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In Silico Study of Quercetin, Isoquercetin, and β-sitosterol Interaction with Acetylcholinesterase (AChE) Enzyme in Moringa Leaves (*Moringa oleifera Lamk*) as Alzheimer Disease Therapy

Khoerunnisa Cahyani Lailani✉, Muhammad Dafrizal Firdaus, Eufrasia Ine Pilianto, Luthfiyyah Muthamainah, Bagus Adhinagoro, Shela Salsabila, Muchtaridi

Department of Pharmaceutical Analysis and Medicinal Chemistry, Faculty of Pharmacy, Universitas Padjadjaran, Jl. Raya Bandung - Sumedang KM. 21, Jatinangor, West Java, 45363, Indonesia

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Abstract

Acetylcholinesterase (AChE) is a cholinergic enzyme which hydrolyzes the neurotransmitter acetylcholine (ACh) into acetic acid and choline. ACh is clinically significant in the development of progressive Alzheimer's disease that impairs memory and mental function. AChE inhibitor compounds are potential candidates for the treatment of Alzheimer's. This research conducted an in-silico study of compounds in Moringa leaves (*Moringa oleifera Lamk*) to obtain potential compounds in treating Alzheimer's using the molecular docking, Lipinski rule prediction, including ADME and toxicity predictions. The research showed that isoquercetin, quercetin, and beta-sitosterol were the best drug candidates based on binding energy and inhibition constant (ki). The binding energy and inhibition constant (ki) values of isoquercetin are -9.21 kcal/mol and 0.1765 μM, respectively. The quercetin binding energy and inhibition constant (ki) values are -8.33 kcal/mol and 778 μM, respectively. Both isoquercetin and quercetin have hydrogen bonds at residue ARG 296. Beta-sitosterol bond energy and inhibition constant (ki) values are -12.87 kcal/mol and 3.7 10-10 μM respectively with Van der Waals interaction at the residue SER 203, GLY 121, PHE 295, and GLY 120.

✉ Correspondence address:
Jl. Raya Bandung - Sumedang KM. 21, Jatinangor, West Java, 45363
E-mail: khoerunnisa22001@mail.unpad.ac.id

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Introduction

A syndrome characterized by a decline in brain function, such as memory loss, disorientation, inability to communicate, overall function decline, and other manifestations, is the hallmark of Alzheimer's disease, a form of dementia. Alzheimer's disease is the result of the impaired function of acetylcholine, a compound that transmits nerve signals. AChE is an enzyme that catalyzes the degradation of acetylcholine (ACh) into inactive forms, specifically acetate and choline. After activation, nerves require this response to allow them to settle down. Nevertheless, this reaction can also cause harm to brain cells if it is conducted in an excessive manner. In 2016, the estimated prevalence of Alzheimer's disease in Indonesia was approximately 1.2 million (Alzi, 2019). This figure is expected to rise to 2 million by 2030 and 4 million by 2050. The current treatment for Alzheimer's disease involves the use of synthetic medications that inhibit AChE, including physostigmine, donepezil, and tacrine. These medications are recognized to have adverse effects and limitations, including liver toxicity, gastrointestinal disturbances, and high cost (Reubun, *et al.*, 2020). Alzheimer's therapy may also involve the utilization of medicinal plants that are accessible in Indonesia, in addition to medications.

In Indonesia, moringa leaves are a widely used medicinal plant. They contain bioactive compounds, including vitamins, carotenoids, polyphenols, flavonoids, alkaloids, and steroids, which have the potential to improve brain memory. Moringa leaves demonstrate nootropic effect in Alzheimer's disease by increasing lipid peroxidation induced by colchicine, which in turn reduces glutathione enzymes, catalase, superoxide dismutase, acetylcholine, and AChE. According to existing research, the AChE enzyme was inhibited by the flavonoid quercetin in a spectrophotometric analysis, with an IC_{50} value of 19.8 µM (Jung & Park, 2007). Additionally, isoquercetin, another flavonoid, exhibited inhibitory activity against the AChE enzyme. Furthermore, the investigation conducted by Ayaz et al. The acetylcholinesterase enzyme activity can be inhibited by the compound β-sitosterol through the formation by hydrogen bonds between its group and the glutamic acid present in the choline pocket at the active site of AChE (Ayaz, *et al.*, 2017). Consequently, moringa leaves that contain β-sitosterol, isoquercetin, and quercetin are capable of inhibiting the AChE enzyme, which is the primary cause of Alzheimer's disease (Reubun, *et al.*, 2020).

The purpose of this study was to examine the potential of quercetin, isoquercetin, and β-sitosterol compounds in *Moringa oleifera Lamk*. leaves as potential drugs for the treatment of Alzheimer's disease. Molecular docking, PreADMET determination, pharmacophore modeling, and Lipinski's Rule of Five were employed to carry out this investigation (Hutabarat & Nurfadly, 2020). Moringa leaves were chosen due to their potential as an Alzheimer's therapy, as they are known to contain a variety of compounds and possess diverse pharmacological properties. Nevertheless, the absence of specific research on moringa leaf compounds that could be used as Alzheimer's therapy led to the selection of quercetin, isoquercetin, and β-sitosterol from Moringa leaves for this study. The acetylcholinesterase (AChE) enzyme with PDB ID code 7E3H was selected as the target protein because it represents the human AChE structure co-crystallized with donepezil, a clinically approved acetylcholinesterase inhibitor, at a resolution of 2.45 Å. The availability of a co-crystallized reference ligand allows reliable validation of the molecular docking protocol through re-docking analysis and ensures accurate identification of the active binding site. In addition, the binding pocket of the 7E3H structure is well defined and biologically relevant for Alzheimer's disease therapy, making it suitable for structure-based virtual screening studies. Donepezil was used as the reference ligand for comparative analysis, as it has been approved for the treatment of mild, moderate, and severe stages of Alzheimer's disease (Lee, *et al.*, 2015).

Methods

The instruments and tools used in this study included hardware in the form of a laptop with Windows 11 Home operating system, equipped with an Intel® Core™ i5-11300H processor running at 3.11 GHz, 8 GB of RAM, an NVIDIA GeForce GTX 1650 graphics card, and a 64-bit x64-based architecture. The software utilized consisted of Windows 10 Home, LigandScout® 4.3, ChemDraw® Ultra 12.0, Chem3D® Pro 12.0, AutoDockTools®, Discovery Studio 3.5 Visualizer®, SwissADME, Protein Data Bank, PubChem, DUD-E, and Pre-ADMET.

The materials used in this research included the 3D structure of the Human Acetylcholinesterase receptor (PDB ID: 7E3H), obtained via X-ray diffraction with a 2.45 Å resolution from the Protein Data Bank. In addition, fourteen secondary metabolites reported from *Moringa oleifera* leaves were initially collected from the literature and PubChem database in .sdf format, including quercetin, isoquercetin, moringin, beta-sitosterol, moringinin, niazimin, niazirinin, kaempferol, 4-[(alpha-L-rhamnopyranosyloxy)benzyl]glucosinolate, anthraquinone, anthocyanin, benzylglucosinolate, alpha-phellandrene, and pterygospermin. These compounds were subjected to an initial screening process based on physicochemical properties, Lipinski's Rule of Five, and ADMET predictions to evaluate their drug-likeness and pharmacokinetic profiles. Subsequently, pharmacophore screening and molecular docking analyses were

conducted to assess their interaction potential toward the AChE receptor. Based on the integrated screening results, quercetin, isoquercetin, and beta-sitosterol were selected as the final lead compounds for further molecular interaction and binding affinity analysis.

Metabolite analysis in *Moringa oleifera* was conducted through Lipinski's Rule of Five (RO5) prediction to determine the physicochemical properties of the test compounds by using the SwissADME platform at http://www.swissadme.ch/index.php (Wulandari, *et al.*, 2023). The SMILES codes for each compound were obtained from PubChem (http://PubChem.nbi.nlh.nih.gov) and uploaded into the Pre-ADMET system to generate the ADMETox profile.

Pharmacophore modeling was performed by preparing active and decoy databases downloaded from the DUD-E website (https://dude.docking.org/tartgets) in .ldb format (Widyasari, *et al.*, 2022). The decoy databases were prepared by downloading 10 active and 50 decoy compounds from DUD-E, followed by clustering and screening using LigandScout®. Ten pharmacophore models were generated, and their performance was evaluated by screening a dataset containing 127 active compounds and 4,803 decoys. The resulting ROC (Receiver Operating Characteristic) curves were analyzed to determine the model with the highest discriminatory power. The best model obtained was then used for pharmacophore-based screening against the compound database.

Molecular docking preparation began by downloading the AChE receptor structure with PDB code 7E3H from the Protein Data Bank (https://www.rcsb.org/) (Widyasari, *et al.*, 2022). The receptor structure was separated from its ligand using BIOVIA Discovery Studio 2021, followed by structure refinement such as the removal of water molecules, addition of hydrogens, and completion of necessary parameters within AutoDockTools®. The receptor was then saved in .pdbqt format. Ligand preparation involved downloading the 3D structures from the PubChem database, followed by geometry optimization using Chem3D Pro 12.0. The optimized structures were then subjected to energy minimization, saved in .mol2 format, and subsequently converted to .pdbqt using AutoDockTools® .

Docking simulations were carried out by generating the Grid Box using AutoDockTools® and adjusting the x, y, and z coordinates to precisely cover the active binding site of the AChE receptor. The docking process used AutoDock Vina to calculate binding affinity and RMSD values. Output files generated from docking, including .dpf, .gpf, .dlg, and additional docking logs, were analyzed to determine hydrogen bonding interactions, amino acid residues at the binding site, ligand conformation, and binding energy values. Lower binding affinity values indicated stronger ligand-receptor interactions. Visualization of docking interactions, including 2D and 3D binding modes, was performed using Discovery Studio 2021 Client to obtain detailed insights into the orientation, bond types, and interacting residues between ligands and the target protein (Widyasari, *et al.*, 2022).

Results and Discussion

Lipinski's Rule of Five states that a compound exhibits properties similar to those of drugs or that this method is valid for a compound if its molecular weight (MW) is < 500 Dalton, the log P partition coefficient value is < 5, the number of hydrogen bond donors (HBD) is < 5, and the number of hydrogen bond acceptors (HBA) is < 10 (Lipinski, 2004). Compounds with a molecular weight greater than 500 Da are unlikely to diffuse across cell membranes (Horn & Neundorf, 2018). A high log P value (> 5) indicates that the compound is excessively hydrophobic and may exhibit high toxicity due to prolonged retention in the lipid bilayer or the membrane's structural base, leading to a broader distribution in the body and reduced binding selectivity to the target enzyme. Excessive hydrogen bond donors and acceptors increase the required energy, thus slowing the compound's ability to reach its target (Wang, *et al.*, 2021). Based on the predicted Lipinski parameters for 14 compounds in Moringa leaves, 11 compounds satisfied all of Lipinski's rules, while 3 compounds violated one rule. These compounds can thus be considered potential oral drug candidates (Guedes, *et al.*, 2019). The results of Lipinski's rule predictions are presented in Table 1.

Table 1. Prediction Results of Lipinski's Rule of Five

No	Compound Name	Molecular Weight (<500 Da)	Log P (<5)	Hydrogen Bond		Description
				Donor (< 5)	Acceptor (< 10)	
1	Quercetin	302.24	1.23	5	7	Complies
2	Moringin	295.35	2.27	2	5	Complies
3	β-sitosterol	428.73	7.55	1	1	Complies with 1 violation
4	Kaempferol	286.24	1.58	4	6	Complies
5	Moringinine	312.31	0.40	4	7	Complies

No	Compound Name	Molecular Weight (<500 Da)	Log P (<5)	Hydrogen Bond		Description
				Donor (< 5)	Acceptor (< 10)	
6	Niazimin	383.40	0.96	3	8	Complies
7	4'-[(α-L-rhamnopyranosyloxy)benzyl]glucosinolate	353.40	2.20	2	8	Complies
8	Niazirinin	305.33	1.53	1	6	Complies
9	Anthraquinone	208.21	2.64	0	2	Complies
10	Anthocyanins	207.25	0	0	1	Complies
11	Isoquercetin	488.48	2.18	6	10	Complies with 1 violation
12	Benzylglucosinolates	408.40	0.30	4	11	Complies with 1 violation
13	Alpha-phellandrene	136.23	2.97	0	0	Complies
14	Pterygospermin	406.52	3.75	0	2	Complies

A total of 14 secondary metabolites from Moringa leaf plants were subjected to ADMETox prediction, aiming to predict the absorption, distribution, metabolism, excretion, and toxicity profiles of these compounds. In the ADMETox prediction, several parameters must be met by the compounds, including Human Intestinal Absorption (HIA), Caco-2, %PPB, BBB, mutagenicity, and carcinogenicity. Human Intestinal Absorption (HIA) is a parameter used to determine the absorption capacity of a compound in the human intestine. A compound is considered to have poor absorption if it falls below 20%, moderate absorption if it is in the range of 20-70%, and good absorption if it is within the 70-100% range. Isoquercetin exhibited poor absorption, while quercetin and moringinin showed moderate absorption. Meanwhile, the other ten compounds exhibited good absorption in the intestine. In addition to HIA, another parameter is Human Colon Adenocarcinoma (Caco-2), which is used to predict gastrointestinal permeability and transport mechanisms through the epithelial cells of the colon adenocarcinoma. Permeability is categorized as good if the value is > 70, moderate within the range of 4-70, and poor if the value is below 4. Among the 14 predicted compounds, quercetin and niazirinin were identified as having poor permeability, while the other compounds had moderate permeability.

The distribution profile parameters include BBB and PPB. BBB (Blood-Brain Barrier) is used to predict the ability of a compound to cross the blood-brain barrier. Compounds with a BBB value greater than 2, have a high ability to penetrate the blood-brain barrier. This is demonstrated by the compounds alpha-phellandrene, anthraquinone, beta-sitosterol, and anthocyanins. Quercetin and kaempferol have BBB values in the range of 0.1-2, indicating that these compounds have moderate ability to cross the blood-brain barrier. Meanwhile, compounds with a BBB value below 0.1 have a low ability to penetrate the blood-brain barrier. Drug compounds targeting the central nervous system need to have a high BBB value to effectively cross the blood-brain barrier.

The PPB (Plasma Protein Binding) parameter indicates the extent to which a drug is unbound from plasma proteins. The higher the plasma protein binding, the better the distribution of the compound in the bloodstream. A compound is considered to bind strongly when its PPB value exceeds 90% as demonstrated by quercetin, alpha-phellandrene, anthraquinone, pterygospermin, beta-sitosterol, anthocyanins, and kaempferol.

The toxicity parameters of the compounds include mutagenicity and carcinogenicity. Among the 14 compounds tested for mutagenic properties, only quercetin, isoquercetin, beta-sitosterol, and pterygospermin were found to be non-mutagenic. This suggests that the other compounds, except for these four, have the potential to cause genetic mutations, warranting further research and possibly elimination from subsequent testing stages. Carcinogenicity testing in rats showed that alpha-phellandrene and beta-sitosterol tested positive for carcinogenic properties, while the other compounds were negative. Meanwhile, carcinogenicity testing in mice revealed that alpha-phellandrene, niazirinin, anthraquinone, and kaempferol exhibited carcinogenic properties. The results of the ADMETox prediction are presented in Table 2.

Table 2. ADMETox Prediction Results

No	Compound Name	Absorption		Distribution			Toxicity	
		HIA (%)	Caco-2 (nm/sec)	PPB (%)	BBB	Mutagen	Carcinogen	
							Mouse	Rat
1	Quercetin	63.49	3.41	93.24	0.17	Non-mutagen	Negative	Negative
2	Alpha-phellandrene	100	23.42	100	7.17	Mutagen	Positive	Positive
3	Niazirinin	79.86	1.58	54.45	0.01	Mutagen	Negative	Positive
4	Isoquercetin	11.78	9.44	59.16	0.03	Non-mutagen	Negative	Negative
5	Moringinine	88.75	21.07	69.71	0.03	Mutagen	Negative	Negative
6	Anthraquinone	99.18	21.99	93.61	2.89	Mutagen	Negative	Positive
7	Pterygospermin	97.83	54.49	94.07	0.05	Non-mutagen	Negative	Negative
8	β-sitosterol	100	52.37	100	19.89	Non-mutagen	Positive	Negative
9	Anthocyanin	100	28.09	100	2.72	Mutagen	Negative	Negative
10	Kaempferol	79.44	9.58	89.61	0.29	Mutagen	Negative	Positive
11	Moringinin	62.48	13.60	46.05	0.10	Mutagen	Negative	Negative
12	Niazimin	73.17	19.71	56.24	0.03	Mutagen	Negative	Negative
13	Glucosinolate	91.38	21.11	75.26	0.01	Mutagen	Negative	Negative

Pharmacophore screening is an initial step in drug compound determination aimed at identifying compounds that differ in shape but still maintain a similar arrangement in 3D form through the interaction of functional groups, referred to as pharmacophore features, by identifying the bioactivation conformation and potential drug candidates. This pharmacophore modeling can also screen compounds for protein structures with unknown configuration (Arba, *et al.*, 2020). LigandScout is a software that allows users to perform 3D pharmacophore modeling of ligand-protein complexes using training and test sets of organic compounds (Fatimah, *et al.*, 2020).

After the pharmacophore modeling process, 10 pharmacophore compounds were obtained, with 8 compounds showing good fit scores. The pharmacophore fit score is the percentage of geometric similarity between a pharmacophore and the 3D model of the active ligand pharmacophore. A higher pharmacophore fit score indicates a better match of the pharmacophore to its receptor molecule. Based on the pharmacophore screening results, the highest pharmacophore fit score was obtained for isoquercetin, with a value of 39.66, indicating that isoquercetin has a high degree of compatibility with the acetylcholinesterase receptor.

Subsequently, pharmacophore validation was performed to assess the accuracy of discrimination between active compounds and decoys by examining the AUC and EF values, which meet the standards derived from the ratio between one set of active compounds and one set of decoy compounds, displayed in a single plot as the ROC curve. The ROC curve illustrates sensitivity and specificity as a threshold, with all values falling within the accepted range. The ideal ROC curve can be visualized as a straight horizontal line extending toward the upper-right corner.

Area Under Curve (AUC) is a metric used to evaluate the performance of classification and is applied for comparison methods. A high AUC value indicates better classification performance. A model is considered capable of distinguishing between two different characteristics—whether a compound is active or inactive—if the AUC value of the ROC curve falls within the range of 0.60 to 1.0. An AUC value lower than 0.50 indicates that inactive compounds are more prevalent than active compounds recognized by the software (Braga & Andrade, 2023).

The results of pharmacophore validation showed that Model 5 exhibited the highest area under the curve (AUC) value (AUC = 0.74) compared to the other generated pharmacophore models, indicating its superior ability to discriminate between active compounds and decoys. Although several pharmacophore models demonstrated acceptable predictive performance, Model 5 consistently identified a higher proportion of active compounds, predicting a total of eight compounds with favorable pharmacophore features. Therefore, Model 5 was selected as the representative pharmacophore model for subsequent screening and analysis. A comparative summary of the validation results for all generated pharmacophore models is provided to illustrate the evaluation process.

Pharmacophore screening aims to identify the key molecular features required for ligand recognition based on biological macromolecular interaction. Since a pharmacophore represents an abstract description of essential interaction features rather than fixed functional groups, these features can serve as a reference

for diverse chemical scaffolds. The results of the pharmacophore screening using the selected model are presented in Figure 1.

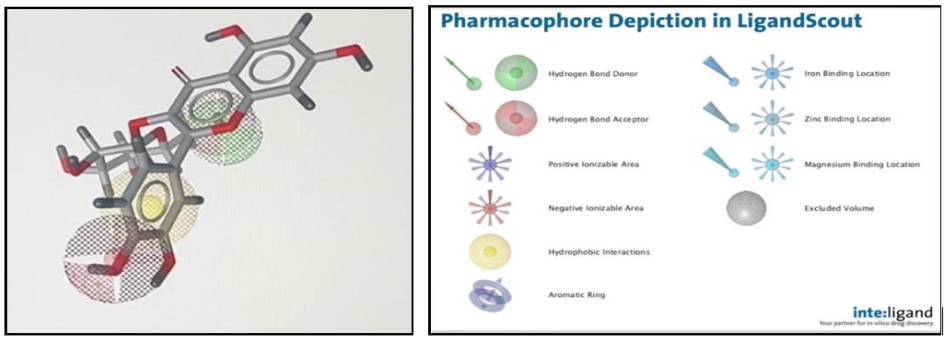

Figure 1. Pharmacophore Screening Results

As shown in the figure above, three pharmacophore features can be observed for the AChE receptor, namely hydrogen bond donor, hydrogen bond acceptor, and hydrophobic interactions.

After obtaining information about the pharmacophore, the procedure of re-docking the co-crystallized reference ligand will be performed, which involves separating the co-crystallized reference ligand from the target protein and re-performing the docking process. The purpose of this procedure is to validate the binding between the receptor and the ligand. The result obtained will be the RMSD value, where a value of 1-2Å is considered very good, 2-3Å is moderate, and >3Å is considered poor (Mateev, *et al.*, 2021). Another parameter indicating the effectiveness of the docking process includes molecular interactions, binding energy, and inhibition constant (KI) (Yuliana, *et al.*, 2023). The results obtained from the re-docking process can be seen in Table 3.

The re-docking results with an RMSD value of less than 2Å, demonstrate that the docking protocol was successfully validated, showing minimal deviation between the docked pose and the co-crystallized reference ligand. The binding energy value of -11.14 kcal/mol reflects the strength and stability of the interaction between the ligand and the active site of the receptor, rather than the ease of binding. More negative binding energy values indicate stronger and more stable ligand-receptor complexes (Forli, *et al.*, 2016). Molecular interactions were visualized using BIOVIA Discovery Studio Visualizer.

Hydrogen bonds and van der Waals interactions play important roles in stabilizing ligand-receptor complexes. These interactions describe the non-covalent contacts formed between the ligand and the amino acid residues within the active site. The re-docking results showed the presence of one hydrogen bond and ten van der Waals interactions between Donepezil and the AChE amino acid residues. The interaction parameters obtained from the re-docking of the reference ligand were subsequently used as a benchmark for evaluating the binding mode and inhibitory potential of the tested ligands against the AChE receptor.

Based on the results of a series of studies, including Lipinski's Rule of Five, ADMETox prediction, and pharmacophore screening, eight compounds identified as hits through pharmacophore screening will proceed to the docking stage as test ligands. The docking process involves a geometry optimization step to obtain the most stable molecular conformation with the lowest energy, as well as to simulate the actual physiological conditions. Next, the test ligands are provided with hydrogen atoms to adjust the docking process, ensuring it aligns with the body's pH environment and allowing the visualization of hydrogen bonds during ligand-receptor interactions. This also aids in guiding the placement of the ligand within the receptor's binding site. The non-polar merge command is applied so that only the polar hydrogen atoms with the receptor. The assignment of Gasteiger charges is used to calculate electrostatic interactions and desolvation energy, ensuring valid and accurate results (Xu, *et al.*, 2017). The ligand's root ligand is identified, and its torsion angles are analyzed to determine the active bonds that can rotate during the docking process. A higher number of active torsions correlates with a longer docking duration. The docking process began with the generation of a grid parameter file, followed by the determination of the grid box center coordinates (x = -43.970, y = 0.409, and z = -80.766), which were defined based on the validated binding site of the co-crystallized reference ligand. The grid box size was set to 40 x 40 x 40 Å to adequately cover the active site region of the AChE receptor. Docking calculations were performed via the Command Prompt using AutoGrid and AutoDock. The docking results for the tested compounds are presented in Table 3.

Table 3. Molecular Docking Results of the Co-crystallized Reference Ligand (Donepezil) and Selected Compounds from *Moringa oleifera* Leaves on AChE (PDB ID: 7E3H) Receptor

No	Compound	Cluster	Binding Energy (kcal/mol)	Ki (μM)	Amino Acid Interaction	Van der Waals Interaction
1	Donepezil co-crystallized reference ligand (re-docking result)	1	-11.14	6.80×10^{-2}	ARG 296	VAL 294 PHE 297 PHE 295 GLY 121 SER 203 TYR 133 ILE 451 GLY 448 TYR 124
2	Quercetin	1	-8.33	778.06	PHE 338 ARG 296 SER 293	-
3	Isoquercetin	1	-9.21	1.765×10^{-1}	PHE 295 ARG 296 GLU 202 GLY 121 TYR 337 SER 125	-
4	Beta-sitosterol	0.00	-12.87	3.70×10^{-10}	TYR 341	ASN 87 SER 125 ASP 74 GLY 126 SER 203 GLY 121 ARG 296 SER 293 PHE 295 GLY 120

In this study, the term amino acid interactions refers to the overall non-covalent contacts formed between the ligand and the amino acid residues within the active site of the AChE receptor. These interactions include hydrogen bonds, van der Waals interactions, and other weak intermolecular forces that collectively contribute to the stability of the ligand-receptor complex. Hydrogen bonds play a key role in determining binding orientation and specificity, whereas van der Waals interactions represent cumulative weak forces arising from close atomic contacts that help stabilize the ligand within the binding pocket. Therefore, the amino acid interaction data presented in Table 3 encompass both hydrogen bonding and van der Waals interactions observed in the docking analysis.

The re-docking process for the test ligands was performed with 100 conformers to obtain the most optimal results. The analysis of the docking results included the binding energy and inhibition constant (Ki) parameters for each ligand with the receptor. Binding energy is the energy required for each ligand to interact with the receptor. A negative binding energy indicates that the ligand can bind directly/spontaneously to the receptor and has good affinity between the receptor and the ligand. All test ligands showed negative binding energy values, indicating good affinity towards the receptor (Pandey & Verma, 2022). The lowest binding energy among the test ligands was observed for beta-sitosterol (-12.87 kcal/mol), which is lower than the value of the co-crystallized reference ligand, -11.14 kcal/mol, suggesting a better affinity between the ligand and the receptor compared to the co-crystallized reference ligand. Quercetin and isoquercetin showed binding energies of -8.33 kcal/mol and -9.21 kcal/mol, respectively, which are good but not better than the co-crystallized reference ligand. The inhibition constant (Ki) of a ligand indicates the concentration required for the ligand to inhibit the receptor. The smaller the Ki value, the stronger the bond between the ligand and the receptor (Pandey and Verma, 2022). The ligand with the smallest Ki among the test ligands was beta-sitosterol ($Ki = 3.7 \times 10^{-10}$ μM), which is even smaller than that of the co-crystallized reference ligand Donepezil ($Ki = 6.6 \times 10^{-2}$ μM), indicating better AChE inhibitory activity than the co-crystallized reference ligand. Thus, beta-sitosterol demonstrated the most favorable docking performance among the tested ligands based on its binding energy and inhibition constant values; however, these findings are limited to in silico predictions and do not directly represent biological efficacy.

Ligand-receptor interactions may involve hydrogen bonds, van der Waals interaction, and other non-covalent forces that collectively stabilize the ligand-receptor complex and contribute to binding specificity and selectivity (Mousavi & Fatemi, 2019). Among the tested ligands, isoquercetin formed the highest number of hydrogen bonds with amino acid residues within the AChE active site, followed by niazirin, moringinin, niazimin, and quercetin. In contrast, kaempferol and beta-sitosterol exhibited a higher number of van der Waals interactions, which are indicative of strong hydrophobic contacts within the binding pocket. A potential drug candidate is characterized by favorable binding affinity, a low inhibition constant, and acceptable safety properties. Based on these criteria, selected test ligands with promising docking performance are visualized in Figure 2.

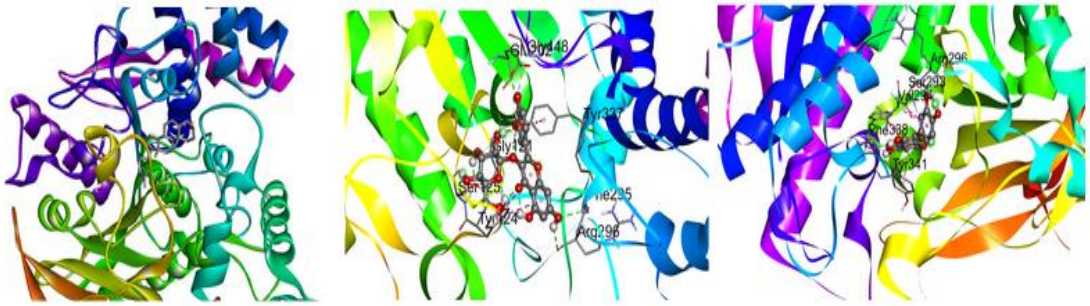

Figure 2. Molecular Docking Visualization of Selected Test Ligands Interacting with the AChE Receptor (PDB ID: 7E3H): (A) Isoquercetin highlighting hydrogen bond interactions, (B) Beta-sitosterol showing dominant van der Waals interactions, and (C) Quercetin illustrating the overall binding orientation within the active site

Based on the results of the testing, there are amino acid interaction between the compounds quercetin and isoquercetin with the AChE enzyme, where hydrogen bonding occurs at ARG 296, similar to the interaction between donepezil and the AChE enzyme. Additionally, the compound beta-sitosterol interacts with the AChE enzyme at the amino acids PHE 295 and GLY 121, which resemble the interaction between donepezil and the AChE enzyme. The similarity in interaction between the compounds and the reference ligand in molecular docking suggests that these compounds have the potential to bind to the same receptor through a similar mechanism. Moreover, based on their binding energy and inhibition constant criteria, all three compounds meet the requirements, and even for beta-sitosterol, the results are better compared to its co-crystallized reference ligand, donepezil. Therefore, these three compounds may exhibit comparable binding potential toward the same receptor based on in silico analysis. In addition to the parameters above, the entire physicochemical profile, ADMETox prediction, and molecular docking of quercetin, isoquercetin, and beta-sitosterol produced favorable and consistent results, indicating that these three compounds have a strong potential to serve as Alzheimer's therapies through AChE enzyme inhibition.

Conclusion

Through a comprehensive series of in silico analyses, including Lipinski's Rule of Five, ADMETox prediction, pharmacophore screening, and molecular docking, three compounds from *Moringa oleifera Lamk* leaves were identified as potential AChE inhibitors for Alzheimer's disease treatment. These compounds (Isoquercetin, Quercetin, and Beta-sitosterol) demonstrated favorable characteristics. The binding energy and inhibition constant (Ki) for Isoquercetin were -9.21 kcal/mol and 0.1765 μM, respectively. Quercetin showed binding energy and Ki values of -8.33 kcal/mol and 778 μM, respectively. Both Isoquercetin and Quercetin formed hydrogen bonds with residue ARG 296. Beta-sitosterol, on the other hand, exhibited a binding energy of -12.87 kcal/mol and a Ki value of 3.7 x 10^{-10} μM, with van der Waals interactions at residues SER 203, GLY 121, PHE 295, and GLY 120. The key contribution of this study lies in the development and application of in silico methodologies to predict the interactions potential of Moringa leaf compounds, specifically quercetin, isoquercetin, and beta-sitosterol, with the AChE enzyme receptor, which may provide novel therapeutic options for Alzheimer's disease. However, the in-silico approach carries theoretical limitations, relying on computational models that might not fully capture the complexity of real biological systems. Consequently, further validation throughand and in vivo experiments is essential to confirm the accuracy of the in silico predictions.

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References

Alzi. 2025. Statistik tentang dementia. Tersedia pada *https://alzi.or.id/statistik-tentang-demensia/*.

Arba, M., Arfan, Trisnawati, A., & Kurniawati, D. 2020. Pemodelan farmakofor untuk identifikasi inhibitor heat shock proteins-90 (HSP-90). *Journal Farmasi Galenika,* 6(2):229-236.

Ayaz, M., Junaid, M., Ullah, F. 2017. Anti-Alzheimer's studies on β-sitosterol isolated from Polygonum hydropiper L. *Front Pharmacol,* 8:697.

Braga, R.C. & Andrade, C.H. 2023. Assessing the performance of 3D pharmacophore models in virtual screening: how good are they?. *Pharmaceuticals*.

Fatimah, D.S., Widiyastuti, S., Oktaviani, D.J., Arnanda, Q.P., Lestari, S., Ramadhan, S.A. 2020. Pharmacophore modeling, virtual screening, and molecular docking metabolite compound from salam leaf extract (Syzigium polyanthum (Wight) Walp.) as antihypertensive compound. *Jurnal Farmasi Galenika*, 7(2):97-107.

Forli, S., Huey, R., Pique, M.E., Sanner, M.F., Goodsell, D.S., & Olson, A.J. 2016. Computational protein-ligand docking and virtual drug screening with the AutoDock suite. *Nat Protoc,* 11(5):905-919.

Guedes, R.A., Aniceto, N., Andrade, M.A.P., Salvador, J.A.R., & Guedes, R.C. 2019. Chemical patterns of proteasome inhibitors: Lessons learned from two decades of drug design. *Int J Mol Sci*, 20(21):5326.

Horn, M. & Neundorf, I. 2018. Design of a novel cell-permeable chimeric peptide to promote wound healing. *Sci Rep*, 8(1):16279.

Hutabarat, R.R. & Nurfadly. 2020. Aktivitas enzim asetilkolinesterase pada larva nyamuk Aedes aegypti di Kecamatan Medan Area. *Jurnal Ilmiah Kohesi*, 4(4):138-143.

Jung, M. & Park, M. 2007. Acetylcholinesterase inhibition by flavonoids from Agrimonia pilosa. *Molecules*, 12(9):2130-2139.

Lee, J.H., Jeong, S.K., Kim, B.C., Park, K.W., & Dash, A. 2015. Donepezil across the spectrum of Alzheimer's disease: dose optimization and clinical relevance. *Acta Neurol Scand*, 131(5):259-267.

Lipinski, C.A. 2004. Lead-and drug-like compounds: the rule-of-five revolution. *Drug Discov Today Technol,* 1(4):337-341.

Mateev, E., Valkova, I., Angelov, B., & Georgieva, M. 2021. Validation through re-docking, cross-docking and ligand enrichment in various well-resoluted MAO-B receptors. *International Journal of Pharmaceutical Sciences and Research,* 13(3):1099.

Mousavi, S.F. & Fatemi, M.H. 2019. Probing the binding mechanism of capecitabine to human serum albumin using spectrometric methods, molecular modeling, and chemometrics approach. *Bioorg Chem*, 90(1):1-12.

Pandey, A.K. & Verma, S. 2022. An in-silico evaluation of dietary components for structural inhibition of SARS-CoV-2 main protease. *J Biomol Struct Dyn,* 40(1):136-142.

Reubun, Y.T.A., Kumala, S., Setyahadi, S., & Simanjuntak, P. 2020. Penghambatan enzim asetilkolinesterase dari ekstrak herba pegagan (Centella asiatica (L.) Urb), ekstrak daun kelor (Moringa oleifera Lam.) dan kombinasinya. *Jurnal Farmasi Indonesia*, 17(2):451-458.

Wang, H., Zhao, Y., Zhang, F., Ke, Z., Han, B., Xiang, J. 2021. Hydrogen-bond donor and acceptor cooperative catalysis strategy for cyclic dehydration of diols to access O-heterocycles. *Sci Adv,* 7(22):396.

Widyasari, A.D., Ramadhan, L.O.A.N., & Dewi, C. 2022. Pemodelan farmakofor dan skrining virtual dari database senyawa bahan alam sebagai inhibitor SARS-CoV-2 RNA-dependent RNA polymerase. *Jurnal Pharmacia Mandala Waluya,* 1(6):247-257.

Wulandari, R.P., Gabriel, K., Nurdin, H.A., Hauna, D., Pakhrul, Harits, S.S. 2023. In silico study of secondary metabolite compounds in parsley (Petroselinum crispum) as a drug therapy for blood cancer (myeloproliferative neoplasm (MPN)) targeting JAK-2. *Indonesian Journal of Chemical Science,* 12(2):212-224.

Xu, X., Yan, C., & Zou, X. 2017. Improving binding mode and binding affinity prediction of docking by ligand-based search of protein conformations: evaluation in D3R grand challenge 2015. *J Comput Aided Mol Des*, 31(8):689-699.

Yuliana, A., Rahmiyani, I., & Kartika, C. 2023. Molecular docking and molecular dynamics simulation using Monascus sp. As a candidate cervical cancer drug. *Journal of Tropical Pharmacy and Chemistry*, 7(1);41-51.