IN-SILICO SCREENING FOR POTENTIAL NATURAL COMPOUNDS AGAINST ACETYLCHOLINESTERASE, CAUSING ALZHEIMER DISEASE

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ABSTRACT

Alzheimer's disease (AD) is a neurodegenerative disorder marked by cognitive decline, memory issues, and behavioral changes. It involves the buildup of amyloid-beta plaques and neurofibrillary tangles in the brain, leading to synaptic dysfunction and neuron loss. Acetylcholinesterase, a key enzyme in AD, regulates acetylcholine levels important for cognitive function. However, there is a growing interest in exploring natural products as potential inhibitors against acetylcholinesterase. In this study, we aimed to explore natural products as potential inhibitors against acetylcholinesterase, a key enzyme implicated in Alzheimer's disease progression. 16 plant-derived compounds were selected and subjected to molecular docking simulations using the YASARA tool. Additionally, the YASARA docking protocol was validated by super positioning between the deposited crystal structure of Acetylcholinesterase in complex with the inhibitor, which was obtained from PDB and the simulated docked Acetylcholinesterase with the same inhibitor. Among the 20 natural compounds docked, zeaxanthin exhibited the highest binding energy of 14.70 kcal/mol with acetylcholinesterase, forming 3 hydrogen bonds, 23 hydrophobic interactions, and 7 π-π stacking interactions. The molecular docking protocol was successfully validated as the superposition value of root mean squared deviation (RMSD) was 0.330 Å which is below 2.0 Å. Therefore, zeaxanthin shows the best potential candidate for inhibiting Acetylcholinesterase.

Keywords: Alzheimer’s disease, Acetylcholinesterase, Phytochemicals, Molecular docking, YASARA.

INTRODUCTION

Alzheimer’s disease is a progressive neurodegenerative disorder characterized by memory loss, cognitive decline, and behavioural changes (Sosa-Ortiz et al., 2012). It is the most common cause of dementia in the elderly population and poses a significant burden on the healthcare system worldwide (Alzheimer's Association, 2021). One of the key enzymes involved in the progress of Alzheimer's disease is cholinesterase, which plays a critical role in the breakdown of acetylcholine, a neurotransmitter essential for memory and cognitive function (Lleó et al., 2006). The relationship between acetylcholinesterase and Alzheimer's disease is significant because the decreased availability of acetylcholine contributes to the cognitive decline and memory impairment characteristic of the disease. Inhibitors of acetylcholinesterase, such as donepezil, rivastigmine, and galantamine are synthetic drugs commonly used as a treatment for Alzheimer's disease. These medications work by boosting acetylcholine levels in the body through the inhibition of its breakdown, which leads to some improvement in cognitive symptoms. However, these cholinesterase inhibitors are associated with several adverse effects, including nausea, vomiting, diarrhoea, and bradycardia, which can limit their use (Hossein, et al., 2023). Apart from that, the inhibition mechanism between the functional group of these compounds towards the active residues of acetylcholinesterase is still unclear. Apart from that, some of these synthetic drugs have consistently shown only modest benefits on cognition after consumed for long term (Zhang et al., 2022).

Therefore, this study aims to address these critical issues by investigating the potential of the natural products as inhibitors towards acetylcholinesterase via molecular docking analysis. Hence, this study shall contribute to the development of the natural product discovery against Alzheimer’s disease.

METHODOLOGY

Molecular docking simulations

YASARA, a bioinformatic tool that implements AutoDock program, was used to conduct these molecular docking simulations. This docking process was performed using a pre-made script that had been downloaded from the link, dock_run.mcr. Initially, the 3D structure of the acetylcholinesterase protein (PDB Id: 4EY4) was downloaded in PDB format from the Protein Data Bank database (https://www.rcsb.org/) (Tallei et al., 2020). Meanwhile, for the phytochemicals compounds, their 3D structures were downloaded from Pubchem database (https://pubchem.ncbi.nlm.nih.gov/) (Tallei et al., 2020). According to Son et al. (2019), the most likely active site of acetylcholinesterase is located from amino acids 202 to 447. Hence, the ligand structures were positioned within the active site range to focus docking with the selected 20 phytochemicals: chamazulene, eucalyptol, menthol, menthone, aloin, anthocyanins, valerenic acid, linalyl acetate, citral, zeaxanthin, leptodactylone, tetrandrine, chrysophanol, physcion, celastrol, tingenone, iguesterin, hirsutenone, wogonin, and cepharanthine. These phytochemicals with the prepared receptor, acetylcholinesterase, were then docked together. The binding energy and the molecular interactions of each ligand were observed and tabulated.

RESULTS AND DISCUSSION

Molecular docking analysis

Twenty phytochemicals as listed above, as well as the target receptor, acetylcholinesterase, were used for the docking studies. The binding affinities and molecular interactions between each ligand and the receptor for the 10 best-fitted phytochemicals were investigated in detail and enumerated in Table 1.

Table 1: Results of docking between acetylcholinesterase and the 10 best-fitted phytochemicals

Comp. No.	Phytochemicals	Binding Energy (kcal/mol)	Contacting Receptor Residues	No. of Hydrogen Bond	No. of Hydrophobic Interaction	No. of π-π Stacking
1	Iguesterin	10.63	**TRP A 286** HIS A 287 LEU A 289 GLN A 291 GLU A 292 SER A 293 VAL A 294 **PHE A 295** ARG A 296 PHE A 297 **TYR A 337** PHE A 338	-	11	5
2	Physcion,	10.08	**TRP A 86** ASN A 87 PRO A 88 **GLY A 120 TYR A 133 GLU A 202 SER A 203 TYR A 337** TYR A 341 **HIS A 447** GLY A 448	2	14	4
3	Wogonin	10.64	**TRP A 86** ASN A 87 PRO A 88 **GLY A 120 GLU A 202 SER A 203 TYR A 337** TYR A 341 **HIS A 447** GLY A 448 TYR A 449 ILE A 451**ASN A 487** CYS A 488	2	7	1
4	Cepharanthine	12.07	**TRP A 286** HIS A 287 **PHE A 295** ARG A 296	-	13	3
5	Celastrol	12.44	**TRP A 286** HIS A 287 VAL A 294 **PHE A 295**	3	9	4
6	Hirsutenone	12.52	**TRP A 86** TYR A 119 **GLY A 120 TYR A 133 GLU A 202 SER A 203 TRP A 286** SER A 293 VAL A 294 **PHE A 295** ARG A 296 PHE A **297 TYR A 337** PHE A 338 TYR A 341 **HIS A 447**	4	13	7
7	Tetrandine	11.47	TYR A 124 **TRP A 286** VAL A 294 **PHE A 295**	-	13	4
8	Tingenone	11.07	**VAL A 288** LEU A 289 4 **PHE A 295** ARG A 296 PHE A 297 **TYR A 337** PHE A 338	-	9	4
9	Aloin	13.05	**TRP A 286** LEU A 289 **PHE A 295** ARG A 296 PHE A 297 **TYR A 337**	3	7	6
10	Zeaxanthin	14.7	**TRP A 86** TRP A 117 TYR A 119 **GLY A 120 TYR A 133 GLU A 202 SER A 203** GLN A 250 LEU A 254 **TRP A 286 PHE A 295** PHE A 297 **TYR A 337** PHE A 338 TYR A 341 GLY A 342 **HIS A 447**	3	23	7

Note: Contacting receptor residues in bold are the active site residues of acetylcholinesterase.

From the results in Table 1, iguesterin, physcion, hirsutenone, tetrandrine, tingenone, aloin, and zeaxanthin showed remarkable binding affinity with acetylcholinesterase, which ranged between 10.63 kcal/mol to 14.70 kcal/mol. Moreover, the intermolecular hydrogen bonding results in a stronger complex formation and more precise docking outcomes. The strong geometric limitation brought about by the presence of hydrogen bonds also helps to improve the ligand's displacement (Wu et al., 2012). Based on the research done by Asamitsu et al. (2000), Harel et al. (1993) and Rhodes & Crabbe (2005), Trp86, Tyr133, Gly121, Glu202, Ser203 and His287 were identified as the major amino acid involved in the protein-ligand bindings which could also be seen from Table 1. Moreover, all 10 phytochemicals showed hydrophobic interactions with the acetylcholinesterase. Furthermore, referring to Table 1, all 10 phytochemicals have π-π stacking with the aromatic ring of acetylcholinesterase.

Best-fitted phytochemical

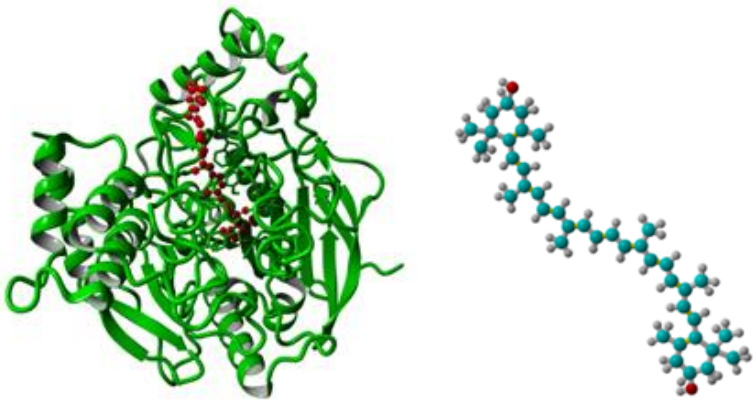
Zeaxanthin, the best-fitting compound among the 10 compounds, was chosen for further analyses based on its high binding energy, the presence of more than one hydrogen bond, hydrophobic interaction and π-π stacking with acetylcholinesterase.

Binding energy

Figure 1 shows the docking of zeaxanthin towards the acetylcholinesterase. Zeaxanthin obtained a 14.70 kcal/mol docking score against acetylcholinesterase, which is considered a high binding affinity compared to the other phytochemicals. YASARA, used in this study has a built-in protocol for calculating binding energies (Derek et al., 2015). The following equation is used to compute the binding energy that results:

Binding Energy = EpotRecept + EsolvRecept + EpotLigand + EsolvLigand – EpotComplex – EsolvComplex

Energy in the YASARA binding energy function was calculated as the difference between the total potential and solvation energies of the separated compounds as well as the total potential and solvation energies of the complex. Hence, a more favourable binding in the context of the selected force field is indicated by the higher positive binding energies obtained in the results (Aamir et al., 2018). Based on the in-silico research carried out by Mohamed et al. (2021), YASARA was used to screen for the potential reactivator against malathion-inhibited human AChE. High binding energy, which was 6.45 kcal/mol, formed between the docking of 4-hydroxybenzohydrazide and malathion-inhibited human AChE showed a stable and strong receptor-ligand interaction. In short, high binding energy values have been demonstrated in the literature to indicate a well-fitted protein and ligand complex structure.



(A) (B)

Fig. 1. (A) Docking of zeaxanthin towards acetylcholinesterase. Green colour represents acetylcholinesterase while red colour denotes zeaxanthin; (B) 3D compound structure of zeaxanthin. Light blue colour represents carbon, red colour signify oxygen, and grey colour indicates hydrogen.

CONCLUSION

In this study, the potential inhibitors were screened based on their binding affinity, molecular interactions, and the distance of the nucleophilic attack of the phytochemicals towards the acetylcholinesterase by using the YASARA. The hydrogen bond, hydrophobic interaction, van der Walls interactions, and the conformational entropy of the ligand were each identified with a distinctive physicochemical contribution to the binding free energy. Thus, zeaxanthin, which obtained a high binding energy value and consists of multiple molecular interactions with the residues of acetylcholinesterase, can fit well and bind more tightly to the active site of this protein. In short, this study has narrowed down the searching for a new potential phytochemical, zeaxanthin which could be used as a lead compound for developing acetylcholinesterase inhibitors. In vitro and in vivo studies are needed to further confirm the inhibition mechanism of zeaxanthin towards acetylcholinesterase.

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