, HMG-CoA reductase, and ACAT proteins with specific codes 2P54, 1HW9, and 6P2P respectively as protein receptors. Anthocyanin PubChem CID 145858 as ligand and positive control Simvastatin with specific codes PubChem CID 54454. The protein receptors were obtained by downloading the protein structure (PDB format) through the Protein Data Bank (PDB) website, while the 3D structure of the ligand was downloaded via the PubChem website.

2.2. Download of receptor proteins and ligand compounds

The proteins were downloaded through the Protein Data Bank (PDB) website (http://www.rscb.org/pdb/). If the website is already open, in the search menu, write down the protein code to be downloaded (2P54; 1HW9 and 6P2P for PPARα; HMG-CoA reductase; and ACAT proteins). The protein file was opened until detailed information of the protein appeared, then the 'Download file' menu was selected. The selected file is a PDB format file.

Anthocyanin compounds and simvastatin are downloaded from the NCBI PubChem website (http://pubchem.ncbi.nlm.nih.gov/). If the website was already open, the name or code of the compounds (Simvastatin CID 54454 and Anthocyanin CID 145858) were written in the search menu. The ligand was selected until detailed information about the compound appeared. Select the 'Download' menu, and save the 3D Conformer in SDF format to download the compound file.

2.3. Molecular Docking

2.3.1. Protein Separation with Accompanying Components

The protein structure downloaded from the Protein Data Bank is a complex structure that contains several

components, including native ligands and heteroatoms [17]. Before initiationg the molecular docking, all components in the protein structure are removed leaving one protein molecule used as a receptor when running docking. Molecular docking only requires one protein molecule as the receptor. Other components need to be removed by clicking on the option to be deleted (including proteins with symbols for the letters B, C, D, E, F, G, and H, heteroatoms, active sites, and ligand group) then click the 'X' option in turn. The remaining protein molecules are receptors that will be used in the docking process.

2.3.2. Receptor Preparation

The first step is opening the PyRx software. Protein molecules are entered by selecting the 'File' menu, then clicking the 'Load Molecules' command. The selected molecule is a protein file separated with its accompanying components. Next, select 'AutoDock' and click on the 'Make Macromolecule' command to change the receptor format from '.pdb' to '.pdbqt'.

2.3.3. Ligand Preparation

The ligand structure file downloaded from the PubChem website was entered into the PyRx worksheet. Once the list of ligands appears in the PyRx, right-click on one of the ligands. The cursor was directed to the 'Minimize All' option to reduce the energy value of the ligand. After the energy value appears behind the ligand name, the cursor is directed to the option 'Convert All to AutoDock Ligand' to change the ligand format to .pdbqt.

2.3.4. Running Molecular Docking using PyRx and AutoDock Vina

In the Vina AutoDock software, the 'Vina Wizard' menu was selected and clicked the 'Start' option. Receptors and ligands were selected in the AutoDock menu Navigator display box. If the number of ligands and receptors was appropriate, the 'Forward' option in the Controls box was clicked, a grid box would appear on the macromolecule display. A description of the distance value and gridbox dimensions would appear. Next, the 'Maximize' menu option was clicked to enlarge the gridbox size to match the size of the macromolecule. Before running Vina, ensure that the exhaustiveness value is multiple of 8 (8-64), the higher the exhaustiveness value, the higher the docking accuracy. The last step in docking was to click on the 'Forward' or 'Run Vina' option. The software will automatically run the docking process, then wait for the process to finish until the binding affinity and RMSD values appear in the Controls box which is displayed in tabular form.

2.3.5. Docking result validation

The docking process including ligand conformation, a prediction of position and orientation within these sites (usually called poses), and assessment of binding affinity. The accuracy of pose prediction can be evaluated by a root mean square deviation (RMSD) calculation that compares the predicted pose to the experimental pose [18].

2.3.6. Visualization of Docking Results using Biovia Discovery Studio 2020

The docked file was opened using Biovia Discovery Studio 2020, a 3D structure of the protein with docked ligands appeared. Hydrogen bonds between ligands and proteins were displayed by clicking the 'Structure' menu on the toolbar, then clicking the 'H Bond' option. Clicking the receptor name in the hierarchy to visualize the docking results in 2D, then clicking the 'Define Receptor' in the 'View Interaction' command box. One ligand with the best docking value was selected by clicking on the selected ligand name, then click the 'Define Ligand' command. The 2D diagram view was created by directly clicking the 'Show 2D Diagram' option in the 'View Interaction' command box. The diagram will automatically appear on the software's main screen, where the diagram shows the various amino acid residues and the types of bonds that occur.

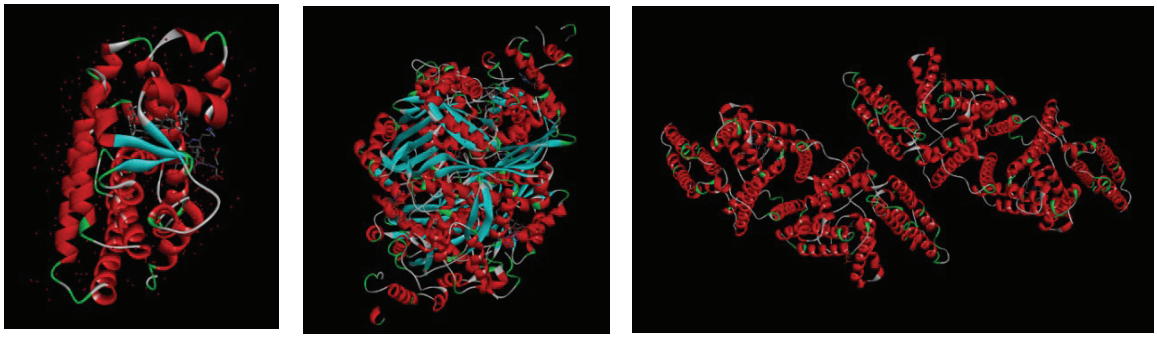
2.3.7. Data Analysis

This research is descriptive. Analysis of molecular docking results was carried out by looking at the affinity energy value (kcal/mol) and the complex conformation between the receptor-ligand. The more negative the affinity energy value indicates a good level of stability between the ligand and the receptor so that the bond formed will be stronger [19].

3. RESULTS

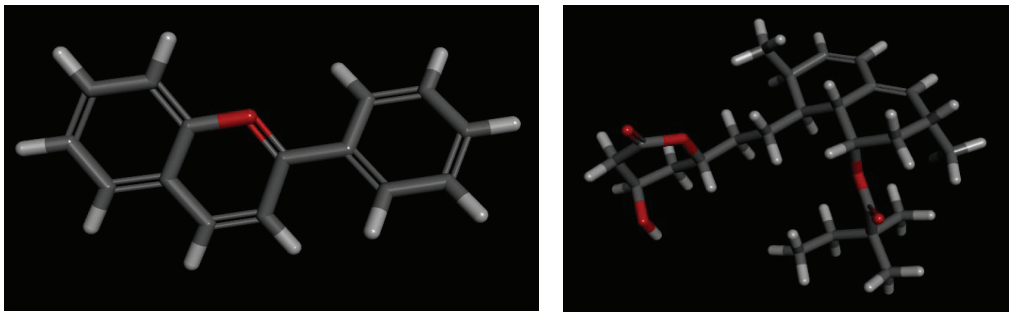
3.1. Data Collection of Receptor Proteins and Ligand Compounds

The three-dimensional structure of the PPARα, HMG Co-A Reductase, and ACAT proteins used in this study were downloaded from the Protein Data Bank (PDB) website with specific codes 2P54, 1HW9,



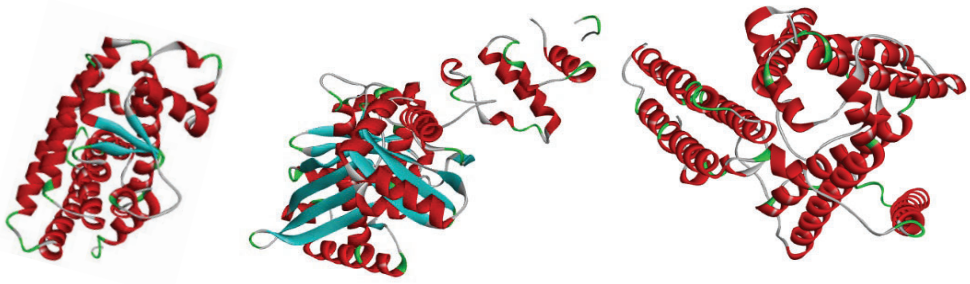
(A (B (C

Figure 1. The 3D structure of proteins (A) PPARα, (B) HMG Co-A, and (C) ACAT



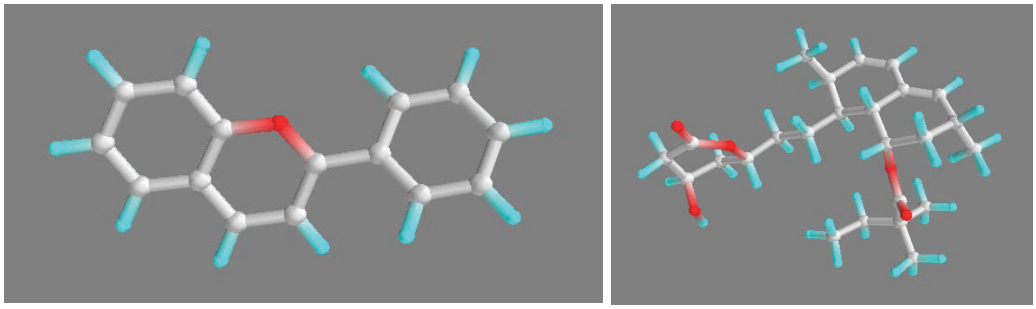
(A) (B)

Figure 2. 3D structure of (A) Anthocyanin and (B) Simvastatin



(A (B) (C)

Figure 3. The 3D structure of protein (A) PPARα, (B) HMG Co-A, and (C) ACAT after preparation



(A) (B)

Figure 4. Structure of prepared ligands (A) Anthocyanin and (B) Simvastatin

and 6P2P, respectively. The following is the structure of the PPARα, HMG Co-A Reductase, and ACAT proteins that have been successfully downloaded (Figure 1). The structure of the compound used in this study was downloaded from the NCBI PubChem website (http://pubchem.ncbi.nlm.nih.gov/) with specific codes 145858 for anthocyanins and 54454 for simvastatin. The structure showed that anthocyanin has a positive charge at the oxygen atom of the C-ring of basic flavonoid structure, which called called the flavylium (2-phenylchromenylium) ion [20]. Simvastain contain a lactone ring within their structure, it act as pro-drug that is administered in its inactive lactone form and converted in the body to an active beta-hydroxy acid metabolite [21]. The four compounds were saved in 3D Conformer format. The following is the compound structure that has been successfully downloaded (Figure 2).

3.2. Receptor Protein and Ligand Preparation

The receptor preparation was done by removing water molecules, ions, and ligands so that these molecules would not interfere with the interaction between target molecules and proteins in AutoDock [22]. The prepared file was saved in .pdb format. Furthermore, the .pdb format was changed to .pdbqt using PyRx software as a special software format used for molecular docking [23]. The following is the structure of the prepared PPARα, HMG Co-A, and ACAT proteins (Figure 3).

The ligand preparation was carried out using PyRx software by selecting "Convert Selected to AutoDock Ligand (pdbqt)." The change in file format aims to match the format of the ligand with the receptors and can be read by the PyRx software [23]. The following is the structure of the ligands that have been successfully prepared (Figure 4).

3.3. Molecular Docking of Receptor Proteins and Ligand Compounds

The results of molecular docking show a comparison of the docking scores of a compound with other compounds, and it can explain whether a compound has potential or not. Molecules with the lowest docking score (minus value) showed a high binding affinity. The value of docking energy produced by anthocyanin compounds was -8.8 kcal/mol; -6.0 kcal/mol; and -7.6 kcal/mol for PPAR-α, HMG Co-A reductase, and ACAT proteins,

Table 1. The docking scores of anthocyanin and simvastatin with the protein related to lipid metabolism

Conformation	Docking Energy / Binding Affinity (kcal/mol)					
	PPARα		HMG Co-A		ACAT	
	Simvastatin	Anthocyanin	Simvastatin	Anthocyanin	Simvastatin	Anthocyanin
(Ligan)_001_conf_1	-7,8	-8,8	-6,4	-6,0	-7,7	-7,6
(Ligan)_002_conf_1	-6,9	-8,1	-6,3	-5,9	-7,4	-7,6
(Ligan)_003_conf_1	-6,8	-8,1	-6,2	-5,8	-7,3	-7,5
(Ligan)_004_conf_1	-6,1	-8,1	-6,0	-5,7	-7,1	-7,5
(Ligan)_005_conf_1	-6,0	-7,9	-6,0	-5,5	-7,1	-7,4
(Ligan)_006_conf_1	-5,9	-7,9	-5,8	-5,5	-7,1	-7,4
(Ligan)_007_conf_1	-5,6	-7,9	-5,8	-5,5	-7,0	-7,3
(Ligan)_008_conf_1	-5,5	-7,8	-5,7	-5,4	-7,0	-7,2
(Ligan)_009_conf_1	-5,3	-7,7	-5,7	-5,3	-6,9	-7,2

respectively. Simvastatin produced docking energy of -7.8 kcal/mol, -6.4 kcal/mol, and -7.7 kcal/mol. The docking scores are listed in Table 1. The best docking score represented the form of interaction between the ligand and the docked receptor protein. The following are the results of the best docking scores from all ligands (Table 2).

Table 2. The result of docking score selection

Receptor	Ligand	The best docking score
PPARα	Simvastatin	-7,8 kcal/mol
	Anthocyanin	-8,8 kcal/mol
HMG Co-A	Simvastatin	-6,4 kcal/mol
	Anthocyanin	-6,0 kcal/mol
ACAT	Simvastatin	-7,7 kcal/mol
	Anthocyanin	-7,6 kcal/mol

The method validation results were obtained an RMSD value of 0.00 Å for anthocyanin compounds and Simvastatin. RMSD is a parameter that describes how much the protein-ligand interaction changes in the crystal structure before and after docking to determine the deviation value. The docking method is valid if the RMSD value is ≤ 2Å, so the docking method can be used for docking the test compound [24]. The smaller the RMSD value indicates that the predicted ligand binding is better because it is getting closer to the native ligand conformation [25]. The validation of the docking method is also determined from the amount of free bond energy, which must be negative [26].

Based on the docking score, it was known that the first conformation was the best mode of the nine generated modes [27]. The results of the validation method obtained an RMSD value of 0.00 Å for all compounds in mode one. The docking results between proteins and ligands are considered a success because of the value ≤ 2Å [28]. The lower the docking score, the more accessible a compound can bind. Therefore, the 3 test ligands used in this study met the criteria as anti-hyperlipidemic agents through a molecular docking approach. This was evidenced by the binding affinity that was negative and had RMSD value ≤ 2Å. The highest affinity value was shown by the docking between PPARα and anthocyanin, which means that the interaction of anthocyanin to PPARα receptors is predicted to be the most effective compared to HMG Co-A reductase and ACAT in reducing lipid levels. The free bond energy on the docking score is directly related to the inhibition constant. Thus, the determination of the value of the free bond energy can predict the ability of compounds to inhibit the activity of proteins or enzymes [29].

3.4. Visualization of Docking Results using Biovia Discovery Studio 2020

The docking results visualization was carried out using the Biovia Discovery Studio 2020 software. The docking results show an interaction between anthocyanin compounds and the active site of PPAR-α, HMG-CoA reductase, and ACAT proteins. The PPARα protein has binding sites Phe273, Ile241, Asn219, Cys276, Leu247, Met220, Gln277, Glu251, Thr279, Ser280, Leu254, Glu282, Tyr314, Ile272, Thr283, Ile317, Cys275, Glu286, Phe354, Leu332, Val330 , Val324, His440, Ile339, Ala333, Val444, Phe343, Tyr334, Leu460, Leu344, Tyr464, and Met355 [30]. While the binding sites on HMG-CoA reductase are Glu559, Ser565, Val683, Ser684, Asp690, Lys691, Lys692, Lys735, Asn755, Asp767, His866, and Val853 [31, 32]. The binding sites on ACAT are His425, Ser456, and His460 [33]. The following is a table of visualization results of amino acids that bind to the target ligand and receptor.

Visualization of the docking results obtained shows the bond between the ligand and protein. The bonding results that occur provide the value of the docking energy for each compound used. Docking PPAR-α and anthocyanin show binding to amino acids CYS275 and CYS276 through hydrogen donor Pi bonds, VAL 332 through Pi Sigma bonds, and ILE241, ILE272, ILE339, ALA333 through Pi Alkyl bonds in the binding site. Meanwhile, PPAR-α and simvastatin docking showed binding to the amino acids THR279 and ALA333 via the Conventional Hydrogen Bond, as well as ILE272, ILE339, CYS275, and VAL332 via Alkyl bonds at the binding site. Docking HMG Co-A reductase and anthocyanin show binding to amino acids LEU737 and ILE746 via Pi Alkyl bonds, and TYR749 and TRP698 via Stacked Pi-Pi bonds. The docking result do not show any binding in the binding site area but are still in the grid box when docking is done. HMG Co-A reductase and simvastatin docking showed binding to the amino acid LYS692 through the carbon hydrogen bond at the binding site.

Docking ACAT and anthocyanins showed binding to the amino acid LEU389 via Pi Sigma bonds, as well as PHE258, PHE384 and TRP388 via Alkyl bonds. Meanwhile, ACAT and simvastatin docking showed binding to the amino acid ARG262 through Unfavorable

Table 3. Binding visualization between amino acid of PPAR-α, HMG-CoA reductase, and ACAT proteins with ligands

Ligands and Target Receptors	Amino Acid Visualization	*Binding Site*
PPARα and Simvastatin	Thr279, Ala333, Ile272, Ile339, Cys275, and Val332	Phe273, Ile241, Asn219, Cys276, Leu247, Met220, Gln277, Glu251, Thr279, Ser280, Leu254, Glu282, Tyr314, Ile272, Thr283, Ile317, Cys275, Glu2861, Phe354, Leu332, Val,430, His440, Ile339, Ala333, Val444, Phe343, Tyr334, Leu460, Leu344, Tyr464, and Met355
PPARα and Anthocyanin	Cys275, Cys276, Val332, Ile241, Ile272, Ile339, Ala333, Ala250, Val255, and Met330	
HMG Co-A and Simvastatin	Pro693, Tyr687, Lys692, Asn686, Thr689, and Tyr644	Glu559, Ser565, Val683, Ser684, Asp690, Lys691, Lys692, Lys735, Asn755, Asp767, His866, and Val853
HMG Co-A and Anthocyanin	Leu737, Ile746, Tyr749, and Trp698	
ACAT and Simvastatin	Arg262, Ile255, Trp388, and Phe258	His425, Ser456, and His460
ACAT and Anthocyanin	Leu389, Phe258, Phe384, and Trp388	

donor-donor binding; ILE255, TRP388 via Alkyl bond, and PHE258 via Pi Sigma bond. ACAT protein docking results are similar to the docking of HMG Co-A reductase and anthocyanin. The docking of ACAT protein with anthocyanin and simvastatin compounds did not show any binding that occurred in the binding site area. Still, it produced amino acid residues that could contribute to the resulting docking value. The visualization results of anthocyanin and simvastatin compounds against PPARα, HMG Co-A and ACAT proteins were shown in Fig. 5; Fig. 6; Fig. 7; Fig. 8; Fig. 9; and Fig. 10 obtained from the Biovia Discovery Studio software.

The interactions formed include hydrogen, pi-sigma and pi-alkyl bonds, which has a different function. These intermolecular interactions will determine the biological properties of the molecules in the cell. The followings are the function of each bond that occurs in the molecular docking of the ligands to the PPARα, HMG Co-A Reductase, and ACAT proteins.

- Conventional hydrogen bonding is an important bond in the formation of protein-ligand complexes because it provides stability to the complex [34].
- The pi-alkyl bond serves to donate the dipole moment of the ligand through electron charge transfer, which is important in the orientation of the molecule when interacting with the bonding site [35, 36].
- Pi-sigma bonds: bonds to assist ligand intercalation at the receptor binding site [37].

The resulting docking score and interaction between anthocyanin compounds and the active site of PPAR-α, HMG-CoA reductase, and ACAT proteins indicate that anthocyanin compounds have potential anti-hyperlipidemia with a mechanism through the inhibition of PPARα, HMG Co-A reductase, and ACAT proteins. The interaction of anthocyanin compounds with PPARα protein is stronger than HMG Co-A reductase and ACAT proteins because they have the lowest docking scores. PPARα is a ligand-inducible transcription factor that belongs to the nuclear receptor (NR) superfamily. It binds DNA as an obligate heterodimer with retinoid X receptors (RXRs). The PPARα act to control the expression of various genes involved in most lipid metabolism pathways, including fatty acid oxidation, triglyceride (TG) synthesis and breakdown, lipoprotein metabolism, gluconeogenesis, bile acid metabolism, and various other metabolic pathways [9]. PPARα is involved in lipid and cholesterol transport by decreasing VLDL production and increasing the catabolism of triglyceride-rich lipoproteins by increasing the activity of LPL, which is a key enzyme in TG hydrolysis.

Moreover, the TG-lowering action of PPARα is also due to enhanced FA uptake, conversion into acyl-CoA derivatives, and further catabolism via the β-oxidation pathway [38]. Indirectly, PPARα lowers LDL particles, increases HDL particle formation, and removes excess cholesterol in the liver. Generally, PPARα has functioned as a major regulator of fatty acid homeostasis [9],

expressed predominantly in the liver, heart, skeletal muscle, and brown adipose tissue (BAT), which have high fatty acid oxidation (FAO) rates [12]. However, it is also expressed in many tissues and cells, including the intestine, vascular endothelium, smooth muscle, and immune cells such as monocytes, macrophages, and lymphocytes [38]. Accordingly, PPARα is a target physiological receptor of the hypolipidemic fibrate drugs and regulates the expression of genes involved in lipid metabolism [39]. One of the hypolipidemic mechanisms is a decrease in triglyceride levels. The hypotriglyceridemic effect of PPARα is directly under the control of LPL and Apo C-III, where PPARα activators repress Apo C-III expression and induce LPL in the liver [40].

Another protein regarded as a major target for regulating hypercholesterolemia is HGM-CoA reductase. Anthocyanin, which belongs to flavonoid compounds, can affect lipid metabolism and cholesterol biosynthesis by

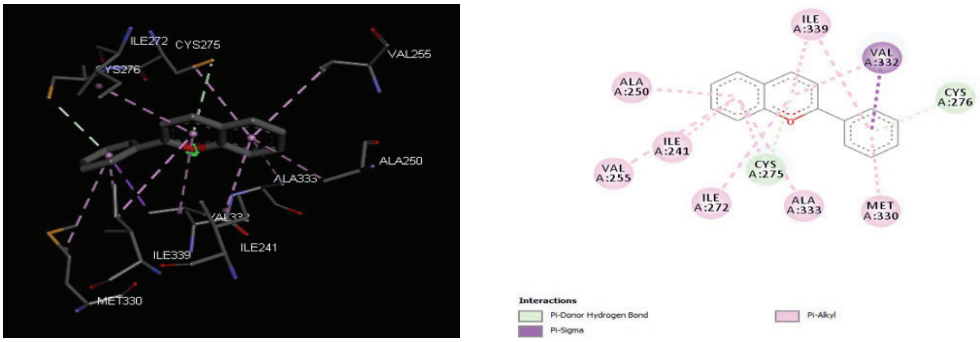


Figure 5. Results of 3D (left) and 2D (right) Molecular Docking of PPARα and Anthocyanins

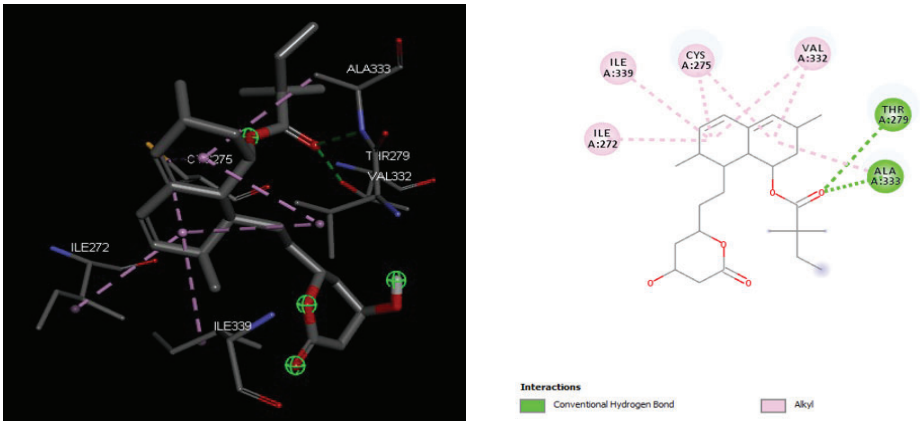


Figure 6. Results of 3D (left) and 2D (right) Molecular Docking of PPARα and Simvastatin

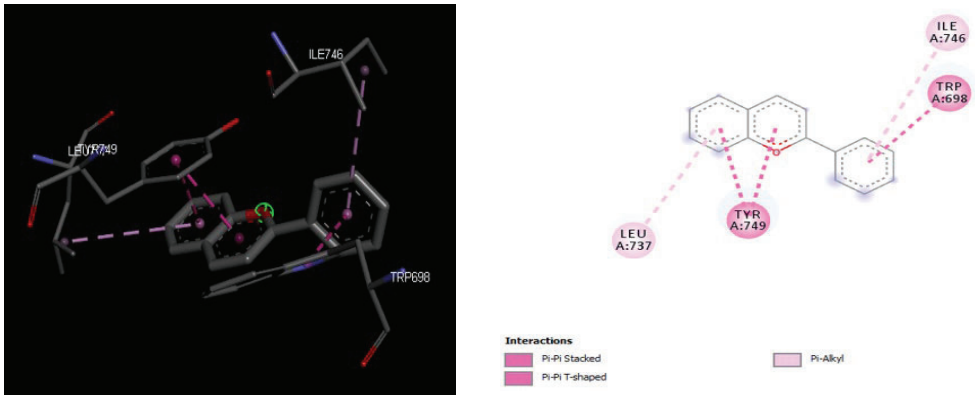


Figure 7. Results of 3D (left) and 2D (right) Molecular Docking of HMG Co-A reductase and Anthocyanin

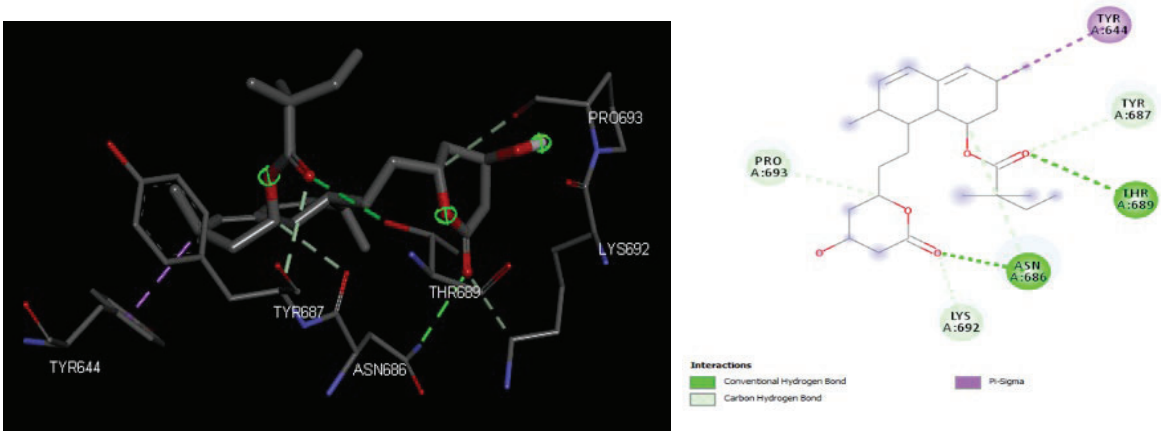


Figure 8. Results of 3D (left) and 2D (right) Molecular Docking of HMG Co-A reductase and Simvastatin

Figure 9. Results of 3D (left) and 2D (right) Molecular Docking of ACAT and Anthocyanin

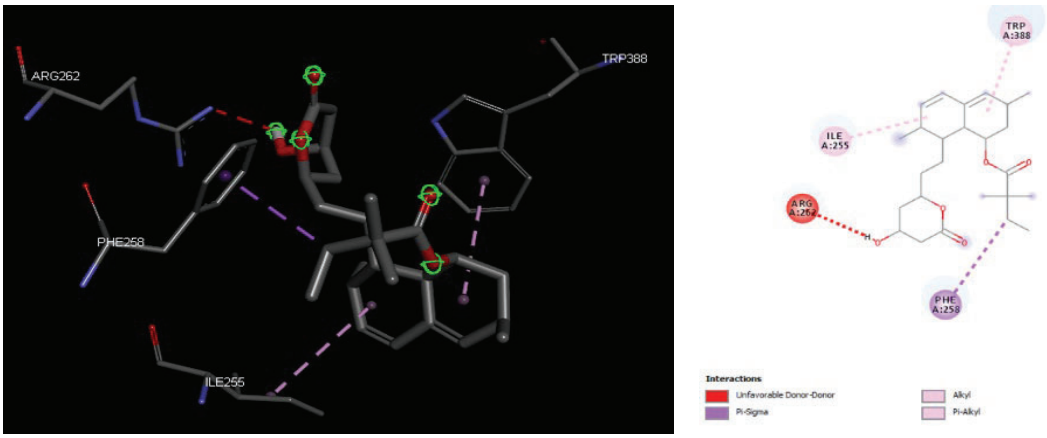


Figure 10. Results of 3D (left) and 2D (right) Molecular Docking of ACAT and Simvastatin

inhibiting HMG-CoA reductase's action that catalyzes the conversion of HMG-CoA to mevalonate [11]. The active form of the reductase inhibitor is a structural analog of HMG-CoA formed by HMG-CoA reductase in the

synthesis of mevalonate. When cholesterol is transported from the intestine to the liver, HMG-CoA reductase that plays a role in converting HMG-CoA to mevalonate in the cholesterol synthesis will be inhibited. Thus, cholesterol synthesis products by the liver will be reduced, and plasma LDL levels will also decrease. Inhibition of HMG-CoA reductase induces an increase in high-affinity LDL receptors. This effect increases the rate of fractional LDL catabolism and the liver's extraction of LDL precursors (remnant VLDL), thereby reducing plasma LDL stores. The present molecular docking showed that the binding affinity between anthocyanin and HMG-CoA reductase was strong. The docking score is almost the same as simvastatin, a mevinic acid-derived antilipemic compound of the statin class used as a cholesterol-lowering drug. Statins have well known as an inhibitor of HMG-CoA reductase enzyme.

Statins bind to the enzyme's active site and induce a conformational change in its structure, thus reducing its activity and reducing the intracellular synthesis of cholesterol. Inhibition of HMG-CoA reductase by statins reduces intracellular cholesterol content increasing SREBP-2-mediated hepatic LDL receptor synthesis. The increasing LDL-R corresponds to an increase in clearance of atherogenic lipoproteins, particularly LDL, chylomicron remnants, and VLDL remnants. Statins are the most efficacious agents for lowering the plasma concentration of LDL cholesterol and apoB-100 [41]. Accordingly, statins reduce the plasma concentrations of total cholesterol, LDL-C, VLDL-C, triglycerides, apo-B, and increase the plasma concentrations of HDL-C [42]. This docking result suggested that HMG-CoA reductase is likely to be the potential target of the cholesterol-lowering effect of anthocyanin.

ACAT is also known as a novel target for treating hypercholesterolemia and atherosclerosis [43]. ACAT inhibitors are reported to have cholesterol-lowering and anti-atherosclerotic effects [44]. ACAT protein catalyzes cholesterol esterification from cholesterol and fatty acyl-coenzyme A to cholesterol esters, followed by subsequent cholesterol absorption. The ACAT inhibition can cause a decrease in cholesterol levels, reduce the assembly and secretion of Apolipoprotein B-containing lipoproteins such as VLDL and LDL, and inhibit the formation of foam cells in the arterial walls [11]. The molecular docking result showed that the docking score between anthocyanin and simvastatin to ACAT protein was not significantly different. There are -7.6 kcal/mol and 7.7 kcal/mol for anthocyanin and simvastatin, respectively. This score showed that anthocyanin and ACAT protein binding was stronger than HGM-CoA reductase but weaker than PPARα. This docking result also suggested that ACAT could be a potential target of the cholesterol-lowering effect of anthocyanin.

This result is similar to the docking score produced by Simvastatin as a positive control. There are -7.8 kcal/mol, -6.4 kcal/mol, and -7.7 kcal/mol. Anthocyanin compounds have been proven to be a potent inhibitor of PPAR-α, HMG Co-A reductase, and ACAT proteins and act as anti-hyperlipidemic in silico, where the strongest binding occurs between anthocyanin and PPARα protein.

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