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

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Abstract

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.

1. Introduction

Alzheimer's disease is a neurodegenerative disorder characterized by the death of brain cells, causing memory loss and cognitive decline. This disease is the most common cause of dementia in people aged 65 years and older. Alzheimer's disease causes brain shrinkage as the number and interactions between nerve cells are reduced [1]. This condition is characterized by progressive memory loss, impaired memory formation, decreased intellectual function and speaking skills, and personality changes [2]. According to Alzheimer's Disease International [3], there were 55 million people worldwide with dementia in 2019. This number is expected to increase to 78 million in 2030 and 139 million in 2050. According to Alzheimer's Indonesia [4], the prevalence of Alzheimer's disease in Indonesia in 2016 reached 1.2 million people, and this number is expected to increase to 4 million in 2050. This shows that people living with Alzheimer's are increasing every year.

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	
Donepezil (reference)	Eugenol
Quercetin	Terpinen-4-Ol
Epicatechin	D-Limonene
Medioresinol	Gamma-terpinene
(+)-syringaresinol	2-Carene
Gamma-Eudesmol	trans-cinnamic acid
Proanthocyanidins	Alpha-terpineol
(+)-ledene	Cinnamic acid
Alpha-calacorene	Camphor
epi-bicyclosesquiphellandrene	Alpha-pinene
Gamma-Muurolene	Eucalyptol
Guaiol	(-)-Beta-pinene
Calarene	1,8 cineole
Gamma-cadinene	Borneol
Ylangene	Cinnamyl alcohol
(-)-spathulenol	Cinnamaldehyde
Alpha -Cubebene	Linalool
(Z)-nerolidol	Benzenepropanal
Aromadendrene	Syringaldehyde
Alpha-muurolene	Benzaldehyde
Caryophyllene Oxide	(Z)-nerolidol
Epicubeno	Linoleic 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 crystallized donepezil. Several molecules attached to the enzyme structure were 1-benzyl-4-[(5,6-dimethoxy-1-indanon-2-yl)methyl]piperidine (Donepezil), 2-acetamido-2-deoxy-beta-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

2.70 [16]. 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 runprocheck.

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 energy 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-iitd.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/admetsar1/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 form, 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 Ki (ΔG_{bind} = binding free energy (kcal/mol)., R = 1.986 x 10^{-3} kcal/mol.K, T= 298.15 K [22]. 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

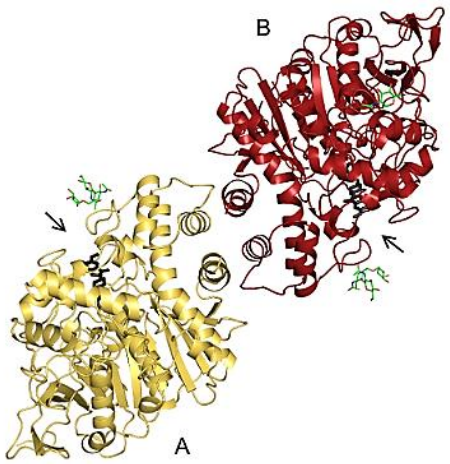

Figure 1. Structure of complexed AChE with donepezil [23]

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 the residues in the generously allowed regions, and the white color shows the residues in the prohibited regions (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.

The Ramachandran diagram analysis shows that the enzyme with code 4EY7 has amino acid residues of 90.9% in the most favored regions, 8.9% in the additionally allowed regions, 0.0% in the generously

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.

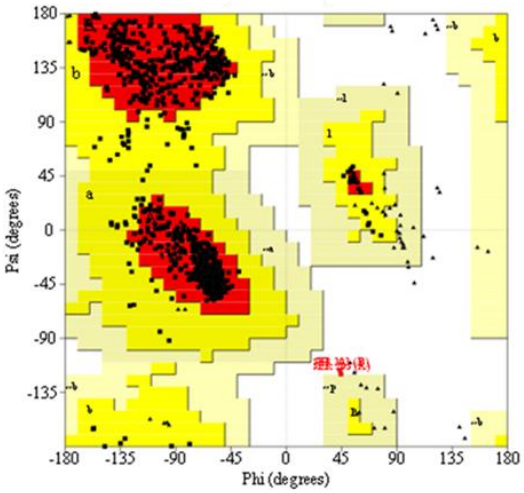

Figure 2. Ramachandran diagram of the AChE enzyme [18]

3.2. Virtual screening

Table 2. The best compounds from 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-bicyclosesquiphellandrene	-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)

The virtual screening results were evaluated based on the order of binding free energy and RMSD [11]. The value of free binding energy (ΔG_{bind}) indicates the stability of a ligand to bind to the receptor. The more negative the G_{bind} value, the better the stability level, so that the bond between the ligand and the receptor is getting stronger [27]. The best ligand resulting from virtual screening is the ligand with the highest binding free energy close to the binding free energy value of the reference ligand. Donepezil has a binding free energy of -12.2 kcal/mol, indicating that the reference ligand has the most robust and most stable affinity for the receptor compared to all test ligands.

Of the 60 ligands, 15 ligands passed the virtual screening based on the binding free energy, with 9 of them having binding free energy approaching the donepezil reference ligand, which is less than -9.0 kcal/mol (Table 2). Meanwhile, the other six compounds are typical cinnamon compounds based on the 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 molars 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.

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

Ligand	Lipinski's rule				
	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**
(+)-Ledene	204	0	0	4.415	64.583
(Alpha-calacorene)	200	0	0	4.541	67.026
epi-bicyclosesquiphellandrene	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	148	1	2	1.784	43.112
Cinnamyl alcohol	134	1	1	1.692	42.562
Cinnamaldehyde	132	0	1	1.899	41.540
Syringaldehyde	182	1	4	1.222	46.598

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 4. The results of the predictive analysis of ligand toxicity

Ligand	Inhibition of Human Ether-A-Go-Go Related Gene (hERG)		Carcinogenic		Acute oral toxicity	
	Category	Score	Category	Score	Category	Score
Donepezil (reference)	Strong inhibitor	0.5386	Non-carcinogenic	0.9528	III	0.5250
Quercetin	Weak inhibitor	0.9781	Non-carcinogenic	0.9450	II	0.7348
Epicatechin	Weak inhibitor	0.9666	Non-carcinogenic	0.9539	IV	0.6433
Medioresinol	Weak inhibitor	0.9418	Non-carcinogenic	0.8939	III	0.6630
(+)- Syringaresinol	Weak inhibitor	0.9494	Non-carcinogenic	0.8036	III	0.6169
Gamma-eudesmol	Weak inhibitor	0.8830	Non-carcinogenic	0.8501	IV	0.4811
(+)-Ledene	Weak inhibitor	0.9643	Non-carcinogenic	0.7765	III	0.8165
Alpha-calacorene	Weak inhibitor	0.9438	Non-carcinogenic	0.7062	III	0.6186
epi-bicyclosesquiphellandrene	Weak inhibitor	0.8089	Non-carcinogenic	0.7928	III	0.7969
Cinnamyl acetate	Weak inhibitor	0.9475	Non-carcinogenic	0.6061	III	0.9005
Coumarin	Weak inhibitor	0.8702	Non-carcinogenic	0.9412	II	0.7019
Cinnamic acid	Weak inhibitor	0.9620	Non-carcinogenic	0.5927	III	0.8487
Cinnamyl alcohol	Weak inhibitor	0.8730	Non-carcinogenic	0.5332	III	0.8179
Cinnamaldehyde	Weak inhibitor	0.9241	Non-carcinogenic	0.5786	III	0.8687
Syringaldehyde	Weak inhibitor	0.9418	Non-carcinogenic	0.8939	III	0.6630

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 (LD_{50}) 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 LD_{50} value. Category I (LD_{50} 50 mg/kg), category II has (LD_{50} >500 mg/kg), category III (LD_{50} 5000 mg/kg), and category IV (LD_{50} 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].

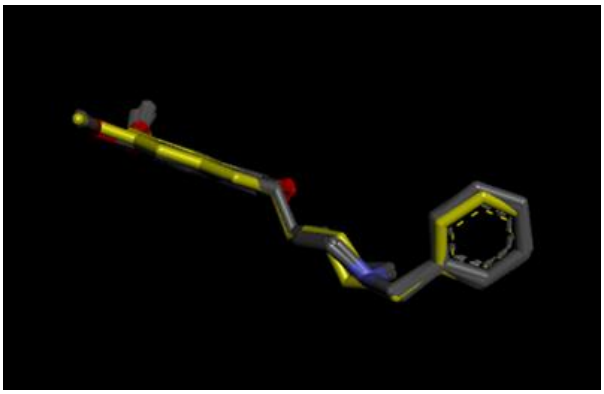

Figure 3. Molecular docking validation 20 times with natural ligand (donepezil) (yellow) and the validated ligand (gray)

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.

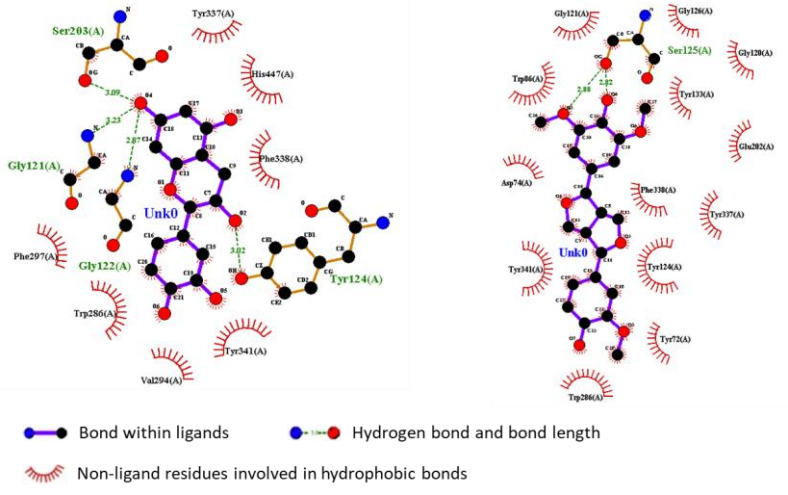

Figure 4. Two-dimensional molecular docking results in (a) *epicatechin*, (b) *medioresinol* [41]

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 value 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 ΔG_{bind} 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**, Glu202, Trp86, Trp286, Phe338, Tyr337, Ser293, Tyr72, Tyr124, Tyr341, Gly121, Gly448, Val294
Epicatechin	-10.0	Gly121	3.23	Tyr337, **His447**, Phe338, Tyr341, Val294, Trp286, Phe297
		Gly122	2.87	
		Tyr124	3.02	
		Ser203	3.09	
Medioresinol	-9.9	Ser125	2.88 2.82	Gly126, Gly120, Tyr133, Glu202, Phe338, Tyr337, Tyr124, Tyr72, Trp286, Tyr341, Asp74, Trp86, Gly121
(+)-Syringaresinol	-9.8			Gly121, Tyr337, Tyr124, Tyr341, Tyr72, Trp286, Leu289, Ser293, Phe297, Phe295, Gly122, **His447**, Phe338
Gamma- eudesmol	-9.7	Glu202 **Ser203**	3.30 2.70	Tyr133, Gly121, Gly122, Tyr124, Phe297, Phe338, Tyr337, **His447**, Trp86, Gly448, Gly121
(+)-Ledene	-9.4			**Ser203**, Tyr341, Tyr337, Phe338, Trp86, Glu202, Gly121, **His447**, Gly122, Phe297, Tyr124
Alpha-calacorene	-9.4			Phe338, Tyr337, Asp74. Ser125, Tyr124, Trp86, Gly448, Glu202, Tyr341
epi-bicyclosesquiphellandrene	-9.2			Tyr124, Gly121, Glu202, **His447**, Tyr337, Phe338, Asp74, Ser125, Trp86
Cinnamyl acetate	-7.5	Arg296 Phe295	3.31 2.90	Val294, Phe297, Tyr341, Tyr337, **His447**, Phe338, Trp286
Cinnamic acid	-7.1	Try337	2.84	Try341, Asp74, Trp86, **His447**, Glu202, Gly448,
Cinnamyl alcohol	-6.6	Arg296	2.96 3.10	Val294, Phe297, Phe295, Tyr124, Tyr341, Tyr337, Phe338, Trp286
Cinnamaldehyde	-6.6	Tyr337	2.97	Asp74, Tyr341, Gly121, Glu202, Gly448, Trp86
Syringaldehyde	-6.4	**Ser203**	2.82 3.06	Gly121, **His447**, Phe295, Phe297, Phe338, Tyr124, Trp86
		Gly122	2.96	

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 ΔG_{bind} and the inhibition constant (Ki) of the test ligand is greater than that of the reference ligand donepezil. Donepezil has a ΔG_{bind} of -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	0.0543	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
Epibicyclosesquiphellandrene	−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 ΔG_{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 ΔG_{bind} is influenced by hydrogen bonding and hydrophobicity. Ligands with many hydrogen bonds can bind 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 ΔG_{bind} value is epicatechin with four hydrogen and seven hydrophobic interactions. The value of ΔG_{bind} is inversely proportional to the value of the inhibition constant (Ki). The more negative the ΔG_{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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