



COMPUTATIONAL ANALYSIS OF BIOACTIVE COMPOUNDS Piper crocatum AS INHIBITORS OF ACETYLCHOLINESTERASE

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Abstract

This study aims to determine the inhibition of natural compounds *P. crocatum* against AChE enzyme activity. Inhibition of natural compounds of *P. crocatum* was analyzed by combining virtual screening and molecular docking methods with two different software, namely Autodock Tools 1.5.6 and YASARA Structure. This method is expected to improve the ability to identify potential natural compounds contained in betel leaf as AD drug candidates with AChE inhibition mechanism. A total of 21 natural compounds from three red betel leaf extract fractions (water fraction, ethyl acetate, and n-hexane) were analyzed for their binding affinity energy to AChE by virtual screening along with 664 active compounds known to have AChE inhibitory activity based on the DUD-E database to identify the potential of natural compounds for each fraction of red betel leaf (*P. crocatum*) inhibits AChE activity

Materials

The amino acid sequences and the 3D structure of AChE (PDB code of 6O4W) were downloaded from the protein data bank database, <https://www.rcsb.org/search>. 5-10 sequences of the homologous gene obtained from the results of BLASTp homology analysis were downloaded by accessing the accession number of these sequences. The 3D structure of natural compounds for each fraction of red betel leaf (*P. crocatum*) in the format *.sdf and *.mol downloaded from Pubchem database site (<https://pubchem.ncbi.nlm.nih.gov/>) and Chemspider (<http://www.chemspider.com/>). 664 active compounds as AChE inhibitor obtained from DUD-E database (A Database of Useful Decoys: Enhanced) in *.sdf format.

1 Identification of the Active Site of Acetylcholinesterase (AChE)

Identification of the active site of the acetylcholinesterase enzyme is important before carrying out virtual screening and molecular docking. The stages of identifying the active site of AChE included the homology analysis of amino acid sequences and multiple sequence alignment. Homology of AChE amino acid sequences was analyzed using the BLASTp protein alignment program (Basic Local Alignment Search Tool of protein), <https://blast.ncbi.nlm.nih.gov/Blast.cgi>, by selecting the protein bank data (PDB) as the database. The amino acid sequences of AChE (PDB code of 6O4W) were downloaded from the protein data bank database, <https://www.rcsb.org/search>, then compared with the amino acid sequences obtained from PDB database through the BLASTp program to determine the homology of these sequences.

As many as 5-10 sequences of the homologous gene obtained from the results of BLASTp homology analysis were downloaded by accessing the accession number of these sequences for the multi-sequence alignment analysis stage. AChE amino acid sequence (PDB code of 6O4W) and its homologous gene sequences were added to the Clustal Omega multi-sequence juxtaposition program, <https://www.ebi.ac.uk/Tools/msa/clustalo>, by selecting the protein set type. The resulting multi-sequence alignment identified the amino acids that played a role in the AChE active site, which was the sequence motive or conserved sequence based on literature (Modified-Kurniasih2018).

2 Preparation of Ligand and Receptor structures

The ligands used in this research consisted of two types: test and comparative ligands. The test ligand was natural compounds found in red betel leaf (*P. crocatum*), water, ethyl acetate, and n-hexane fractions obtained from LC-MS results (Table 1). The 3D structure of natural compounds for each fraction was downloaded in the format *.sdf and *.mol on the Pubchem database site (<https://pubchem.ncbi.nlm.nih.gov/>) and Chemspider (<http://www.chemspider.com/>) by inputting the name of the molecule. The comparative ligand to compare the activity of natural compounds of red betel leaf against AChE inhibition was selected as active compounds obtained from DUD-E database (A Database of Useful Decoys: Enhanced) totaling 664 compounds in *.sdf format, which were known to play an active role against AChE. The geometry of the test ligand was optimized using YASARA structure software with the energy minimization method using NOVA force field. Energy minimization was conducted by using the em_runclean.mcr program on YASARA Structure. This resulted in the energy values between atoms reaching the minimum energy and forming a more stable and optimal molecular conformation. The 3D structure of test ligand from the geometry optimization result was saved in *.pdb file format. The file can be used for the analysis stage of virtual screening and molecular docking using Autodock Vina or YASARA Structure. Advanced preparation of the test ligand of 3D structure in the molecular docking stage using Autodock Vina was carried out by adjusting the atomic bond torsion of the molecule composition and converting to a *.pdbqt file.

The 3D structure of acetylcholinesterase (AChE) enzyme as a target receptor was obtained from the database of Protein Data Bank (PDB) code 6O4W. It was a homodimer docked by an inhibitor in the form of donepezil. Donepezil was separated from AChE three-dimensional structure and stored in *.pdb format to be used in the virtual screening grid box validation stages and molecular docking through redocking. The preparation of the three-dimensional AChE structure was conducted by removing water, adding hydrogen atoms to the correct amino acid, and removing the subunit of B polypeptide chain and other ligands without important roles in molecular docking. The preparation utilized Autodock Tools 1.5.6 and YASARA Structure software. The files of AChE three-dimensional structure prepared using Autodock Tools 1.5.6 software were saved in *.pdbqt format for virtual screening and molecular docking with Autodock Vina. The files prepared by YASARA Structure software were saved in *.sce format for molecular docking with YASARA.

3 Validation of Docking Areas (grid boxes) for Virtual Screening and Molecular Docking

The docking areas (grid box) of test and comparative ligands were constructed by adjusting the natural ligand docking areas (inhibitor: Donepezil) on AChE, through selecting the center on ligand in Autodock Tools 1.5.6, and making a grid box around selected atoms: Donepezil in YASARA structure. Donepezil natural ligand was used for determining the best grid box by redocking the natural ligand to AChE. Grid box size validation through redocking using Autodock Vina was repeated 100 times, while redocking using YASARA structure was repeated 999 times. A number of grid box sizes were analyzed for the accuracy of the donepezil redocking by assessing the position and conformation of the molecules with the Root Means Square Deviation (RMSD) value parameter compared with ligands that were docked as the result of redocking, and with ligands that were naturally docked to the three-dimensional AChE structure (PDB 6O4W). The smaller the RMSD value ($<2.0 \text{ \AA}$), the more precise the position and conformation of the ligand molecule would be than its original position and conformation.

The dimensions of the best molecular docking areas obtained from grid box validation using Autodock Vina were x, y, z of 60 at coordinates $x = 88.991$, $y = 85.361$, and $z = -5.291$. Meanwhile, the validation of docking areas utilized the YASARA structure in which the best molecular docking area size was $7.2 \text{ \AA}$ with angles x, y, and z, each of 27.26° . The obtained docking area size was then used for analyzing the interaction of the test and comparison ligands through virtual screening and molecular docking using Autodock Vina and YASARA structure methods (modified-Weni 2020).

4 Virtual Screening

Virtual screening was the initial step in identifying the potential inhibition of natural compounds of red betel leaves from water, ethyl acetate, and n-hexane fractions against AChE (6O4W). Identification was carried out by comparing the natural compounds contained in betel leaves (Table 1) with 664 active compounds known to have AChE inhibitory activity based on DUD-E database as a comparative ligand. The geometry optimized for the test ligand (Table 1) and 664 comparative ligands that had been prepared were docked to AChE through virtual screening using PyRx software.

The virtual screening was carried out on a grid box with the coordinates of the validation results based on Autodock Vina; i.e. coordinates of $x = 88.991$, $y = 85.361$, and $z = -5.291$ with 10 repetitions, and the results were the binding affinity energy and RMSD values.

5 Molecular Docking with Autodock Vina

Receptor and ligand preparations were the initial steps in molecular docking with Autodock Vina. Receptor preparation was the removal of water molecules, docked ligands, and homodimer polypeptide subunits, as well as the addition of hydrogen atoms using the Discovery studio program and Autodock Tools 1.5.6, and they were saved in a *.pdbqt file format. Ligand preparation included the geometry optimization with YASARA Structure program, the addition of hydrogen atoms, and the bonding torque of the ligand constituent atoms using the Autodock Tools 1.5.6 program, then saved in *.pdbqt file format. The results of receptors and test ligand preparation were then used in the analysis of potential test ligands in AChE inhibition using Autodock Vina.

Molecular docking with Autodock vina utilized the validated grid box size and coordinates of x , y , and z , each of 60 with the coordinates of $x = 88.991$, $y = 85.361$, and $z = -5.291$. The ligands docked to AChE (6O4W) were test ligands, namely natural compounds contained in the water, ethyl acetate, and n-hexane fractions of red betel leaf (*P. crocatum*) (Table 1). The molecule docking was carried out with 20 exhaustiveness. Exhaustiveness was a function to control the accuracy in finding the minimum global. The higher the exhaustiveness used in the docking process, the more extensive the search results were; however, the docking process took longer duration. The results obtained were in the forms of free binding energy (ΔG) and RMSD values.

6 Molecular Docking with YASARA Structure

Molecular docking with YASARA Structure utilized the receptor file prepared in a format of *.sce; water molecules, docked ligands, and polypeptide B subunits were removed, and appropriate hydrogen atoms were added to amino acids. In the 3D AChE structure, a grid box was formed with the size of the validation results of 7.2 Å. Molecular docking was performed using the AMBER14 force field to calculate the energy value of the ligand-receptor interaction.

7 Analysis of Docking Results

The molecular docking using Autodock Vina and YASARA Structure were analyzed for ligand interaction on AChE in two dimensions with the LigPlot+ program, and in three dimensions with the PyMol program. Visualization of the ligand docking position on AChE was shown in three dimensions with the PyMol program.