International Journal of Life Sciences and Biotechnology
Bektas, I. and S. Akmese, Molecular Docking Studies of Selected Polyphenols Against Acetylcholinesterase as Potential Anti-Alzheimer Agents. International Journal of Life Sciences and Biotechnology, 2026 9(1): p. 279-289.
https://doi.org/10.38001/ijlsb.1841666
Araştırma Makale/ Research article

Molecular Docking and ADMET Analysis of Selected Polyphenols Targeting Acetylcholinesterase: An In Silico Approach

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ABSTRACT
Alzheimer's disease (AD) is a multifactorial, progressive neurodegenerative disorder, primarily caused by disorders in the cholinergic system. Inhibition of the acetylcholinesterase (AChE) enzyme forms the basis of current symptomatic treatment. The aim of this study is to investigate the potential inhibitory effects of several different polyphenols, whose biological activities are known in the literature, on AChE using *in silico* methods. In this study, molecular docking simulations were performed using the crystal structure of the AChE enzyme (PDB code: 4EY7) and the structures of selected natural compounds and the reference drug donepezil. The binding affinities (kcal/mol) and binding modes of the compounds to the enzyme's active site were determined using AutoDock Vina software. In addition, *in silico* ADMET (Absorption, Distribution, Metabolism, Excretion, Toxicity) analyses were performed using ADMETlab 3.0 to predict the drug similarity, pharmacokinetic, and toxicological profiles of these compounds. Molecular docking analyses revealed that the reference drug donepezil showed the highest binding affinity (-11.9 kcal/mol), followed by naturally occurring compounds such as rosmarinic acid (-10.5 kcal/mol) and apigenin (-10.4 kcal/mol). Structural analyses showed that all compounds with strong affinity, like donepezil, exhibited dual-site inhibitory behavior, interacting simultaneously with both the catalytic active site and the peripheral anionic site of AChE. ADMET analyses demonstrated that all compounds studied conformed to Lipinski's rule and possessed generally acceptable pharmacokinetic and toxicity profiles. The results indicate that the polyphenols studied, particularly rosmarinic acid and apigenin, are promising AChE inhibitor candidates in the treatment of AD, exhibiting high affinity and dual-site binding potential. These findings form the basis for *in vitro* and *in vivo* validation studies for the development of natural compounds.

ARTICLE HISTORY
Received
13 December 2025
Accepted
08 Januarv 2026

KEY WORDS
Alzheimer's Disease,
Acetylcholinesterase,
Molecular Docking,
ADMET,
Polyphenols

Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disorder among the elderly worldwide, accounting for a large proportion of dementia cases[1, 2]. The disease is characterized by memory loss, impaired cognitive function, and behavioral changes[3]. AD accounts for approximately 60–80% of known dementia subtypes. Furthermore, around 50 million people worldwide live with dementia, and this number is projected to triple by 2050 due to the aging population.[3, 4].

The pathogenesis of AD is a progressive and irreversible neurodegenerative process shaped by the complex interaction of numerous molecular and cellular mechanisms. At the heart of the disease lies the accumulation of amyloid-beta (Aβ) plaques and neurofibrillary tangles (NFTs) composed of hyperphosphorylated tau protein in the brain[5]. The formation of Aβ proteins as a result of the abnormal proteolytic degradation of amyloid precursor protein (APP) and their extracellular accumulation in the brain parenchyma leads to impaired synaptic function and increased local oxidative stress. This causes disruptions in synaptic communication and neuronal damage, while also triggering chronic inflammatory responses[1]. Hyperphosphorylation of tau protein leads to destabilization of microtubules and disruption of intracellular structural integrity, accelerating neuronal dysfunction[5, 6].

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Mitochondrial dysfunction plays a significant role in the pathogenesis of the disease. Impairments in energy production within mitochondria and increased reactive oxygen species (ROS) production enhance cellular damage due to oxidative stress. This process damages DNA, proteins, and lipids, ultimately leading to neuronal death. Furthermore, chronic neuroinflammation is characterized by the overactivation of microglia and astrocytes; these glial cells release inflammatory cytokines, further impairing synaptic function and promoting disease progression[4, 7].

Disruptions in the cholinergic system, particularly decreased acetylcholine (ACh) levels and irregularities in acetylcholinesterase (AChE) activity, lead to significant functional impairment in learning and memory processes. This cholinergic deficiency plays a central role in the development of the clinical symptoms of AD[8]. Generally, cholinergic neurons in the hippocampus and cerebral cortex are damaged; this reduces ACh synthesis capacity and leads to significant impairment of cognitive function[7]. A decrease in choline acetyltransferase (ChAT) activity, responsible for ACh synthesis, coupled with the preservation or increase of AChE and butyrylcholinesterase (BChE) activities, which degrade ACh, creates an additional deficiency in synaptic transmission[9]. Because neurons have a very limited capacity for self-renewal, treatment has shifted towards preserving existing ACh rather than restoring lost ACh production. Accordingly, AChE inhibitors, which temporarily enhance cholinergic transmission by preventing ACh degradation in the synaptic cleft, are used as the primary pharmacological approach in current symptomatic treatment[10]. Existing AChE inhibitors such as donepezil, rivastigmine, and galantamine are used in clinical practice; however, these drugs have various side effects including nausea, hepatotoxicity, and gastrointestinal disturbances. This situation necessitates the discovery of newer, safer, and more effective AChE inhibitors[11].

Naturally derived compounds and medicinal plants constitute a rich source for the discovery of new drug candidates. In particular, phytochemicals obtained from plants are attracting attention as potential therapeutic agents against neurodegenerative diseases due to their wide structural diversity and low toxicity profiles[12].

Polyphenols offer an advantage in AD as they are natural compounds that can target multiple pathogenic mechanisms, whereas traditional drugs only alleviate symptoms[13]. In AD treatment, polyphenols modulate the main pathological markers of the disease by inhibiting Aβ production, preventing oligomerization and remodeling toxic structures, and enhancing Aβ clearance. They also regulate Tau protein hyperphosphorylation through kinase inhibition and phosphatase activation[14]. With its antioxidant and anti-inflammatory effects, it reduces oxidative stress and neuroinflammation, while increasing the expression of neurotrophic factors, thus supporting cognitive function and synaptic plasticity[13, 15]. In conclusion, polyphenols offer a potential therapeutic approach by comprehensively targeting the complex pathophysiology of AD.

Currently, Computer-Aided Drug Design (CADD) methods are widely used in drug discovery processes to save time and cost. Molecular docking, one of the leading methods, is a powerful tool for predicting how small molecule ligands (polyphenols) bind to the active site of the target protein (AChE) and their binding affinity[16].

The aim of this study is to investigate the inhibitory effects of several different polyphenols, known in the literature for their various biological activities, on the AChE enzyme, a potential target in the treatment of AD, using *in silico* methods. Using molecular docking simulations, the interactions and binding energies of these compounds with the enzyme's active site were analyzed and compared with a reference drug.

Material and Methods

Preparation of protein and ligand structures

The three-dimensional crystal structure of the AChE enzyme was downloaded from the Protein Data Bank (PDB) (https://www.rcsb.org), and the structure coded 4EY7 was used in this study. Protein preparation was performed using AutoDock Tools 1.5.7 software; the protein chains were separated, and only the A chain was processed. Water molecules, donepezil, and all other cofactors/metabolites on the A chain were removed; missing atoms were completed, polar hydrogen atoms were added, and Kollman coupled charges were assigned. The prepared protein structure was saved in PDBQT format for use in molecular docking analyses.

The chemical structures of the seven ligands and the reference drug (RD) donepezil were obtained from the PubChem database in SDF format (Table 1). The creation of the three-dimensional conformations of the ligands, format transformations, and determination of the appropriate protonation states were performed using Open Babel GUI and AutoDock Tools 1.5.7 software. Subsequently, geometry optimization for each ligand was performed using the UCSF Chimera 1.17.3 program, and the optimized structures were saved in PDBQT format for docking studies.

Molecular docking protocol

Molecular docking simulations were performed using AutoDock Vina software to evaluate the possible binding modes and binding affinities of ligands with the AChE enzyme. The exhaustiveness parameter was set to 8 to ensure computational accuracy and efficiency. Ten binding positions were generated for each ligand and ranked based on their binding affinities (kcal/mol).

A grid box was created for docking studies; its center coordinates were set as x = –14.108464, y = –43.832714, and z = 27.669929; and the spacing was set to 0.5 Å. A 50 × 50 × 50 grid box was used for the molecular docking process. The binding positions obtained from the simulations were analyzed using Discovery Studio Visualizer software. In the analyses, hydrogen bonds, hydrophobic interactions, π–π stacking, π–cation interactions, and other complementary ligand-protein interaction types were evaluated in detail[17].

Absorption, distribution, metabolism, excretion, toxicity (ADMET) estimates

ADMETlab 3.0, an *in silico* prediction tool developed to characterize the pharmacokinetic and toxicological properties of a candidate molecule in drug discovery and development processes, was used in our study[18]. SMILES (Simplified Molecular Input Line Entry System) codes, which are text-based line encodings of compounds, were obtained from the PubChem database and used as the input format to the ADMETlab 3.0 web server in the analyses.

Drug similarity assessment was estimated based on a concept previously established by Lipinski et al. (MW≤500; logP≤5; H-bond acceptor≤10; H-bond donor≤5)[19]. Pharmacokinetic and toxicological profiles were predicted using ADMETlab 3.0. Through this platform, absorption parameters (HIA, Caco-2 permeability), distribution characteristics (blood-brain barrier crossing (BBB), plasma protein binding rate), potential metabolic interactions (interaction with CYP450 isoforms), and excretion trends (LogS and LogP values) were evaluated. In toxicity analysis, AMES mutagenicity, hERG channel inhibition risk, and hepatotoxicity indicators were predicted. Thus, the drug similarity, pharmacokinetic behavior, and safety profile of the compounds were predicted holistically.

Results

Molecular docking results

When molecular docking scores were examined (Table 1), donepezil showed the highest binding affinity with a score of -11.9 kcal/mol; followed by rosmarinic acid (-10.5), apigenin (-10.4), chrysin (-9.7), genistein (-9.6), quercetin (-9.4), tangeretin (-9.0), syringic acid (-6.6), respectively.

Table 1 Docking Scores and PubChem ID Information for Compounds

Compound	Ligand Short Name	PubChem ID	Docking Score
Donepezil	RD	3152	–11.9
Rosmarinic acid	L1	5281792	–10.5
Apigenin	L2	5280443	–10.4
Chrysin	L3	5281607	–9.7
Genistein	L4	5280961	–9.6
Quercetin	L5	5280343	–9.4
Tangeretin	L6	68077	–9.0
Syringic acid	L7	10742	–6.6

Molecular docking analyses revealed various hydrogen bonds, electrostatic interactions, and hydrophobic contacts between the AChE enzyme and the reference drug donepezil and the selected natural compounds (Table 2). Donepezil formed a conventional hydrogen bond with Tyr124 in its binding site (Figure 1A); a π-cation interaction with Trp337; and multiple π-π stacking bonds with aromatic residues such as Trp86 and Tyr341. It has been determined that it forms π-σ, π-π stacking and π-alkyl interactions with Trp286. In addition, it has been shown to form a π-alkyl interaction with Tyr72 and an alkyl interaction with Leu289 (Figure 1B and 1C).

Table 2 Details of the interaction between AChE and ligands

Ligand	Category	Types	Residues (Distance Å)
Donepezil	Hydrogen Bond	Conventional	Tyr124 (3.02)
	Electrostatic	Π-Cation	Trp337 (4.89)
	Hydrophobic	Π- σ	Trp286 (3.68)
		Π-Π Stacked	Trp86 (3.75), Trp286 (4.12), Tyr341 (5.28)
		Alkyl	Leu289 (5.10)
		Π-Alkyl	Tyr72 (5.14), Trp286 (4.09)
Rosmarinic Acid	Hydrogen Bond	Conventional	Tyr124 (3.11), Ser203 (2.87), Tyr341 (2.98), Arg296 (2.77), Glu202 (2.01), Ser203 (2.55), Tyr124 (2.78)
	Hydrophobic	Π-Π T-shaped	Trp86 (5.34), Tyr337 (5.88), His447 (4.60)
Apigenin	Hydrogen Bond	Conventional	Gly122 (2.91), Tyr341 (2.77), Ser203 (2.35)
		Π-Donor	Tyr124 (3.50)
	Hydrophobic	Π- σ	Tyr341 (3.83)
		Π-Π Stacked	Trp286 (4.89), Tyr341 (4.48)
		Π-Π T-shaped	Tyr341 (5.21)
Genistein	Hydrogen Bond	Conventional	Tyr124 (2.94)
		Carbon Hydrogen Bond	His447 (3.63)
		Π-Donor	Tyr124 (3.91)
	Hydrophobic	Π-Π Stacked	Trp286 (5.11), Trp286 (3.95), Tyr341 (4.42), Tyr341 (5.17)
		Π-Π T-shaped	Tyr337 (5.71), Tyr341 (5.25)
Quercetin	Hydrogen Bond	Conventional	Phe295 (2.94)
		Carbon Hydrogen Bond	Val294 (3.25)
		Π-Donor	Tyr124 (3.61)
	Hydrophobic	Π-Π Stacked	Trp286 (4.48), Trp286 (3.69), Tyr341 (4.45), Tyr341 (5.45)
		Π-Π T-shaped	Tyr341 (4.83)
Syringic Acid	Hydrogen Bond	Conventional	Tyr124 (3.18), Ser125 (3.07)
		Carbon Hydrogen Bond	Trp86 (3.68)
	Hydrophobic	Π- σ	Tyr337 (3.68)
		Π-Π Stacked	Trp86 (4.33)
Tangeretin	Hydrogen Bond	Conventional	Phe295 (2.93)
		Carbon Hydrogen Bond	Tyr124 (3.13), Arg296 (3.52), Tyr72 (3.45), Tyr341 (3.56)
		Π-Donor	Tyr341 (4.13)
	Hydrophobic	Π- σ	Trp86 (3.94)
		Π-Π Stacked	Trp286 (5.29), Trp286 (5.56), Tyr337 (4.76), Tyr341 (4.64), Tyr341 (4.82)
		Π-Alkyl	Tyr337 (4.37)
Chrysin	Hydrogen Bond	Conventional	Tyr124 (3.27), Phe295 (3.22)
	Hydrophobic	Π-Π Stacked	Trp286 (5.26), Trp286 (3.85), Phe338 (4.20), Tyr341 (3.99), Tyr341 (4.70)
		Π-Π T-shaped	Tyr337 (5.26), Tyr341 (5.29)

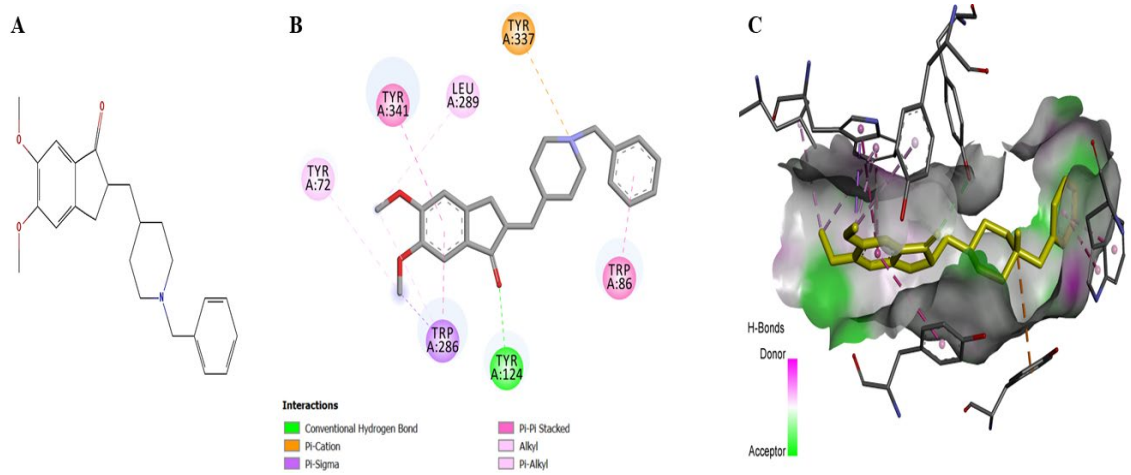

Fig. 1 The structure of donepezil and its interaction with the active site of AChE; A: 2D structure, B: Two-dimensional view of the interaction between the enzyme's amino acids and the ligand, C: H-bond interactions

The naturally occurring compound rosmarinic acid (Figure 2A) exhibited intense hydrogen bonding interactions in its binding site; in particular, multiple conventional hydrogen bonds were identified with Tyr124, Ser203, Tyr341, Arg296, Glu202. This compound also showed π-π T-shaped hydrophobic interactions with Trp86, Tyr337, His447 (Figure 2B and 2C).

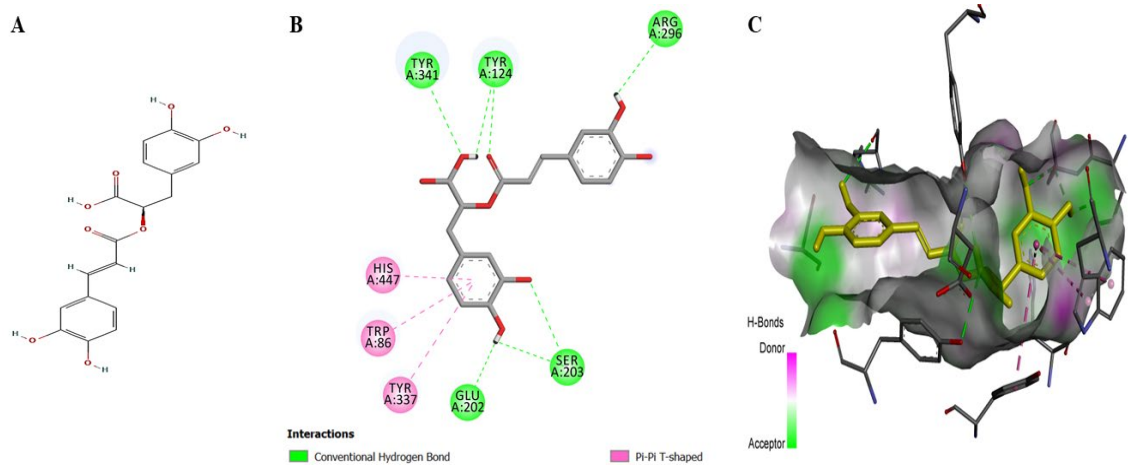

Fig. 2 The structure of rosmarinic acid and its interaction with the active site of AChE; A: 2D structure, B: Two-dimensional view of the interaction between the enzyme's amino acids and the ligand, C: H-bond interactions

The apigenin compound (Figure 3A) formed conventional hydrogen bonds with Gly122, Tyr341, Ser203; and a π-donor hydrogen bond with Tyr124. Hydrophobic interactions occurred with Tyr341 and Trp286 residues in the form of π-σ and π-π stacking-based contacts (Figure 3B and 3C).

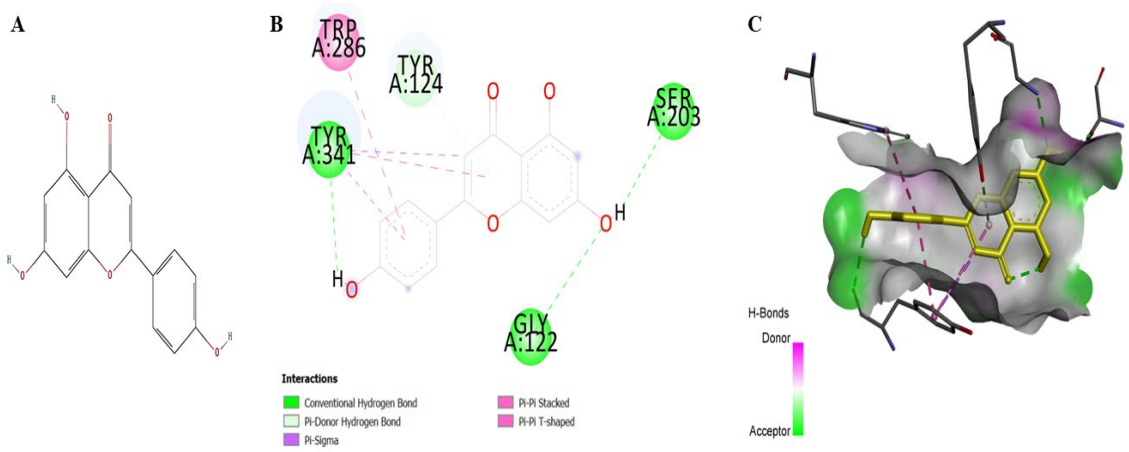

Fig. 3 The structure of apigenin and its interaction with the active site of AChE; A: 2D structure, B: Two-dimensional view of the interaction between the enzyme's amino acids and the ligand, C: H-bond interactions

For genistein (Figure 4A), hydrogen bonds were identified with Tyr124, as well as carbon-hydrogen bonds with His447; multiple π-π stacking and T-shaped hydrophobic interactions were recorded with aromatic residues such as Trp286 and Tyr341. A π-π T-shaped interaction was also observed with Tyr337 (Figure 4B and 4C).

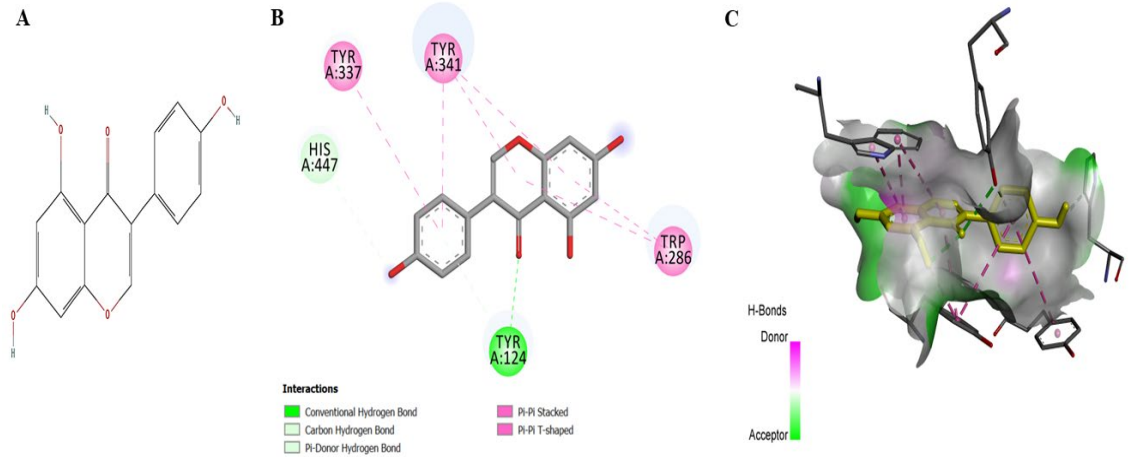

Fig. 4 The structure of genistein and its interaction with the active site of AChE; A: 2D structure, B: Two-dimensional view of the interaction between the enzyme's amino acids and the ligand, C: H-bond interactions

Quercetin (Figure 5A) formed conventional hydrogen bonds with Phe295 and carbon-hydrogen bonds with Val294; it also developed π-donor type hydrogen bonds with Tyr124. Furthermore, multiple π-π stacking and T-shaped interactions were observed with Trp286 and Tyr341 (Figure 5B and 5C).

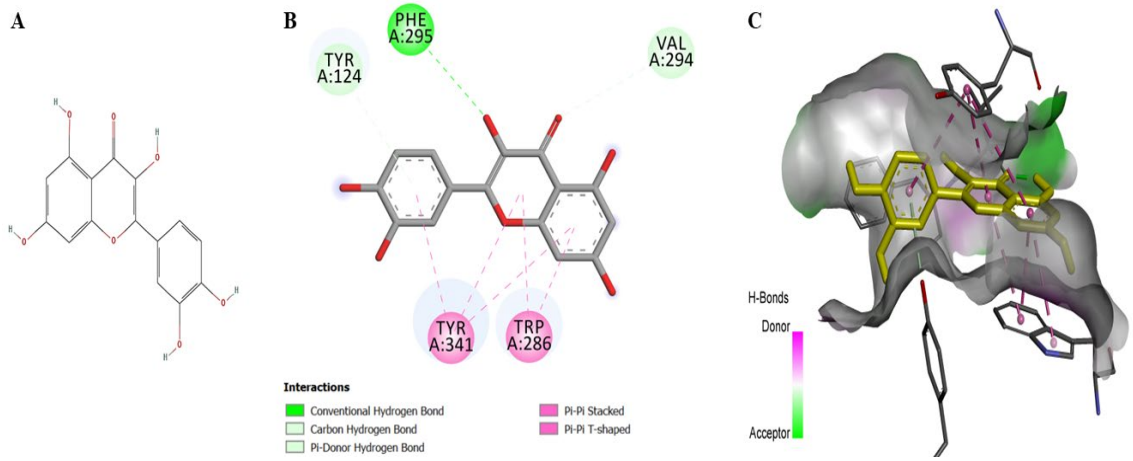

Fig. 5 The structure of quercetin and its interaction with the active site of AChE; A: 2D structure, B: Two-dimensional view of the interaction between the enzyme's amino acids and the ligand, C: H-bond interactions

It was determined that syringic acid (Figure 6A) forms conventional hydrogen bonds with Tyr124 and Ser125; and carbon-hydrogen bonds with Trp86. Hydrophobic interactions were recorded as π-σ stacking with Tyr337 and π-π stacking with Trp86 (Figure 6B and 6C).

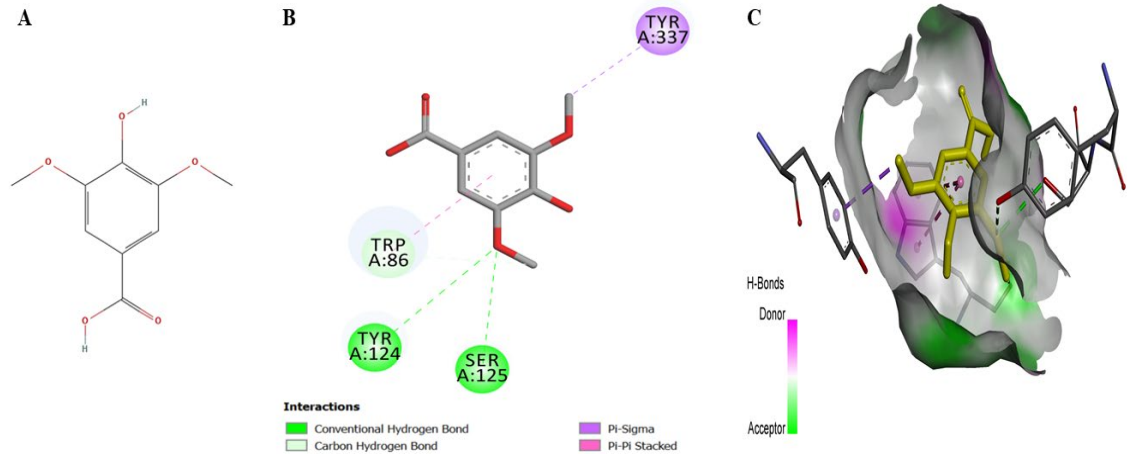

Fig. 6 The structure of syringic acid and its interaction with the active site of AChE; A: 2D structure, B: Two-dimensional view of the interaction between the enzyme's amino acids and the ligand, C: H-bond interactions

Tangeretin (Figure 7A) formed conventional hydrogen bonds with Phe295, carbon-hydrogen bonds with Tyr124, Arg296, Tyr72; and carbon-hydrogen bonds, π-donor hydrogen bonds and π-π stacking interactions with Tyr341. Furthermore, intense hydrophobic π-σ, π-π stacking and π-alkyl interactions were observed with Trp86, Trp286, Tyr337 residues (Figure 7B and 7C).

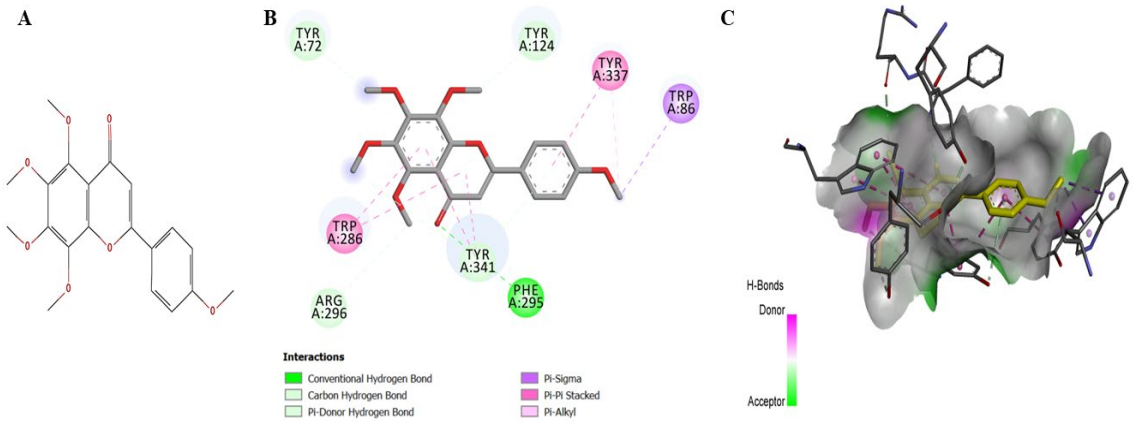

Fig. 7 The structure of tangeretin and its interaction with the active site of AChE; A: 2D structure, B: Two-dimensional view of the interaction between the enzyme's amino acids and the ligand, C: H-bond interactions

Chrysin (Figure 8A) bonded to Tyr124 and Phe295 by forming conventional hydrogen bonds; it also showed multiple π-π stacking interactions with residues Trp286, Phe338, Tyr341. Furthermore, it exhibited π-π T-shaped interactions with Tyr337 and Tyr341 (Figure 8B and 8C).

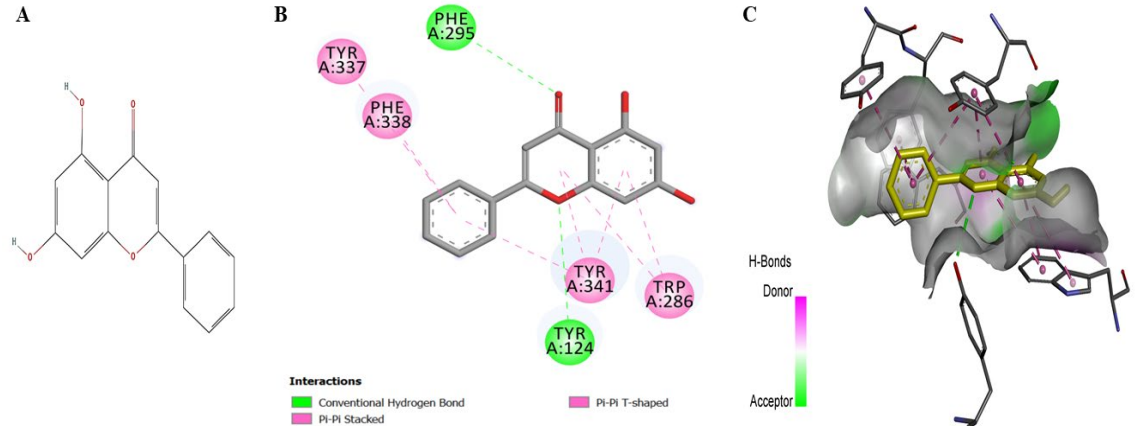

Fig. 8 The structure of chrysin and its interaction with the active site of AChE; A: 2D structure, B: Two-dimensional view of the interaction between the enzyme's amino acids and the ligand, C: H-bond interactions

ADMET results

Pharmacokinetic and toxicological properties of the compounds evaluated in the study were obtained by ADMET analyses (Table 3). The data obtained show that all ligands conform to the Lipinski's rule (Lipinski violation: 0.0). Regarding absorption parameters, HIA probabilities were calculated as low for apigenin, chrysin, donepezil, genistein, and tangeretin; and relatively higher for quercetin, rosmarinic acid, and syringic acid. Caco-2 permeability values were in the negative log cm/s range for all compounds, and were determined as follows: apigenin (-5.129), chrysin (-4.98), donepezil (-4.863), genistein (-5.051), quercetin (-6.177), rosmarinic acid (-6.513), syringic acid (-5.199), tangeretin (-4.5).

Distribution data show that BBB crossing probabilities were calculated as low for apigenin, genistein, and rosmarinic acid, moderate for chrysin, donepezil, syringic acid, and tangeretin, and 0.0 for quercetin. Plasma protein binding rates were reported as apigenin (96.535%), chrysin (97.753%), donepezil (91.953%), genistein (95.865%), quercetin (98.66%), rosmarinic acid (77.277%), syringic acid (76.526%), tangeretin (93.367%).

Metabolic parameters showed variability in interactions with CYP enzymes among ligands. Apigenin, chrysin, genistein, quercetin, and tangeretin exhibited high inhibitory or substrate probabilities for various CYP isoenzymes, while rosmarinic acid and syringic acid showed mostly low levels of these values.

When the LogS and LogP values related to excretion were evaluated, it was determined that the water solubility values of the compounds ranged from -2.166 to -6.513 and the lipophilicity values ranged from 1.209 to 3.6.

When toxicity parameters were examined, it was observed that AMES probabilities ranged from 0.246 to 0.628, hERG inhibition probabilities reached their highest value for donepezil at 0.937, and remained at lower levels for other ligands. Hepatotoxicity probabilities were calculated as apigenin (0.435), chrysin

(0.467), donepezil (0.763), genistein (0.461), quercetin (0.337), rosmarinic acid (0.699), syringic acid (0.484), tangeretin (0.428).

Table 3 The results of the ADMET test with AdmetLab3.0 (I: Inhibitor, S: Substrate)

Ligands	RD	L1	L2	L3	L4	L5	L6	L7
Lipinski's rule	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
HIA	0.001	0.025	0.002	0.002	0.008	0.134	0.049	0.04
Caco-2 (Log cm/s)	-4.863	-6.513	-5.129	-4.98	-5.051	-6.177	-4.5	-5.199
BBB	0.353	0.0	0.013	0.277	0.031	0.0	0.193	0.216
PPB (%)	91.95	77.28	96.54	97.75	95.87	98.66	93.37	76.53
AMES	0.246	0.549	0.618	0.628	0.556	0.586	0.418	0.322
hERG	0.937	0.056	0.1	0.105	0.128	0.053	0.207	0.035
Hepatotoxicity	0.763	0.699	0.435	0.467	0.461	0.337	0.428	0.484
LogS	-3.866	-3.033	-4.216	-4.886	-3.471	-3.722	-4.135	-2.166
LogP	3.245	2.005	2.981	3.6	2.075	1.448	2.446	1.209
CYP1A2 (I)	0.0	0.0	1.0	1.0	0.999	0.998	0.739	0.0
CYP1A2 (S)	0.994	0.0	0.47	0.904	0.995	0.384	0.485	0.02
CYP2C19 (I)	0.0	0.0	0.112	0.312	0.86	0.006	0.527	0.011
CYP2C19 (S)	1.0	0.0	0.0	0.0	0.0	0.0	0.127	0.041
CYP2C9 (I)	0.0	0.0	0.002	0.004	0.309	0.432	0.821	0.004
CYP2C9 (S)	0.272	0.218	0.673	0.921	0.867	0.03	0.201	0.107
CYP2D6 (I)	0.933	0.0	0.957	0.973	0.997	0.0	0.024	0.008
CYP2D6 (S)	1.0	0.0	1.0	0.998	1.0	0.978	0.99	0.001
CYP3A4 (I)	0.175	0.0	1.0	0.995	0.992	0.937	0.887	0.006
CYP3A4 (S)	0.999	0.0	0.0	0.011	0.0	0.0	0.981	0.001
CYP2B6 (I)	0.0	0.106	0.112	0.614	0.968	0.001	0.895	0.0
CYP2B6 (S)	0.001	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CYP2C8 (I)	0.23	0.235	0.99	0.985	1.0	1.0	1.0	0.079

Discussion

AChE is an enzyme that plays a critical role in terminating synaptic signal transmission by accelerating the hydrolysis of the ACh neurotransmitter. The enzyme's catalytic activity site is a narrow, deep region extending from the surface to the active site, called the active site gate. The gate is approximately 20 Å long and 5 Å wide[20]. This narrow and deep throat region is divided into two main binding sites: the Peripheral Anionic Site (PAS) at its entrance and the Catalytic Active Site (CAS) deep within, which is vital for catalysis. The enzymatic mechanism and substrate/inhibitor binding of AChE are based on highly conserved aromatic amino acid residues surrounding this throat. This binding is facilitated by the amino acid residues Trp86, Tyr133, Tyr337, Phe338[21]. In addition, Ser203, His447, Glu334, Trp86, Tyr337 residues also play a role in the CAS region[22]. In the PAS region of AChE, Tyr72, Tyr124, Trp286, Tyr341 residues facilitate substrate recognition and orientation towards the active site gate, establish electrostatic and hydrogen bonds that facilitate substrate pre-orientation, and mediate π-π interactions with ligands[22].

One of the most important symptomatic strategies in the treatment of AD is AChE inhibition. Recent studies have shown that AChE inhibitors not only regulate cholinergic signaling but also modulate Aβ aggregation via the PAS domain[23]. Therefore, inhibitors developed for AD treatment focus on dual-site strategies, aiming to both raise ACh levels and slow the formation of neurotoxic Aβ plaques by binding not only to CAS but also to PAS[22, 23].

Findings from molecular docking analyses showed a high degree of agreement with AChE structural features and binding sites described in the literature. The majority of interactions observed in the docking data showed a distribution consistent with these residues.

The reference inhibitor donepezil exhibited a typical dual-site inhibitor profile capable of interacting simultaneously with the PAS and CAS regions, particularly establishing a strong localization to the CAS region via π-cation interaction with Trp337 and π-π stacking interactions with Trp86. Simultaneously, its π-alkyl and aromatic interactions with PAS residues such as Tyr124, Tyr72, Trp286 explain donepezil's stable localization along the throat and its high binding score. This is consistent with the dual-site inhibitor design strategies targeted in AD treatment, and donepezil's -11.9 docking score is a structural indicator of this bidirectional binding behavior.

When the binding profiles of natural compounds were examined, it was determined that rosmarinic acid showed the closest affinities to donepezil by forming numerous hydrogen bonds in both the PAS and CAS regions. In particular, its bindings to residues bridging the two regions, such as Tyr124, Ser203, Arg296, are consistent with the positioning principle of inhibitors along the pathway described in the literature.

In flavonoid compounds such as apigenin, genistein, quercetin, and chrysin, intense π-π stacking and T-shaped aromatic interactions were observed with residues such as Trp286, Trp86, Tyr337, Tyr341, consistent with the aromatically rich throat architecture of AChE. This suggests that flavonoids can influence the substrate's entry pathway by forming strong interactions in the PAS region, and parallels reports in the literature related to the modulation of PAS-mediated Aβ aggregation.

Tangeretin, syringic acid, and other phenolic compounds, despite more limited hydrogen bonding interactions, exhibited binding stability due to π-σ and π-alkyl contacts with aromatic residues specific to the PAS region. The ranking was donepezil > rosmarinic acid > apigenin > chrysin > genistein > quercetin > tangeretin > syringic acid, consistent with the aromatic stacking density, hydrogen bond diversity, and interaction continuity between PAS and CAS of the ligands. In conclusion, comparisons with donepezil confirm the accuracy of the binding modes, while the unique interaction profiles exhibited by rosmarinic acid and flavonoids along the aromatic throat highlight the innovative contribution of this study to the field. In this respect, the study advances existing knowledge by providing a new structural and pharmacokinetic perspective on the design of AChE-targeted dual-domain inhibitors.

The obtained ADMET data show that compounds exhibiting strong binding tendencies in docking analyses show a distribution consistent with their pharmacokinetic profiles. The conformity of all ligands to Lipinski rules supports the idea that these molecules possess drug-like properties. Absorption and distribution parameters indicate a favorable bioavailability potential for molecules that can bind to the PAS and CAS regions of AChE, particularly compounds such as quercetin, rosmarinic acid, and syringic acid, with higher HIA and moderate BBB translocation. The concentration of CYP enzyme interactions in flavonoids suggests that their aromatic structures play an active role in metabolic processes; LogP and LogS values support the excretion tendencies of ligands. Toxicity profiles are generally acceptable, and hepatotoxicity and hERG data provide a safety margin that does not contradict the docking scores.

Conclusion

Molecular docking and ADMET analyses conducted within the scope of this study comprehensively revealed the interaction potential of naturally occurring compounds with the characteristic active site architecture of AChE, consisting of PAS and CAS regions. The results show that the vast majority of the compounds examined exhibit contact motifs compatible with the aromatic-rich binding gate of AChE and possess high-affinity binding ability to the enzyme through both hydrogen bonds and aromatic stacking interactions. In particular, the dual-site binding behavior of compounds extending between the PAS and CAS regions similar to donepezil, supports the idea that these molecules are potential inhibitor candidates that can be evaluated in AD treatment. The ADMET results, showing that all ligands have drug-like properties, exhibit acceptable pharmacokinetic profiles, and have recommendable toxicity parameters, strengthen the docking findings from a pharmacological perspective. Overall, this study demonstrates the structural suitability of natural compounds for AChE inhibition and provides important data that will form the basis for future validation studies at *in vitro* and *in vivo* levels.

Scientific Responsibility Declaration

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Ethics Approval and Consent

This study does not require ethical approval.

Conflict of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author Contributions

All stages of this study were carried out by the author.

Financial Support/Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

Data Availability Declaration

The datasets used and/or analyzed during the present study are available from the relevant author upon reasonable request.

Declaration of Generator AI and AI-Powered Technologies in the Writing Process

The authors used Grammarly and ChatGPT to improve the language and readability of the text. The authors reviewed and edited the content and are fully responsible for its accuracy. No data was generated or modified using AI tools.

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