In Silico Screening of Cinnamon (Cinnamomum burmannii) Bioactive Compounds as Acetylcholinesterase Inhibitors

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1. Introduction

Alzheimer’s is a progressive and neurodegenerative disease that mainly affects people aged 65 years and older. The pathophysiology of Alzheimer’s is possibly related to the depletion of the neurotransmitter acetylcholine (ACh) due to beta-amyloid plaques and neurofibrillary tangles. Secondary metabolites found in cinnamon bark (Cinnamomum burmannii) have the potential as anticholinesterases to treat Alzheimer’s symptoms. This study aimed to identify the potency of bioactive compounds from cinnamon bark as AChE inhibitors in silico through analysis of binding energy, inhibition constants, and types of interactions. The research was conducted by screening virtually 60 test ligands using the PyRx program and molecular docking using the Autodock Tools program. The results of the ligand–receptor interaction analysis showed that 12 of the 15 tested ligands had potential as AChE inhibitors. Epicatechin and medioresinol are the ligands with the best potential for AChE inhibition with affinity close to the natural ligand or donepezil. Epicatechin has a binding energy of −10.0 kcal/mol and inhibition constant of 0.0459 M, with four hydrogen bonds and seven hydrophobic bonds. Meanwhile, medioresinol has −9.9 kcal/mol binding energy and inhibition constant of 0.0543 M, with one hydrogen bond and thirteen hydrophobic bonds.

Several hypotheses regarding the pathophysiology of Alzheimer’s disease include the cholinergic hypothesis, beta-amyloid (Aβ) plaque formation, tau protein, oxidative stress, and inflammation. Acetylcholine (ACh) is the primary neurotransmitter of the cholinergic system, which can be hydrolyzed to choline and acetic acid catalyzed by acetylcholinesterase (AChE). The cholinergic hypothesis assumes that AChE inhibition can prevent ACh hydrolysis, leading to increased ACh levels at cholinergic synapses [5]. Therapeutic attempts developed to treat Alzheimer’s are inhibiting cholinesterase activity, thereby decreasing ACh levels at synaptic junctions. Some cholinesterase inhibitors approved by the US Food and Drug Administration for treating Alzheimer’s disease are donepezil, rivastigmine, galantamine, and tacrine. These four drugs have different pharmacological properties in inhibiting AChE [6].
Alzheimer’s drugs can cause several side effects: hepatotoxicity, nausea, vomiting, diarrhea, fainting, and bradycardia [7]. As a result, biomedical innovation is required to develop and utilize alternative AChE inhibitor compounds with fewer side effects and are safer and effortless to obtain. One of the possible alternative sources of AChE inhibitors is the cinnamon bark and leaves (Cinnamomum burmannii). The main components contained in Cinnamomum sp. are cinnamaldehyde, cinnamic acid, cinnamyl acetate, eugenol, caryophyllene, caryophyllene oxide, -cubebene, -terpineol, coumarin, and camphor. Cinnamon bark essential oil contains high cinnamaldehyde content, while the leaf contains mainly eugenol as the main compound [8].

One of the computational drug discovery methods that have been developed is virtual screening. Virtual screening is a computational technique for identifying the potency of many bioactive compounds against specific macromolecules such as proteins [9]. Virtual screening offers short processing times and cost-effectiveness involved in screening compounds and does not require the physical presence of molecules [10]. Virtual screening is conducted using the PyRx program, which can perform molecular docking quickly and accurately. Vina Wizard–based virtual screening results were evaluated based on the binding free energy [11].

Cinnamon leaves and bark contain bioactive compounds that have the potential as antibacterial, antifungal, antioxidant, antidiabetic, anticancer, anticholinesterase, and hypouricemic [12]. According to Okello and Mather [13], the inhibitory activity of AChE in vitro indicates that green tea epicatechin has inhibitory effects on AChE. The interaction that occurs in the bioactive compound of cinnamon as anticholinesterase in vitro has not been studied yet. This study aimed to determine bioactive compounds found in the bark and leaves of cinnamon (Cinnamomum burmannii) in silico using virtual screening and molecular docking methods. These methods were used to predict the interaction of the bioactive compounds of cinnamon bark and leaves as inhibitors of the AChE enzyme through the analysis of binding free energy, inhibition constants, and ligand interactions with AChE receptors (PDB ID: 4EY7).

2. Methods

2.1. Tools and Materials

The tools used in the research were computer hardware with specifications of TOSHIBA Intel Core i3, 2 GB RAM with Windows 7 operating system. Molecular docking simulation using AutoDock Tools software version 1.5.6, Discovery Studio Visualizer version 16.1.0.15350 (free), Ligplot+ version 1.5.4, PyRx, and PyMOL version 4.60. The material used was the three-dimensional structure of the enzyme AChE, complexed with donepezil was downloaded from the RCSB Protein Data Bank database (https://www.rcsb.org/) [14] with code 4EY7 on October 14, 2020. Sixty three-dimensional structures of cinnamon bioactive compounds and natural ligands donepezil were downloaded from the PubChem database (accessed on October 14, 2020) in *.sdf format (Table 1).

Table 1. Active compounds of cinnamon bark and leaves

| Ligands                           | Percentage |
|----------------------------------|------------|
| Donepezil (reference)            | Eugenol    |
| Quercetin                        | Terpinen–4-OH |
| Epicatechin                      | D–Limonene |
| Medioresin                       | Gamma–terpine |
| (+)-syringaresinin               | 2–Carene   |
| Gamma–Eudesmol                   | trans–cinnamic acid |
| Proanthocyanidins                | Alpha–terpineol |
| (+)-ledene                       | Cinnamic acid |
| Alpha–calacorene                 | Camphor    |
| epi–bicycloesquiphellandrene     | Alpha–pinene |
| Gamma–Murolene                   | Eucalyptol |
| Guaiol                           | (–)-Beta–pinene |
| Calarene                         | 1,8 cineole |
| Gamma–cadinene                   | Borneol    |
| Yangene                          | Cinnamyl alcohol |
| (–)-spathulenol                  | Cinnamaldehyde |
| Alpha–Cubebene                   | Linalool   |
| (Z)-nerolidol                    | Benzene propanol |
| Aromadendrene                    | Syringaldehyde |
| Alpha–murolene                   | Benzaldehyde |
| Caryophyllene Oxide              | (Z)-nerolidol |
| Epicuberno                       | Linoleric Acid |
| Elemol                           | Gamma–elemene |
| Copaene                          | Bornyl acetate |
| Alpha–amorphene                  | Humulene   |
| Beta–guaiene                     | Alpha–humulene |
| Santalene                        | Alpha–terpinyl acetate |
| Beta–caryophyllene               | Cinnamyl Acetate |
| Isoledene                        | Cinnamyl alcohol |
| Caryophyllene                    | Coumarin   |
| p-cymene                         |            |

2.2. Preparation of receptor structure and stability

The structure used was the three-dimensional structure of AChE (GDP ID: 4EY7) in *.pdb format. AChE consisted of two subunits, A and B, joined by two disulfide bonds from the cysteine residue to form a protein homodimer [15]. The three-dimensional structure of the human AChE enzyme forms a complex with the crystalized donepezil. Several molecules attached to the enzyme structure were 1-benzyl-4-((5,6-dimethoxy-1-indanone-2-y)methyl)piperidine (Donepezil), 2-acetamido-2-deoxy-β-D-glucopyranose, 1,2-ethanediol, and the nitrate ion. Donepezil is a natural ligand functioning as an inhibitor of the AChE enzyme. The enzyme with this code has a resolution of 2.35. The resolution of the protein crystal structure is declared good if the value is not more than...
The structure was prepared using the Discovery Studio Visualizer program by removing water molecules and other ligands which still attached to the structure. Polar hydrogen atoms were added to the structure. The prepared structure was saved in *.pdbqt format using Autodock Tools version 1.5.6 [17].

The stability of the three-dimensional receptor structure was analyzed online with PROCHECK on http://servicesn.mbi.ucla.edu/ [18]. The three-dimensional structure of the receptor downloaded from PDB was uploaded to the page and clicked on runcheck.

### 2.3. Ligand structure preparation

The three-dimensional structure of the test ligand compound in *.sdf format was downloaded from the PubChem website (https://pubchem.ncbi.nlm.nih.gov/) and converted using Discovery Studio into *.pdb format. Furthermore, the ligand was optimized using Autodock Tools version 1.5.6 with the addition of hydrogen bonds and saved in *.pdbqt format.

### 2.4. Virtual screening

Sixty optimized and pre-prepared ligands were attached to AChE through virtual screening using PyRx software. Virtual screening was conducted on a grid box with the coordinates of the optimum validation results based on AutoDock Vina. The resulting binding free energy and Root Mean Standard Deviation (RMSD) values were then sorted based on the smallest to the most significant binding free energies values for the next step.

### 2.5. Prediction of physicochemical and ligand toxicity

The physicochemical of ligand were predicted online by accessing the site http://www.scfbio-ltd.res.in/software/drugdesign/lipinski.jsp [19]. The prediction of online-based ligand toxicity was carried out using admetSAR on the http://lmmd.ecust.edu.cn/admetSAR/predict/ by uploading the SMILES structure of the predicted ligand [20].

### 2.6. Molecular docking validation

Validation was performed by specifying the grid box $x = 38, y = 20, z = 28$; central point coordinates at $x = -13,988, y = -43,906, z = 27109$; and the exhaustiveness value was set at 20 in the natural ligand using AutoDockTool v.1.5.6 and AutoDock Vina. Validation was repeated 20 times to obtain the RMSD < 2. The grid box was created by adjusting the dimensions of the natural ligand–binding pocket (donepezil) in AChE by selecting the center on the ligand in AutoDockTool v.1.5.6. RMSD values were checked using the Discovery Studio Visualizer 2016 program. Molecular docking is declared valid if the RMSD value is less than 2 [21].

### 2.7. Molecular docking

The structure of ligands and receptors in *.pdbqt format was stored in the same vina folder. The docking was done in the best grid box area as validation results using the AutoDock Vina program by running CMD (Command Prompt) with the command “vina.exe –config config.txt –log log.txt”. The molecular docking result was in log.txt format, containing the binding free energy and RMSD value and a document in *.pdbqt format [21].

### 2.8. Analysis and visualization of molecular docking results

Analysis of the results of the binding of the ligand molecule with the AChE enzyme in the form of bonding free energy in log.txt format. Based on this value, the value of the inhibition constant was obtained based on the equation $G_{bind} = -RT \ln K_i \ (AG_{bind} = \text{binding free energy (kcal/mol), } R = 1.986 \times 10^{-3} \text{kcal/mol.K, } T = 298.15 \text{K})$. In these results, hydrogen bond and hydrophobic interactions were visualized using LigPlot+ v.1.5.4 (2D) and PyMol v.4.60 (3D).

### 3. Results and Discussion

#### 3.1. Receptor structure and stability

![Figure 1. Structure of complexed AChE with donepezil](image)

The stability of the receptor structure was analyzed to determine the feasibility of the receptor in the molecular docking process using the Ramachandran diagram. The diagram shows the distribution of amino acids in a protein that can be classified into four quadrants. The red color in the Ramachandran diagram shows residues in the most favored regions, and the yellow color shows residues in the additionally allowed regions. The orange color shows residues in the generously allowed regions, and the white color shows residues in the disallowed regions. All amino acids are prohibited in this region, excluding glycine [24]. The amino acid glycine has only a hydrogen atom and no reactive carbon atom, which can cause many of the steric movements in the Ramachandran diagram; consequently, these amino acids have a low steric effect against other amino acids residues [25]. The structure of complexed AChE with donepezil is depicted in Figure 1. No.
allowed, and 0.0% in the disallowed regions. A protein structure is declared good if the number of plots of non-glycine amino acid residues present in the most favored regions is more than 90% and in the disallowed regions less than 15% [26]. The AChE enzyme can be declared stable and of reasonably good quality. Ramachandran diagram of the AChE enzyme from the previous study can be seen in Figure 2.

![Figure 2. Ramachandran diagram of the AChE enzyme](image)

### 3.2. Virtual screening

| Ligand                   | Binding free energy (kcal/mol) |
|--------------------------|--------------------------------|
| Donepezil (natural and reference) | -12.2                          |
| Quercetin                | -10.2                          |
| Epicatechin              | -10.0                          |
| Medioresinol*            | -9.9                           |
| (+)-Syringaresinol*      | -9.8                           |
| Gamma-eudesmol          | -9.7                           |
| Proanthocyanidins        | -9.5                           |
| (+)-Ledene               | -9.4                           |
| (Alpha-)calacorene       | -9.4                           |
| epi-bicyclosessquisphellandrene | -9.1                       |
| Cinnamyl acetate*        | -7.5                           |
| Coumarin*                | -7.4                           |
| Cinnamic acid*           | -7.1                           |
| Cinnamyl alcohol*        | -6.6                           |
| Cinnamaldehyde           | -6.6                           |
| Syringaldehyde*          | -6.4                           |

* Typical compound of cinnamon based on IJAH (Indonesian Jamu Herbs) website (http://ijah.apps.cs.ipb.ac.id) will be determined their potential as AChE inhibitors.

#### 3.3. Prediction of physicochemical and ligand toxicity

The physicochemical analysis is a crucial step used in analyzing the effectiveness of oral drugs. The effectiveness can be predicted based on Lipinski’s rule or Lipinski’s Rule of Five. Lipinski’s rule states that an oral drug compound has excellent absorption and permeation if it has a molecular mass of less than 500 Daltons, a Log P value of less than 5, the number of hydrogen bond donors less than 5, no more than 10 hydrogen bond acceptors, and the value of refractivity molar in the 40–130 range [28].

The molecular mass is related to the absorption of the drug in the body. The smaller the relative atomic mass of a compound, the easier it will be for the compound to passively diffuse through cell membranes in the small intestine and into target tissues [29]. The number of donors and acceptors of hydrogen ligands affects the absorption rate of the active compound into the body. A large number of hydrogen bond donors can reduce the ability of the molecule to penetrate the membrane bilayer. Compounds with many hydrogen bond donors and acceptors will form stronger hydrogen bonds to solvents (water) than to the lipophilic environment in the cell membrane [28].

The partition coefficient value (Log P) represents the compound’s solubility in octanol or water, ideally below 5. Ivanovic [30] stated that lipophilicity, expressed by Log P, is related to the rate of drug distribution in the body. A positive value for Log P means that the compound has a higher affinity for the nonpolar phase; therefore, the drug will bind strongly to lipids and have difficulty penetrating biological membranes. This phenomenon makes the compound unable to bind to the receptor. The value of the molar refractivity shows the size of the spread of a derived molecule per one mole of the compound. A compound has potential as an oral drug if it has a molar refractivity value ranging from 40 to 130 [31]. The results of Lipinski’s rule for active compounds in cinnamon bark and leaves are shown in Table 3. According to Lipinski’s Rule of Five, the compounds that do not violate more than two of the rules are potential candidates for oral drugs. All ligands complied with Lipinski’s rules except for proanthocyanidins which violated four rules; therefore, these compounds were not classified as good oral drug candidates. From the results, only 14 meet the five criteria of Lipinski’s rule so that they could be used as drug candidates.
Drug development determines the level of toxicity based on the virtual structure of the compound. This process ensures compounds that have the potential as drugs can work effectively without causing damage to organs. Toxicity is the ability of a compound to cause harmful effects when it enters the body. Toxicity prediction used three parameters: inhibition of Human Ether-A-Go-Go Related Gene (hERG), carcinogenicity, and acute oral toxicity (Table 4). Prediction results of hERG inhibition indicate that donepezil is an hERG strong inhibitor.

Meanwhile, the other test ligands were classified as weak hERG inhibitors. hERG is a gene encoding a pore-forming subunit in K+ channel protein that plays a role in repolarizing cardiac action potentials. Blockage or disruption of K+ channels found in cardiac cells can cause cardiac arrhythmias and be toxic to the heart [32].

**Table 3.** The results of the stability analysis of the test ligands using Lipinski's rule

| Ligand                        | Molecular mass | Hydrogen bond donor | Hydrogen bond acceptor | Log P | Molar refractivity |
|-------------------------------|----------------|---------------------|------------------------|-------|-------------------|
| Donepezil (reference)         | 350            | 0                   | 4                      | 1.106 | 87,089            |
| Quercetin                     | 302            | 5                   | 7                      | 2.011 | 74,050            |
| Epicatechin                   | 290            | 5                   | 6                      | 1.546 | 72,623            |
| Medioresinol                  | 388            | 2                   | 7                      | 3.199 | 100,236           |
| (+)-Syringaresinol            | 418            | 2                   | 8                      | 3.203 | 106,788           |
| Gamma-eudesmol               | 222            | 1                   | 1                      | 4.064 | 68,297            |
| Proanthocyanidins             | 592            | 9                   | 12                     | 3.298 | 148,272           |
| (Alpha-calacorene)            | 204            | 0                   | 0                      | 4.415 | 64,583            |
| epi-bicycloesquiphellandrene | 204            | 0                   | 0                      | 4.581 | 66,673            |
| Cinnamyl acetate             | 176            | 0                   | 2                      | 2.263 | 52,109            |
| Coumarin                      | 146            | 0                   | 2                      | 1.619 | 41,111            |
| Cinnamic acid                 | 142            | 1                   | 2                      | 1.784 | 43,112            |
| Cinnamyl alcohol              | 134            | 1                   | 1                      | 1.562 | 42,562            |
| Syringaldehyde                | 132            | 0                   | 1                      | 1.899 | 41,540            |
| Syringaldehyde                | 182            | 1                   | 4                      | 1.222 | 46,598            |

**Table 4.** The results of the predictive analysis of ligand toxicity
Carcinogenicity is the ability of a compound to cause new tissue growth or neoplasia [33]. The International Agency for Research on Cancer (IARC) classifies carcinogenicity into four groups: I (carcinogenic), II-A (probably carcinogenic), II-B (possibly carcinogenic), III (unclassified), and IV (non-carcinogenic) [34]. The carcinogenicity prediction results showed that the reference and test ligands were included in the non-carcinogenic category, so all of these ligands are safe to be used as drugs.

Acute oral toxicity refers to side effects that occur in a short time due to single or repeated doses of a drug for 24 hours [35]. Acute oral toxicity is based on the Lethal Dose 50 (LD₅₀) value, the amount of the chemical administered orally in mg/kg body weight, causing the death of 50% of the experimental animal population [36]. According to the United State Environmental Protection Agency (US EPA), acute oral toxicity of compounds is classified into four categories based on the LD₅₀ value. Category I (LD₅₀ >50 mg/kg), category II has (LD₅₀ >500 mg/kg), category III (LD₅₀ 5000 mg/kg), and category IV (LD₅₀ 5000 mg/kg) [37]. Prediction results of acute oral toxicity showed that quercetin and coumarin were included in category II (moderately toxic), and almost all test ligands were included in category III (slightly toxic) and IV (non-toxic). Based on these results, quercetin and coumarin are predicted to be toxic to the digestive tract, which can cause damage to cells and organ tissues in the body, so they are not safe to be used as drug candidates.

3.4. Molecular docking validation

Validation was conducted to obtain a grid box that includes the active site of the AChE enzyme as a binding target by re-docking the natural ligand to the AChE enzyme. The validation parameters are based on the RMSD value, indicating the degree of deviation of the ligand re-docking results from the crystallographic ligands at the same binding site. The lower the RMSD value, the closer the ligand was to the native conformation, and this is considered a good position. The RMSD value is confirmed to be valid if it is less than or equal to 2. RMSD values greater than 2.5 indicate that the ligand molecule is far shifted from the docking position; thus, the ligand does not bind to the receptor’s active site [38].

The validation results showed that the binding side with the best binding pose was at position x = 38; y = 20; z = 28 and center area x = 13.988; y = −43.906; z = 27.109 (Figure 3). The average RMSD value obtained in 20 repetitions was less than 2. The molecular docking is declared valid if the RMSD value is 2.00 [39]. The validation results indicate that the grid box area used for docking is valid and can be used for molecular docking of the test ligand because it has an RMSD value below 2Å [39].

3.5. Molecular docking and visualization of molecular docking results

Molecular docking simulations can predict interactions, binding free energies, and conformation of protein–ligand complexes. The ligand binds to the target protein’s active site to generate an optimal interaction pose and docking score based on the binding free energy. This study utilized a targeted docking method carried out on the active site area of the receptor that binds to a particular ligand; therefore, it only predicts the interaction between the ligand and the active site of the receptor [40]. Two–dimensional visualization of the interaction between cinnamon compounds with hydrogen bonds and their hydrophobicity with receptor amino acid residues can be seen in Figure 4.

Figure 3. Molecular docking validation 20 times with natural ligand (donepezil) (yellow) and the validated ligand (gray)

Figure 4. Two–dimensional molecular docking results in (a) epicatechin, (b) medioresinol [41]
AChE inhibitors can inhibit AChE by interacting with the active sites of the CAS catalytic triad (Ser203, Glu334, and His447) or the Peripheral Anionic Site (PAS) sites, namely Tyr72, Asp74, Tyr124, Ser125, Trp286, Tyr337, and Tyr341, or by binding to both sides CAS and PAS [42]. The active sites of AChE, namely Catalytic Anionic Site (CAS) and Peripheral Anionic Site (PAS), play a prominent role in ligand binding. PAS propels ligands into the gorge, particularly positively charged species such as choline. Once the ligand enters the gorge, the acyl binding site and choline–binding site are located near the catalytic triad, assisting in positioning the ligand for catalysis by CAS [43]. CAS of serine, glutamic acid, and histidine residues, also known as the catalytic triad. The primary catalytic function performed by AChE is the hydrolysis of the neurotransmitter ACh into acetic acid and choline [44].

Epicatechin and medioresinol have more potential as AChE inhibitors because they have a low binding free energy and interact more than the other test compounds. Epicatechin has the highest affinity value of −10.0 kcal/mol (Table 5).

Epicatechin, a polyphenol group compound, possibly has a more negative ΔGbind value than other test ligands because it has more hydrogen bonds in the structure of the compound. The value of the binding free energy of a ligand toward the receptor is influenced by hydrogen bonds and hydrophobic bonds [45]. The distance between a hydrogen bond affects the free energy of the bond present. The close distance of the hydrogen bond between the ligand and the target amino acid residue leads to a strong and stable interaction that occurs. According to Jeffrey [46], hydrogen bonds are divided into several criteria according to the interaction distance, namely strong, medium, and weak hydrogen bonds. Strong hydrogen bonds have an interaction distance of < 2.5, medium (almost electrostatic) hydrogen bonds have an interaction distance of 2.5–3.2, and weak hydrogen bonds have an interaction distance of 3.3–4.0. The hydrogen–bonding distance in the test ligands and receptors is in the weak and moderate categories.

In contrast to medioresinol, the structure of this compound has fewer hydroxyl groups than epicatechin. Each hydrogen bond increases the affinity by 0.48 to 1.55 kcal/mol for neutral bonds and 2.39 to 4.78 kcal/mol for charged bonds or salt bridges [47]. In addition, epicatechin compounds also bind to two amino acid residues at the CAS catalytic site and partly bind to the PAS site. Meanwhile, medioresinol only binds to the PAS active site. Dual binding inhibitors such as donepezil bind to CAS and PAS sites, thus exhibiting potent AChE inhibitory activity [5]. Inhibition of the CAS of AChE can reduce the degradation of ACh. At the same time, inhibition of the PAS site can reduce the formation of Aβ plaques. PAS can form stable complexes with beta-amyloid peptides, thereby accelerating oligomerization of Aβ peptides and senile plaque aggregation that will lead to Alzheimer’s [48].

**Table 5. The results of the two-dimensional visualization of the ligand to the receptor**

| Ligand                  | Binding free energy (kcal/mol) | Hydrogen bonded amino acid residue | Bond length (Å) | Hydrophobic bonded amino acid residue |
|-------------------------|--------------------------------|-----------------------------------|-----------------|---------------------------------------|
| Donepezil (reference)   | −12.3                          | Phe295                            | 2.80            | Ser203, His447, Gly202, Trp286, Phe295, Tyr337, Ser293, Tyr72, Tyr124, Tyr341, Gly121, Gly448, Val294 |
| Epicatechin             | −10.0                          | Gly121                            | 3.23            | Gly126, Gly120, Tyr113, Gly202, Phe338, Tyr137, Tyr124, Tyr72, Trp286, Ser254, Asp74, Trp286, Gly121 |
| Medioresinol           | −9.9                           | Ser125                            | 2.88            | Gly121, Tyr337, Tyr124, Tyr341, Tyr72, Trp286, Leu289, Ser293, Phe297, Phe295, Gly121, His447, Phe338 |
| (+)-Syringaresinol      | −9.8                           | Glu202                            | 3.10            | Gly121, Gly121, Tyr124, Tyr297, Phe338, Tyr337, His447, Trp286, Gly448, Gly121 |
| Gamma- eudesmol        | −9.7                           | Ser203                            | 2.70            | Ser203, Tyr341, Tyr337, Phe338, Trp286, Gly120, Gly121, His447, Gly122, Phe297, Tyr124 |
| (+)-Ledene             | −9.4                           | Gly338                            | 2.80            | Gly338, Tyr337, Ser254, Tyr124, Tyr297, Gly120, Gly448, Gly202, Tyr341 |
| Alpha- calacorene      | −9.4                           | Arg296                            | 3.31            | Ty337, Val294, Gly121, Gly202, Phe295, Tyr137, Phe338, Asp74, Ser254, Trp286 |
| epi- bicycloquphellandrene | −9.2                         | Arg296                            | 3.06            | Gly121, His447, Phe295, Gly120, Phe338, Tyr124, Trp286 |
3.6. Antibacterial activity of fractions

The molecular docking result parameter is binding free energy. Molecular docking results obtained from AutoDock Vina (Table 6) indicate that the value of $\Delta G_{\text{bind}}$ and the inhibition constant (Ki) of the test ligand is greater than that of the reference ligand donepezil. Donepezil has a $\Delta G_{\text{bind}}$ of $\sim 12.3$ kcal/mol, indicating that all the tested ligands can bind to the receptor, although not as strongly as donepezil.

**Table 6. Binding free energy and ligand inhibition constant**

| Ligand          | Binding free energy (kcal/mol) | Inhibition constant (µM) | Number of hydrogen bonds | Number of hydrophobic bonds |
|-----------------|--------------------------------|--------------------------|--------------------------|-----------------------------|
| Donepezil (reference) | -12.3                          | 0.0009                   | 1                        | 14                          |
| Epicatechin     | -10.0                          | 0.0459                   | 4                        | 7                           |
| Medioresinol    | -9.9                           | 0.0533                   | 1                        | 13                          |
| (+)-syringaresinol | -9.8                          | 0.0644                   | 0                        | 13                          |
| Gamma-eudesmol  | -9.7                           | 0.0762                   | 2                        | 11                          |
| (+)-ledene      | -9.4                           | 0.1265                   | 0                        | 11                          |
| (Alpha-calacorene) | -9.4                         | 0.1265                   | 0                        | 9                           |
| Epibicyclosesquihe llandrene | -9.2                      | 0.1774                   | 0                        | 9                           |
| Cinnamyl acetate | -7.5                           | 3.1359                   | 2                        | 7                           |
| Cinnamic acid    | -7.1                           | 6.1643                   | 1                        | 6                           |
| Cinnamyl alcohol | -6.6                           | 14.3482                  | 1                        | 8                           |
| Cinnamaldehyde   | -6.6                           | 14.3482                  | 1                        | 6                           |
| Syringaldehyde   | -6.4                           | 20.1169                  | 2                        | 7                           |

The binding free energy represents the most negligible energy used by the receptor to interact with the ligand. The smaller the $\Delta G_{\text{bind}}$ value leads to a stronger binding affinity between the receptor and the ligand, causing the compound to be better at inhibiting the receptor. The value of $\Delta G_{\text{bind}}$ is influenced by hydrogen bonding and hydrophobicity. Ligands with many hydrogen bonds can bond more strongly to the enzyme’s active site \[45\]. The interaction between the ligand and the target amino acid residue is stronger and more stable when the hydrogen bond distance gets closer \[49\]. The test ligand with a high $\Delta G_{\text{bind}}$ value is epicatechin with four hydrogen and seven hydrophobic interactions. The value of $\Delta G_{\text{bind}}$ is inversely proportional to the value of the inhibition constant (Ki). The more negative the $\Delta G_{\text{bind}}$ value, the lower the Ki value. When the value of Ki gets lower, lower concentrations of ligand would be required to inhibit the activity of the enzyme \[50\].

4. Conclusion

Cinnamon has potential as an alternative treatment for Alzheimer’s related to the mechanism of inhibition of the AChE enzyme. Based on the in silico method, epicatechin, medioresinol, and gamma eudesmol compounds were predicted to bind almost as strongly as the reference ligand donepezil. However, these compounds are not potentially better than donepezil. The inhibition of AChE by epicatechin was dominated by hydrogen bonding with more enzyme active sites than other compounds, whereas medioresinol was dominated by hydrophobic bonds. Epicatechin has the best potential among other compounds in cinnamon for Alzheimer’s treatment because it has the most negative affinity compared to other compounds and the lowest Ki value. In addition, epicatechin has dual binding to AChE, namely CAS and PAS.

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