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In Silico Study of Antioxidant Compounds in *Ipomoea aquatica* Forsk as Acetylcholinesterase (AChE) Enzyme Inhibitor in Alzheimer's Disease

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Abstract

Alzheimer's Disease is a form of dementia characterized by a progressive decline in cognitive function, encompassing various aspects of intellectual abilities. Acetylcholinesterase (AChE) is an enzyme that degrades the neurotransmitter acetylcholine and plays an important role in regulating neurotransmission at synapses across the nervous system. Disruption of this cholinergic transmission significantly impacts the development of Alzheimer's disease. Compounds with AChE inhibitory activity are known to be promising drug candidates for treating this disease. In this study, an in-silico analysis was conducted on antioxidant compounds, specifically carotenoid compounds from *Ipomoea aquatica* Forsk, to determine whether these compounds could be potential drug candidates for Alzheimer's treatment using molecular docking methods. The 3D structure of the Acetylcholinesterase (AChE) receptor was obtained from the Protein Data Bank, while the structure of the carotenoid compounds was obtained from the PubChem database. The software used for the molecular docking simulation was AutoDock Vina, with additional supporting software such as AutoDockTools 1.5.6, PyMOL, and LigPlot. The analysis results showed that β-carotene is the best antioxidant, with binding energy and inhibition constant values of -10.2 kcal/mol and 20.06 μM, respectively, with hydrogen bonding occurring at the amino acid residue PHE 295.

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Introduction

Alzheimer's Disease is a form of dementia characterized by a progressive decline in cognitive function, encompassing various aspects of intellectual abilities. Cognitive function, as one of the higher-level activities of the human brain, plays a crucial role in an individual's social and psychological life. The decline in this function can lead to difficulties in carrying out daily activities (Suaka *et al*. 2022). In the neuropathology of Alzheimer's disease, there is a loss of neurons and atrophy in the frontotempora cortex, leading to inflammation, the accumulation of amyloid plaques, and the formation of protein fragments and abnormal tangled fibers (intracellular neurofibrillary tangles). Four main hypotheses are proposed as possible causes of this condition: the tau protein hyperphosphorylation hypothesis, the oxidative stress hypothesis, the metal ion hypothesis, and the cholinergic hypothesis. According to the cholinergic hypothesis, there is a reduction in receptor binding with cholinergic neurons in specific areas of the brain, resulting in decreased acetylcholine-mediated neurotransmission. In healthy adults, low receptor binding levels can slow down the speed of information processing (Thakur *et al*. 2018)

Acetylcholinesterase (AChE) is an enzyme that degrades the neurotransmitter acetylcholine and plays an important role in regulating neurotransmission at synapses across all areas of the nervous system. Disruption of this cholinergic neurotransmission significantly impacts the development of Alzheimer's disease (Az-Zahra *et al*. 2022). Treatment for Alzheimer's typically involves compounds that act as acetylcholinesterase inhibitors, which form the core of Alzheimer's therapy (Santos *et al*. 2018). Therefore, further discovery and development of anti-Alzheimer's that can inhibit the activity of this enzyme are necessary to enhance the effectiveness of the treatment. Several therapies known as acetylcholinesterase inhibitors include galantamine, rivastigmine, and donepezil. Donepezil, in particular, has been used for over 20 years as the basis for symptomatic treatment of Alzheimer's.Based on this information, the selected target protein is the AChE enzyme with the PDB code 4EY7. The AChE enzyme (4EY7) is classified as an enzyme that hydrolyzes carboxylates in ester bonds (EC 3.1.1.7) (Az-Zahra *et al*. 2022).

Ipomoea aquatica Forsk is known to have acetylcholinesterase inhibitor effects. *Ipomoea aquatica* Forsk has relatively high antioxidant activity due to its content of various nutrients and antioxidants, such as carotenoids, terpenoids, phenolics, flavonoids, alkaloids, polyols, organic acids, sterols, and amino acids (Supardi *et al*. 2023). Carotenoids are compounds known to have the highest antioxidant activity compared to other compounds. *Ipomoea aquatica* Forsk contains three types of carotenoids: violaxanthin, lutein, and β-carotene (Shah *et al.* 2021). According to the study by Suaka *et al*. (2022), *Ipomoea aquatica* Forsk extract capable of improving cognitive function in animal models *in vivo*. Therefore, the initial hypothesis in this study is that carotenoid compounds have antioxidant properties that can improve cognitive function in animal models.

This study aims to identify the most effective carotenoid compounds in *Ipomoea aquatica* Forsk that can be used as potential drug candidates for Alzheimer's disease. The carotenoid compounds tested in this study include violaxanthin, lutein, and β-carotene (Shah *et al.* 2021). As a comparative compound, donepezil is used because it is well-established and commonly used in Alzheimer's therapy. Donepezil is also known as the only medication that can be used for Alzheimer's treatment across a wide spectrum, from mild to severe stages (Lee *et al*. 2015).

Method

Materials and Equipment

The device used in this study is an Asus laptop with the following specifications: 64-bit operating system, x64-based processor, 4.00 GB RAM, and Windows 11 operating system. The software used includes Discovery Studio Visualizer 2023, AutoDock Vina, AutoDockTools 1.5.6, PyMOL application, and PyRx software. The 3D structure of the receptor protein was obtained from the Protein Data Bank (PDB) (https://www.rcsb.org), and the 3D structure of the ligand was obtained from PubChem (https://www.pubchem.com).

Receptor Protein and Ligand Preparation

The receptor used is acetylcholinesterase (AChE) with PDB ID 4EY7. The 3D structure of the receptor was downloaded from the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB) website at https://www.rcsb.org/ using the search code. The 3D structure of AChE was downloaded and saved by selecting "PDB Format." The receptor protein was then prepared by removing unnecessary molecules, such as water molecules and any bound ligands, using Discovery Studio Visualizer 2023 software. The structure was saved in PDB format (.pdb*).* Hydrogen atoms were added using Autodock Vina Tools 1.5.6, and the prepared file was saved in PDBQT format (.pdbqt) (Kamble *et al*. 2022). The 3D structures of the carotenoid compounds from *Ipomoea aquatica* Forsk, namely violaxanthin (CID: 448438), lutein (CID: 5281243) and β-carotene (CID: 5280489), as well as the comparative ligand (donepezil), were obtained from

PubChem at https://pubchem.ncbi.nlm.nih.gov/ and saved in SDF format (*.sdf). These structures were then converted into PDB file format using Discovery Studios Visualizer 2023 (Kamble *et al.* 2022).

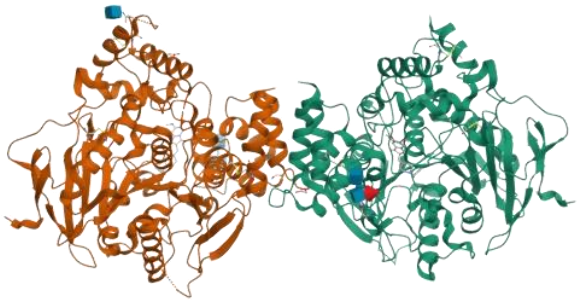
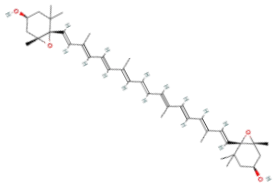


Figure 1. Structure of AChE Enzyme (PDB ID: 4EY7)



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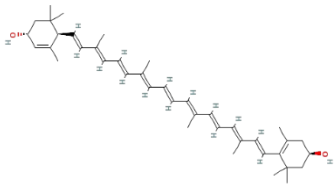

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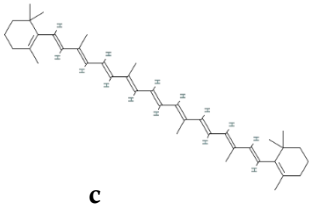


c

Figure 2. Structure of carotenoid compounds in *Ipomoea aquatica* Forsk
(a. violaxanthin, b. lutein, c. β-carotene)

Validation of Molecular Docking Method

The accuracy of the molecular docking method used in this study depends on the precision of ligand positioning and the suitability of the gridbox size that has been defined. To assess the appropriateness of the gridbox to be used in molecular docking, a redocking process was performed between the protein and the native ligand obtained from the PDB using AutoDock Vina. The native ligand from redocking will be compared with the native ligand from the PDB using PyMOL software to calculate the root mean square deviation (RMSD) value (Ummah *et al*. 2024).

Molecular Docking

The analysis of the inhibitory activity of the ligand (carotenoid compounds (violaxanthin, lutein, β-carotene)) against the acetylcholinesterase receptor was conducted using AutoDock Vina software. The results of the molecular docking process were then analyzed with PyMOL software to visualize the position of the ligand in the active site pocket of the acetylcholinesterase protein. The types of interactions occurring between the ligand and the active site of the protein can be analyzed using LigPlot software (Ummah *et al*. 2024).

Results and Discussion

Molecular docking is an *in silico* method used to analyze the interactions between a receptor and a ligand. The purpose of this method is to predict the binding conformation that occurs and the binding affinity formed. This prediction is important as it serves as virtual screening for compounds with potential to be developed into new drugs (Tahir *et al*. 2024). In this study, the target protein used as the receptor is acetylcholinesterase (AChE) and ligands in the form of carotenoid compounds (violaxanthin, lutein, and β-carotene) with antioxidant properties were used. Medicinal plants with remarkable antioxidant and AChE inhibitory properties could therefore offer benefits in the therapy of neurodegenerative diseases. The relationship between antioxidants and acetylcholinesterase (AChE) in Alzheimer's disease is closely linked to the role of oxidative stress in disease progression. Oxidative stress, caused by an imbalance between reactive oxygen species (ROS) and the body's antioxidant defense system, can damage cellular components such as lipids, proteins, and DNA. In Alzheimer's, oxidative stress contributes to neuronal dysfunction, amyloid plaque formation, and neurofibrillary tangles. Increased AChE activity is often observed in Alzheimer's patients, which accelerates acetylcholine degradation and exacerbates cholinergic neurotransmission deficits. Antioxidants can help reduce oxidative stress and protect neurons from further damage. Additionally, some antioxidant compounds have shown potential as AChE inhibitors, thereby not only reducing ROS but also enhancing acetylcholine availability, which is crucial for cognitive function. Therefore, antioxidants can play a dual role in Alzheimer's therapy, both as neuroprotective agents and as modulators of AChE activity (Adetuyi *et al*. 2024).

The selection of the receptor with this code was based on the fact that the enzyme is non-mutagenic, and the crystal structure of the receptor contains a natural ligand, Donepezil, which is known to be a drug for Alzheimer's disease. This allows the location of the active pocket and the amino acids involved in the interaction between the ligand and the receptor to be more easily identified (Az-Zahra *et al*. 2022). The crystal structure of the receptor with code 4EY7 has a resolution of 2.35 Å. The stability of the receptor's crystal structure is determined based on its resolution, a smaller resolution value indicates that the crystal structure in the PDB has high-resolution quality because the atomic arrangement of the structure closely approximates the actual state. A resolution is considered stable if it is less than 2.5 Å (Ferdian *et al*. 2021).

The receptor structure downloaded from the PDB is typically bound to ligands, solvents, and other non-standard residues. This structure is then separated from the non-standard residues, such as removing water molecules, as they may interfere with the docking process, and checked for the possibility of missing residues. The separation process was performed using PyMOL software, and no missing residues were found in the protein's amino acid chain, confirming that the receptor structure is suitable for use (Tahir *et al*. 2024).

The molecular docking validation process in this study was performed using AutoDock Vina through a redocking process between the native ligand compound (donepezil) and the acetylcholinesterase (AChE) protein. This test was also used to determine the suitability of the grid box size used during the molecular docking process. Based on the validation results, it was observed that the redocked structure of donepezil overlapped with the original donepezil structure, with an RMSD value of 0.3109 Å. This result indicates that the grid box size used in this study is appropriate, and the molecular docking method applied has been proven to be valid (Ummah *et al*. 2024).

The analysis of the binding site or active site of the receptor was performed using Discovery Studio software. Since the AChE receptor in the PDB is bound to the natural ligand donepezil, the active site is already known. Therefore, the grid box for the docking process was adjusted according to the position of the natural ligand from the PDB. Visualization using Discovery Studio revealed the coordinates of the AChE receptor's active site as X = -13.988, Y = -43.906, and Z = 27.108. Donepezil, as the natural ligand of AChE commonly used in Alzheimer's treatment, was used as a reference ligand against the test ligand, which is a carotenoid compound in *Ipomoea aquatica* Forsk (violaxanthin, lutein, β-carotene).

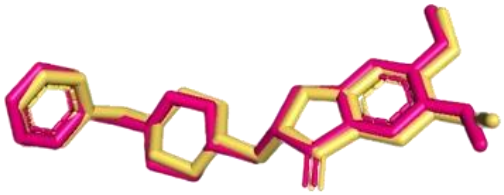


Figure 3. Visualization of the molecular docking method validation results (yellow: initial native ligand, pink: redocked native ligand).

Molecular docking analysis is performed for in silico screening of compounds using the PyRx software, which is an efficient tool in terms of time for conducting in silico screening, with high accuracy

and prediction of binding modes (Santoso 2019). This analysis is based on the binding affinity data or Gibbs free energy (ΔG), obtained after the docking simulation is completed. The binding affinity indicates the strength of the bond formed from the interaction between the ligand and the receptor and serves as a parameter for the stability of the conformation between the ligand and the receptor. The more negative the value, the greater the potential of the ligand to form a strong bond with the receptor (Lu *et al*. 2024). Ligand and receptor interactions tend to be in the lowest energy condition, which leads to a stable molecular state, meaning that the smaller the docking score between the ligand and receptor, the more stable the interaction (Manalu *et al.* 2021). The inhibition constant (IC) analysis is also performed to determine the inhibitory power of a compound against its receptor. A smaller Ki value indicates a stronger inhibition by the compound. A smaller ΔG value from molecular docking results also leads to a smaller Ki value. Both parameters can serve as references in identifying the best compound (Kalontong *et al.* 2022).

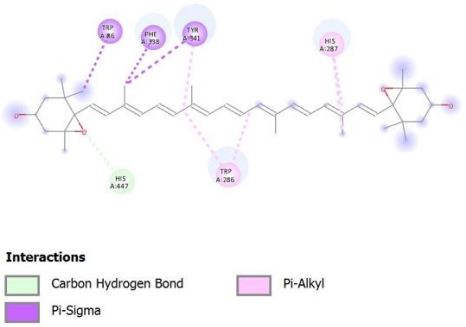
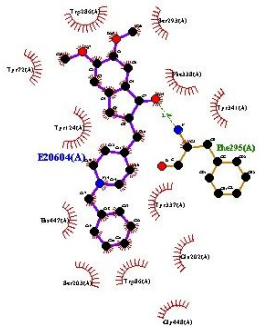
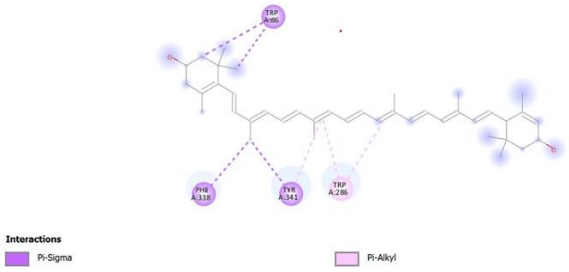
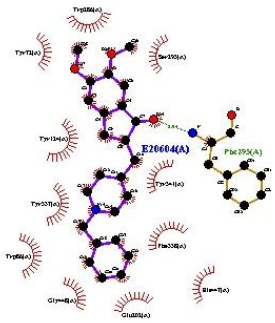
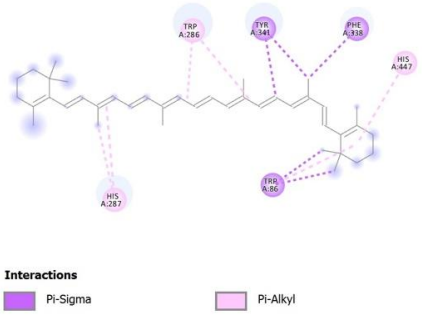
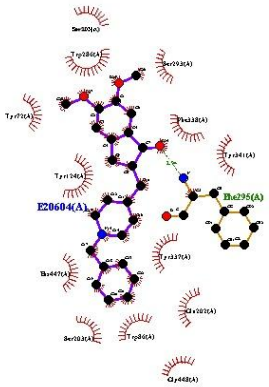
Table 1. Molecular docking results of carotenoid compounds from *Ipomoea aquatica* Forsk against the AChE receptor.

Ligand	IC (μM)	Binding Affinity ΔG (kkal/mol)	Hydrogen Bond	Hydrophobic Bond
Donepezil (Native Ligand)	1.99	-12.1	PHE295 (2.94)	TRP286 (3.82) TYR341 (5.06)
Violaxanthin	117	-10.0	HIS447 (3.03)	TRP86 (3.37) PHE338 (3.73) TYR341(3.79) HIS287 (4.70) TRP286 (4.22)
Lutein	206	-9.6	-	TRP86 (3.47) PHE338 (3.83) TYR341 (3.82) TRP286 (4.12)
β-carotene	20.6	-10.2	-	HIS287 (4.79) TRP286 (3.99) TYR341 (3.69) PHE338 (3.73) HIS447 (5.26) TRP86 (3.64)

Based on the molecular docking results in Table 1, three carotenoid compounds tested have binding energies that are similar between one compound and another, ranging from -9.6 to -10.2 kcal/mol. The binding energy of these carotenoid compounds is close to the binding energy of donepezil, which has been used as an Alzheimer's drug. This suggests that the complex formed between the carotenoid compounds and AChE can be considered stable. The inhibitory strength against the AChE receptor is also determined by the interactions that occur between the ligand and the active site of AChE. Interactions such as hydrogen bonds and hydrophobic interactions can strengthen the complex formed between the ligand and AChE (Ummah *et al.* 2024)

The compound with the lowest ΔG and Ki values, besides the natural ligand, is β-carotene, with a ΔG value of -10.2 and Ki of 20.6 μM. However, the hydrogen bond amino acid residues between the ligand and receptor of the natural ligand (PHE 295) do not match those of β-carotene. The same type of bond as in the natural ligand is found in β-sitosterol only in the pi interactions (TRP 286, TYR 341). According to the literature, sub-sites for amino acid residues such as TRP 286, TYR 337, PHE 338, and TYR 341 can cause interactions between the ligand and receptor through hydrophobic bonding. It is thus known that the compound piperine has the best potential as a candidate drug for treating Alzheimer's through the inhibition of the enzyme acetylcholinesterase (Az-Zahra *et al*. 2022).

Table 2. Molecular docking visualization results of carotenoid compounds

Carotenoid Compounds	Visualization	Interaction of carotenoid compounds with the active site of AChE
Violaxanthin		
Lutein		
β-carotene		

Conclusion

Based on the molecular docking results, it can be concluded that the three antioxidant compounds in the form of carotenoids contained in *Ipomoea aquatica* forsk (violaxanthin, lutein, β-carotene) have potential as inhibitors of the acetylcholinesterase (AChE) enzyme, with the best binding energy and inhibition constant values of -10.2 kcal/mol and 20.06 μM, respectively, with hydrogen bonding occurring at the amino acid residue PHE 295.

References

Adetuyi AR, Ayenero ME, Olaleye MT, Akindahunsi AA, Akinmoladun AC. 2024. Antioxidant and acetylcholinesterase inhibitory activities, in silico analyses, and anti-Alzheimer's disease potential of leaf extracts of three nigerian endemic medicinal plants (*Spondias mombin*, *Carica papaya* and *Kalanchoe crenata*). *Future Journal of Pharmaceutical Science*. 10(6): 1-17

Az-Zahra F, Afidika J, Diamantha SDA, Rahmani AE, Fatimah S, Aulifa DL, Elaine AA, Sitinjak BDP. 2022. Studi in silico senyawa dalam daun sirih (*Piper betle* L.) Sebagai inhibitor enzim asetilkolinesterase (AChE) pada penyakit alzheimer. *Indonesian Journal of Biological Pharmacy*. 2(2): 44-58

Ferdian PR, Elfirta RR, Ikhwani AZN, Kasirah K, Haerul H, Sutardi D, Ruhiat G. 2021. Studi in silico senyawa fenolik madu sebagai kandidat inhibitor Mpro SARS-CoV-2. *Media Penelitian dan Pengembangan Kesehatan*. 31(3): 213–232

Kamble RP, Ghosh P, Kulkarni AA. 2022. Identification of α-amylase inhibitory compounds from leaves of Careya arborea Roxb. and in silico docking studies. *South African Journal of Botany*. 00:1–11.

Lee JH, Jeong SK, Kim BC, Park KW, Dash. 2018. Donepezil across the spectrum of Alzheimer's disease: dose optimization and clinical relevance. *Acta Neurol Scand*. 131(5): 259-267

Lu W, Zhang J, Huang W, Zhang Z, Jia X, Wang Z, Shi L,Li C, Wolynes PG, Zheng S. 2024. DynamicBind: predicting ligand-specific protein-ligand complex structure with a deep equivariant generative model. *Nature Communications*. 15(1071): 1-13

Manalu, R.T., Safitri, H., Sari, H.P., Devina, R., Irnawati, I. & Liliwana, E.A., 2021, Analisis in-silico penghambatan main protease (Mpro) pada SARS-CoV-2 oleh senyawa aktif teh hijau (*Camelia sinensis*). *Jurnal Farmagazine*. 8(2): 1-7

Santos TCD, Gomes TM, Pinto BAS, Camara AL, Paes AMDA. 2018. Naturally occurring acetylcholinesterase inhibitors and their potential use for alzheimer's disease therapy. 9(01192): 1-14

Santoso B. 2019. In silico study of selected molecules of sea cucumber as antimitotic using pyrx-vina program. *Indonesian Journal of Pharmaceutical Science and Technology*. 1(2): 33-38

Shah P, Awasthi H, Kunwar K, Kalauni SK. 2021. Phytochemical analysis and antioxidant activity of ipomoea aquatica from ghodaghodi wet land area, nepal. *International Journal of Herbal Medicine*. 9(2): 23-27

Suaka NI, Nugraha SEP, Pratiwi RA, Nurafifah R, Darmawan E. 2022. Modulasi neurotransmitter pada gangguan kognitif hewan coba dengan nanoemulsi ekstrak *Ipomoea aquatica* Forsk. *Jurnal Surya Medika*. 7(2): 159-165

Supardi IW, Rupiasih NN, Putra IK. 2023. Application of hydroponic plants watering automations based on atmega328 microcontroller on water spinach (*Pomea Aquatic* Forsk). *International Journal of Physics & Mathematics*. 6(1): 34-38

Tahir M, Maryam St. 2024. Studi in silico senyawa aktif dari jahe (*Zingiber officinale*) dan kersen (*Muntingia calabura* L.) Sebagai inhibitor main protease (Mpro) dari SARS-CoV-2. *As-Syifaa Jurnal Farmasi*. 16(1): 35-44

Thakur AK, Kamboj P, Goswami K, Ahuja K. 2018. Pathophysiology and management of alzheimer's disease: an overview. *Journal of Analytical & Pharmaceutical Research*. 9(2): 226-235

Ummah K, Jariyah IA, Rumengan SM. 2024. Uji in silico senyawa flavonoid kulit buah delima sebagai antikanker payudara. *Jurnal Kimia (Journal of Chemistry).* 18(2): 195-200