Molecular Docking of Red Betel (Piper crocatum Ruiz & Pav) Bioactive Compounds as HMG–CoA Reductase Inhibitor

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Abstract
Cholesterol plaque buildup in artery walls occurs due to oxidation of Low-Density Lipoprotein (LDL) molecules by free radicals, which are a risk factor for coronary heart disease. Piper crocatum contains active compounds that can act as HMG–CoA reductase inhibitors, such as flavonoids, alkaloids, polyphenols, tannins, and essential oils. This study aimed to predict the potential of Piper crocatum extract and fraction compounds as HMG–CoA reductase inhibitors by investigating the ligand affinity to the HMG–CoA reductase enzyme. Ligand and receptor preparation was conducted using BIOVIA Discovery Studio Visualizer v6.1.0.15350 and AutoDock Tools v.1.5.6. Molecular docking used AutoDock Vina, while ligand visualization and receptor binding used BIOAVIA Discovery Studio Visualizer v6.1.0.15350 and PyMOL (TM) 1.7.4.5.Edu. The receptor used was HMG–CoA reductase (PDB code: 1HWK) with atorvastatin as a control ligand. Catechin, schisandrin B, and CHEMBL216167 had the highest inhibition with affinity energies of −7.9 kcal/mol, −8.2 kcal/mol, −8.3 kcal/mol, respectively. Amino acid residues that played a role in ligand and receptor interactions were Ser684, Asp690, Lys691, Lys692.

1. Introduction
Hypercholesterolemia is a clinical symptom characterized by increased levels of total cholesterol (≥220 mg/dL) and low-density lipoprotein (LDL) cholesterol in the blood [1]. Hypercholesterolemia is a risk factor for cardiovascular disease, namely coronary heart disease (CHD). WHO data [2] showed that the number of deaths due to coronary heart disease was 8.9 million/year. Basic Health Research 2018 [3] showed the total prevalence of CHD and stroke in Indonesia was 1.5% and 10.9%, respectively. Significantly high intake of exogenous cholesterol increases cholesterol, triglyceride, and LDL levels. Coronary heart disease (CHD) is caused by an accumulation of cholesterol plaque on the walls of blood vessels, which causes the narrowing or blockage of blood vessels. The accumulation of cholesterol plaque in artery walls can occur due to the oxidation of LDL molecules by free radicals [4].

The enzyme 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG–CoA) reductase is a key enzyme in cholesterol biosynthesis. This enzyme reduces HMG–CoA to mevalonate, which is then converted into cholesterol [5]. On the other hand, red betel is known to have properties in treating various diseases, such as diabetes mellitus, cholesterol, gout, and hypertension [6]. Red betel contains flavonoids, alkaloids, polyphenols, tannins, and essential oils, which are useful as medicinal ingredients. Flavonoids have been reported to reduce LDL oxidation, suppress lipid peroxidation, and reduce atherosclerotic lesions’ progression in cardiovascular disease [7].

Hasibuan et al. [8] conducted a study on the effect of giving red betel leaf extract. The results showed that the red betel leaf extract could maintain the levels of triglycerides, LDL, and normal total cholesterol in diabetic rats. Betel leaf methanol extract at a 256 mg/kg dose decreased total cholesterol by 42%, LDL 26%, and VLDL by 40% in mice [9]. Also, Raghukutti and Lubis [10] showed that red betel leaf nanoparticles at a dose of 100 mg/kg BW on day 21 could reduce cholesterol levels in guinea pigs by 59.73%. Based on these results, it is
concluded that red betel can be a herbal alternative as an inhibitor of HMG–CoA reductase.

The discovery of drug design is a process that involves many disciplines, such as medicinal chemistry, pharmacy, and biochemistry, through an experimental approach. Many computational drug developments have been done to save costs and time, so the computational method to support drug design becomes more effective. Computational research on HMG–CoA reductase inhibition by herbal plants has been carried out from various plants, such as a water extract formulation of polyherbal [11], banana peel [12], and Azaricta indica [13]. However, computational research on the inhibition of the HMG–CoA reductase enzyme by red betel has not been carried out, so it is unknown how the interaction of active red betel compounds in inhibiting HMG–CoA reductase. This study aims to examine the potential of red betel extract and fraction compounds as HMG–CoA reductase inhibitors in silico by knowing the ligand affinity to the HMG–CoA reductase’s active site enzyme.

2. Methodology

2.1. Tools and materials

This study was designed using a computer device with AMD A9–9420 Radeo R5, 5 Compute Cores 2C+3G processor specifications. The software used was MarvinView 6.0.0, BIOVIA Discovery Studio Visualizer v16.1.0.15350, AutoDock Tools v.1.5.6, AutoDock Vina, and PyMOL (TM) 1.7.4.5 Edu. The materials used were ligands of the extracted compound and the Piper crocatum fraction [14, 15, 16, 17, 18] shown in Table 1.

2.2. Prediction of Ligand Toxicity

The prediction of ligand structure toxicity was carried out online using admetSAR by accessing the page http://lmm.d.uec.edu.cn/admetsar/predict/. The ligand SMILES structure that would be predicted was uploaded to that page and then clicked on the predict option. The results of the prediction of toxicity appeared on that page [19].

2.3. Molecular Docking Method Validation

Method validation was conducted by determining the grid box using AutoDockTools v.1.5.6 and AutoDock Vina. The grid box dimensions were carried out at x = 18, y = 18, z = 18 with a distance between the atoms of 1 Å. Molecular docking was validated until the root mean standard deviation (RMSD) was less than 2 Å [20].

2.4. Ligand and Receptor Preparation

The ligands’ three-dimensional structure was obtained from the Protein Data Bank (PDB) (pubchem.ncbi.nlm.nih.gov). The ligand structure was saved in sdf format and then converted into pdb format using MarvinView 6.0.0. The three-dimensional structure of the HMG–CoA reductase enzyme receptor (PDB code: 1HWK) was taken from the Protein Data Bank (www.rcsb.org/pdb) in PDB format. The HMG–CoA reductase enzyme receptor is a tetramer protein (A, B, C, and D chains), the A and B chains used in docking. Ligand preparation was conducted by adding polar hydrogen atoms using Discovery Studio Visualizer v16.1.0.15350 and bond rotation using AutoDock Tools v.1.5.6. The receptor preparation used BIOVIA Discovery Studio Visualizer v16.1.0.15350 by eliminating water molecules, heteroatoms, and native ligands. The pdbqt protein file added polar hydrogen atoms and Gasteiger partial charges using AutoDock Tools v.1.5.6 [21].

| Compound                      | PubChem ID |
|-------------------------------|------------|
| Glabrescione                  | 44257338   |
| Catechin                      | 73160      |
| Caryophyllene                 | 5281515    |
| Germacrène                    | 5317570    |
| Elemicin                      | 10248      |
| Propionic acid                | 1032       |
| Neophytadiene                 | 10446      |
| Butyl ethanoate               | 31272      |
| Alfa pinene                   | 82227      |
| Limonene                      | 22311      |
| Cineole–1,8                   | 2758       |
| Terpinene–4-ol                | 11230      |
| 6XO32ZSPtD                    | 75019      |
| Ethyl L-serinate hydrochloride| 2729185    |
| Schisandrin B                 | 108130     |
| Columbin                      | 188289     |
| ZINC8756459                   | 6070252    |
| MLS000557666                  | 1077234    |
| Oprea1_462146                 | 2865476    |
| CHEMBL216163                  | 44418672   |
| 1,1’-(1,4-Butanediyl)bis(2,6-dimethyl-4’-[(3-methyl-1,3-benzothiazole-2(3H)-ylidene)methyl]pyridinium) | 3416657 |
| Methyl eugenol                | 7127       |
| 4’-methoxyindole              | 138363     |
| Leucyleucinamide hydrochloride| 16219591   |
| 5-isopropyl-3-pyrazolidinecarboxyhydrazide hydrochloride (1:1) | 61440504 |
| 1H-pyrazole-1-carboximidamidimidhydrochloride | 2734672 |
| Protocatechuc acid            | 72         |
| Ni–(5-methyisoaxazole-3-yl)ethanediamide | 28096655 |
| CHEMBL321736                  | 90665169   |
| 2-4’-morpholinylmethyl)aniline sulfate hydrate | 45595316 |
| SCHEMBL69003                  | 16839452   |
| L-Arginine hydrochloride      | 66250      |
| 1-(1,4-Dithian-2-ylmethyl)-3-(3-methoxypropyl)thiourea | 11650220 |
| ALBB–026042                   | 1511955    |
2.5. Molecular Docking

Molecular docking was conducted using AutoDock Vina. The prepared ligand and receptor structures were saved in .pdbqt format and copied to the Vina folder. Vina’s AutoDock Program was run via Command Prompt (CMD). The programming command that was executed was “vina --config conf.txt --log log.txt.” Molecular docking results obtained out documents in .pdbqt format and log in ‘txt’ format containing ligand affinity energy [20].

2.6. Visualization of ligand and receptor binding

Two-dimensional visualization of hydrogen bonds and hydrophobic interactions of amino acid residues was carried out using BIOVIA Discovery Studio Visualizer v16.1.0.15350 [21] and three-dimensional visualization using PyMOL(TM) 1.7.4.5 Edu [22].

3. Results and Discussion

3.1. Prediction of Ligand Toxicity

It is essential to identify the toxicity early in drug development. This is to ensure that the compound’s potential as a drug can work effectively without causing damage to organs. Toxicity studies were carried out based on ADMET properties with the parameters taken, namely inhibition of hERG, carcinogenicity, and acute oral toxicity in rats. The hERG inhibition test results showed that the compound 2-(4-morpholinylmethyl) aniline sulfate hydrate was included in the strong inhibitor category for hERG. Meanwhile, control ligands and other test ligands are weak inhibitors of hERG. hERG is associated with K⁺ channels in the normal repolarization of cardiac action. Blockage or other disruption of the K⁺ channels in heart cells can cause cardiac arrhythmias and fatal cardiac toxicity [23].

Carcinogenicity prediction indicated that the test ligands belonging to group I (carcinogenic) include propionic acid, neophytadiene, and butyl ethanoate. While the control ligands and other test ligands were included in group 4 (non-carcinogenic), they are safe to be used as drugs. Acute toxicity in mice is based on the amount of the chemical administered orally in mg/kg body weight resulting in mortality in 50% of the rat population. The prediction of acute oral toxicity indicates that the control ligands and all test ligands fall into category III (LD₅₀ <5000 mg/kg body weight), except for catechin ligands and L-(+)-arginine hydrochloride belongs to category IV (LD₅₀ > 5000 mg/kg body weight) [24].

3.2. Molecular Docking Validation

Redocking complexed native ligands validated the molecular docking method into the HMG-CoA reductase crystal structure on the binding site. Molecular docking in this study was carried out on the active site of HMG-CoA reductase, formed on the surface of two different subunits bound together to form dimers [25] (Figure 1b). In this case, molecular docking is carried out on the A and B chains that make up the dimers. The active site residues of the enzymes targeted were Ser684, Asp690, Lys691, Lys692 [26]. The IHWK structure contains one mutation. This did not affect the binding side of validation because mutations did not occur at the enzyme’s residual active site [27]. Re-docking was conducted by comparing the native conformation of the ligands and the ligands from the redocking results. Assessment of validation is based on Root Mean Square Deviation (RMSD). The RMSD value shows the atomic distance’s value at one conformation, with the nearest atom having the same type as the atom in another conformation [28].

Validation shows that the mean RMSD value is 0.9274 ± 0.01 Å with average affinity energy of -9.3 ± 0.1 kcal/mol. The literal tethering method is considered accurate if the RMSD value for heavy atoms is ≤ 2.00 Å [29]. These results indicate that the validated ligands and receptors have met the valid criteria, so the method can be used to determine the test compound. The visualization results show that the native hydrogen ligand interactions with the receptors are on the amino acids Ser565, Glu559, Arg590, Ser661, Ser684, Lys691, Lys692, Lys735, Ala751, and Asn755. Meanwhile, the resulting hydrophobic interactions showed amino acid residues Cys561, Arg568, Leu562, Val683, His752, Leu853, Ala856, and Leu857. The redocking visualization is shown in Figure 1.

![Figure 1. Visualization of the structure of (a) HMG-CoA reductase tetramer before preparation, (b) HMG-CoA reductase dimer consists of the A chain (blue) and B chain (green) after preparation, (c) overlap of native ligands (magenta) and ligands redocking results (yellow), (d) Binding pocket HMG CoA reductase](image_url)

3.3. Molecular Docking and Visualization

From the molecular docking process, affinity energy was obtained as a direct output from AutoDock Vina. The increasingly negative affinity energy value indicated the highest inhibitory activity. The affinity energy values of all the compounds range from -3.6 to -8.3 kcal/mol, as shown in Table 2. The highest affinity energy is found in the water extract, which is a catechin compound of 7.9 kcal/mol with a Ki value of 1.60 µM, and the ethyl acetate fraction, which is a schisandrin B compound -8.2 kcal/mol with a Ki value of 0.96 µM and CHEMBL216163 of 8.3 kcal/mol with a value Ki of 0.81 µM. However, these results were still lower than that of atorvastatin which...
was -9.5 kcal/mol with a Ki value of 0.13 µM. The inhibition constant value is calculated by using the equation $\Delta G = kT \ln(K_i)$, where $\Delta G$ = Gibbs free energy (kcal/mol), $R = 1.986 \times 10^{-3}$ kcal/mol K, $T = 298.15$ K [30]. The inhibition constant is directly proportional to the affinity energy.

**Table 2. Affinity Energy and amino acid residues of binding between ligands and receptors**

| Ligand                  | Affinity energy (Gibbs free energy (kcal/mol)) | Hydrogen bond in ligands | Amino acid residues which form hydrogen and hydrophobic bonds | Hydrophobic interactions |
|-------------------------|-------------------------------------------------|--------------------------|---------------------------------------------------------------|-------------------------|
| Allosteratim (control)  | -9.5 - 0.13                                     | Ser565, Ser570           | Asn658, Asp690, Met655, Met657, Arg590, Asp690, Ser684, Asp690, Lys692, Ala751, Leu853 | -                        |
| Catechin                | -7.9 + 1.60                                     | Gly510, Ser565           | Glu559, Ser570, Arg590, Ser684, Lys735, Ala751, Leu853    |                          |
| Carboxymethyl benzylidene | -5.3 - 129.61                                  | Arg590, Ser565           | Glu559, Ser570, Arg590, Ser684, Lys735, Ala751, Leu853    |                          |
| Germacrine              | -5.5 - 93.48                                    | Arg590, Ser565           | Glu559, Ser570, Arg590, Ser684, Lys735, Ala751, Leu853    |                          |
| Einnic acid             | -5.2 - 135.48                                   | Arg590, Ser565           | Glu559, Ser570, Arg590, Ser684, Lys735, Ala751, Leu853    |                          |
| Propionic acid          | -3.6 - 2288.66                                  | Ser684, Ser686, Lys692   | Glu559, Ser570, Arg590, Ser684, Lys735, Ala751, Leu853    |                          |
| Norphthalimides         | -4.9 - 234.74                                   | Ser684, Ser686, Lys692   | Glu559, Ser570, Arg590, Ser684, Lys735, Ala751, Leu853    |                          |
| Butyl ethanoate         | -4.1 - 983.68                                   | Ser684, Ser686, Lys692   | Glu559, Ser570, Arg590, Ser684, Lys735, Ala751, Leu853    |                          |
| Alkaine                 | -4.0 - 1044.66                                  | Ser684, Ser686, Lys692   | Glu559, Ser570, Arg590, Ser684, Lys735, Ala751, Leu853    |                          |
| Luminol                 | -4.9 - 234.74                                   | Ser684, Ser686, Lys692   | Glu559, Ser570, Arg590, Ser684, Lys735, Ala751, Leu853    |                          |
| Cinchon-1-L              | -4.4 - 592.68                                   | Ser684, Ser686, Lys692   | Glu559, Ser570, Arg590, Ser684, Lys735, Ala751, Leu853    |                          |
| Terpenos-4-ol            | -5.3 - 129.63                                   | Arg590, Ser565           | Glu559, Ser570, Arg590, Ser684, Lys735, Ala751, Leu853    |                          |

**Red betel compounds interact with the receptors via amino acid residues which form hydrogen and hydrophobic bonds. Visualization of ligand-binding**
amino acid residues with the receptor using BIOVIA Discovery Studio Visualizer v16.1.0.15350 and binding pocket HMG–CoA reductase using PyMOL PyMOL (TM) 1.7.4.5 Edu is shown in Figure 4. The visualization results showed that the three compounds, catechin, schisandrin, and CHEMBL216163, interact with Ser684, Asp690, Lys691, Lys692. This is consistent with Itsvan and Deisenhover [26], who stated that HMG–CoA reductase’s binding pocket is present in amino acids 682–694, forming the cis loop, the active site of the enzyme. Based on this, the active compound of red betel is expected to act as a competitive inhibitor by binding to HMG–CoA reductase’s active site.

Figure 2. Molecular Docking Visualization of atorvastatin: A) 2D diagram of hydrogen bonding and hydrophobic interactions between ligands and receptors; B) Binding pocket of HMG–CoA reductase

Figure 3. Visualization of Molecular Docking of catechins: A) 2D diagram of hydrogen bonding and hydrophobic interactions between ligands and receptors; B) 3D binding pocket structure of HMG–CoA reductase

Figure 4. Visualization of the molecular docking of Schisandrin B: A) 2D diagram of hydrogen bonding and hydrophobic interactions between ligands and receptors; B) 3D binding pocket structure of HMG–CoA reductase
4. Conclusion

The computational interaction of red betel active compounds to predict ligands, which can inhibit the HMG-CoA reductase enzyme activity, is based on the energy affinity illustrated by the ideal ligand pose the active site of the enzyme. Red betel water extract compounds, namely catechins and ethyl acetate fraction; schisandrin and CHEMBL216163, have the highest energy affinity, namely -7.9 kcal/mol, -8.2 kcal/mol, and -8.3 kcal/mol, respectively. They all interact with the active site of Ser684, Asp690, Lys691, and Lys692.

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Figure 5. Visualization of the molecular docking of catechins: A) 2D diagram of hydrogen bonding and hydrophobic interactions between ligands and receptors; B) 3D binding pocket structure of HMG-CoA reductase
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