

# Investigating flavonoids derived from *Tribulus terrestris* L. as prospective candidates for Alzheimer's disease treatment: Molecular docking modeling of their interactions with physiological system receptors

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**Abstract.** Alzheimer's disease (AD) stands as the primary cause of dementia, marked by its neurodegenerative essence, which results in cognitive impairment associated with memory and a decline in functionality. Currently there is no drug which can permanently cure the nervous lesions as well as completely eradicate this pathogenesis. The aim of this research is to examine the acetylcholinesterase activity of flavonoids identified in *Tribulus terrestris* L. (Tt) by predicting ligand-receptor binding. The research process begins with the preparation of protein and ligand structures. Subsequently, docking is performed, interaction between protein-ligands is then analyzed and visualized. Four phytoconstituents of Tt were chosen, and molecular docking simulations revealed that all four compounds exhibited good binding affinities. Based on the predicted ADMET values using the Lipinski rule, compounds with potentially good activity were identified. The results suggest that these compounds may exhibit anti-acetylcholinesterase activity.

**Keywords:** AChE, ADME prediction, In-silico analysis, Kaempferol, Physiology-binding receptor

## 1. Introduction

Current treatments for Alzheimer's disease (AD) suffer from drawbacks such as serious adverse effects, prolonged duration of action, and patient dissatisfaction. Hence, finding effective drug candidates is crucial. Herbal medicines, derived from natural sources, contain a diverse range of phytoconstituents with various pharmacological actions. The discovery of new drugs from remedies used in traditional medicine represents an appealing approach that is continuously advancing. Previous research has directed attention towards developing therapies from natural products that could help prevent and manage neurodegeneration seen in Alzheimer's disease (Wang et al., 2022). For instance, phytochemical compounds like curcumin and resveratrol have demonstrated neuroprotective activities against key features of Alzheimer's disease, including beta-amyloid accumulation, oxidative stress, and neuroinflammation. Some of these compounds have shown high efficacy accompanied by low toxicity (Kim et al., 2010). Therefore, employing plants in the quest for new Alzheimer's disease treatments is a suitable strategy (Goozee, 2016; Yan, 2015). Tt, used since ancient times in Indian and Chinese medicine, offers potential for managing various diseases. Tt, commonly referred to as Gokshur or Gokharu, belongs to the Zygophyllaceae family. The genus *Tribulus* encompasses approximately 25 species (Chartare et al., 2014). Recently, Tt has garnered increasing interest due to its diverse pharmacological potential, including anti-cancer (Bouabdallah et al., 2016; Patel et al., 2019), antileishmanial (Bouabdallah et al., 2018), antifungal (Zhang et al., 2006), antibacterial (Al bayath et al., 2008), and neuroprotective activities (Bouabdallah et al., 2024).

Phytochemical analysis of Tt has revealed its abundance in a wide range of structurally diverse compounds, including steroidal saponins, flavonoids, tannins, glycosides, phytosterols, and terpenoids (Khalid et al., 2021; Zhao et al., 2023; Bouabdallah et al., 2024).

Steroidal saponins (Bouabdallah et al., 2023) and flavonoids (Stefanescu et al., 2020) are the predominant metabolites that confer pharmacological activities to Tt. Over a hundred steroidal saponins have been identified from Tt, including protodioscin, diosgenin, dioscine, gracillin, and trillin (Zhu et al., 2017). Recently, it has been demonstrated that these steroidal saponins possess neuroprotective properties against Alzheimer's disease (Guan et al., 2022; Yang et al., 2020; Cai et al., 2020). Bouabdallah et al. investigated the neuroprotective effects of an ethanolic leaf extract in a zebrafish (*Danio rerio*) model of scopolamine-induced memory impairment and brain oxidative stress. They demonstrated that treatment with Tt extract reversed the changes in locomotion patterns induced by scopolamine administration. Biochemical analyses further confirmed *Tribulus*'s ability to inhibit AChE, enhance antioxidant enzyme activities, and reduce oxidative stress markers (Bouabdallah et al., 2024).

Computer-Aided Drug Design (CADD) provides a valuable approach for identifying and optimizing leads, offering cost-effectiveness and time efficiency in drug discovery (Rao et al., 2011). Structure-based drug design (SBDD), ligand-based drug design (LBDD), and sequence-based approaches represent common computational methods used in drug discovery (Ou-Yang et al., 2012). Molecular docking analysis employing computational techniques stands as one of the fundamental and crucial strategies in drug discovery (Silakari and Sign, 2021).

Docking studies aid in predicting the most probable type of interaction between proteins and ligands, determining binding affinities, and providing insights into the orientations of docked ligands at the active site of the target protein (Pintilie and Stefaniu, 2019). The objective of this investigation is to explore the interactions of the ethanolic extract of Tt, which has been previously analyzed via UPLC-ESI/MS by Bouabdallah et al., for its in-silico anti-acetylcholinesterase activity. Our study focuses on assessing the interactions between the four flavonoids of Tt and AChE in-silico using the ADME model, aiming to determine the minimum binding energy (kcal/mol) between them.

## 2. Methods

### 2.1. Software and hardware

We utilized various resources and tools for our research, including the PubChem database, ADMET lab 2.0, and Swiss ADME for compound analysis and properties assessment (Kar and Leszczynski, 2020). Additionally, we employed the RCSB Protein Data Bank for protein structure data. For molecular docking studies, we utilized PyRx Auto Dock Vina 0.8 (Dallakyan and Olson, 2015), and for protein visualization and analysis, we utilized BIOVIA Discovery Studio.

### 2.2. In silico docking studies

#### 2.2.1. Protein structures

As a first step, the crystal structure of acetylcholinesterase (PDB ID: 2WHP) was selected for the study. The 3D structure was downloaded from Protein Data Bank (PDB) (Beg and Athar, 2020) and prepared using ADT server (Williams et al., Citation 2018). The Preparation involved adding hydrogens to it, applying selected flips to residues, analyzing all-atom contacts, and geometry, running the job, and downloading the final protein file.

#### 2.2.2. Ligand structures

The 3D Structure of phytoconstituents of the *Tribulus* were downloaded from PubChem. In the final step, molecular docking simulation was performed using the Autodock-4.2.6 program. The grid box dimensions, in grid settings, were 60x60x60 in x, y, and z directions. Grid points spacing was settled at 0.375 Å in each case. Then, Auto grid-4.2 was used for generating map files. For setting docking parameters, A genetic algorithm (GA) was used for search parameters. The number of GA runs was 50, the number of evaluations was 2500000 and the population size was 150. All docked complexes were analysed and visualized using BIOVIA (Opo et al., 2021).

### 2.3. Drug-likeness and ADMET profiling

ADME/Tox evaluation is an important process for selecting a good drug candidate (Li et al., 2001). The drug-likeness and toxicity of any compound can be evaluated using web servers, then, some properties of the molecule should be calculated based on its structure. In this study, we used two web servers: The Swiss ADME server (<http://www.swissadme.ch/index.php>) (Daina et al., 2017) (accessed on 15 January 2023) and the ADMET SAR 2.0 server <http://lmmd.ecust.edu.cn/admet-sar2>) (Yang et al., 2019) (accessed on 15 January 2023). We obtained The SMILES codes of the studied compounds from the PubChem Database (Kim et al., 2021).

## 2.4. Chemoinformatic

Rutin, quercetin, kaempferol and luteolin, were retrieved from PubChem Compound Database (Bolton et al., 2008) and were tested.

## 3. Result and discussion

### 3.1. Phytochemical analysis of *Tribulus terrestris* extract

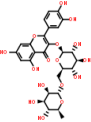
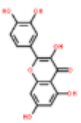
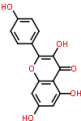
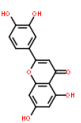
Ethanol leaf extract of Tt was analysed by UPLC-LC-ESI/MS as described by (Bouabdallah et al., 2024). The chromatogram analysis depicted the presence of multiple compounds within the sample, as indicated by Bouabdallah et al. The Tt extract underwent UPLC-PDA analysis to ascertain its chemical composition. The findings unveiled the existence of diverse flavonoids and saponins, notably including rutin, quercetin, kaempferol, luteolin, cynaroside, trillin, hecogenin, terreside B, trillarin, protodioscin, and saponin C.

### 3.2. Docking analysis

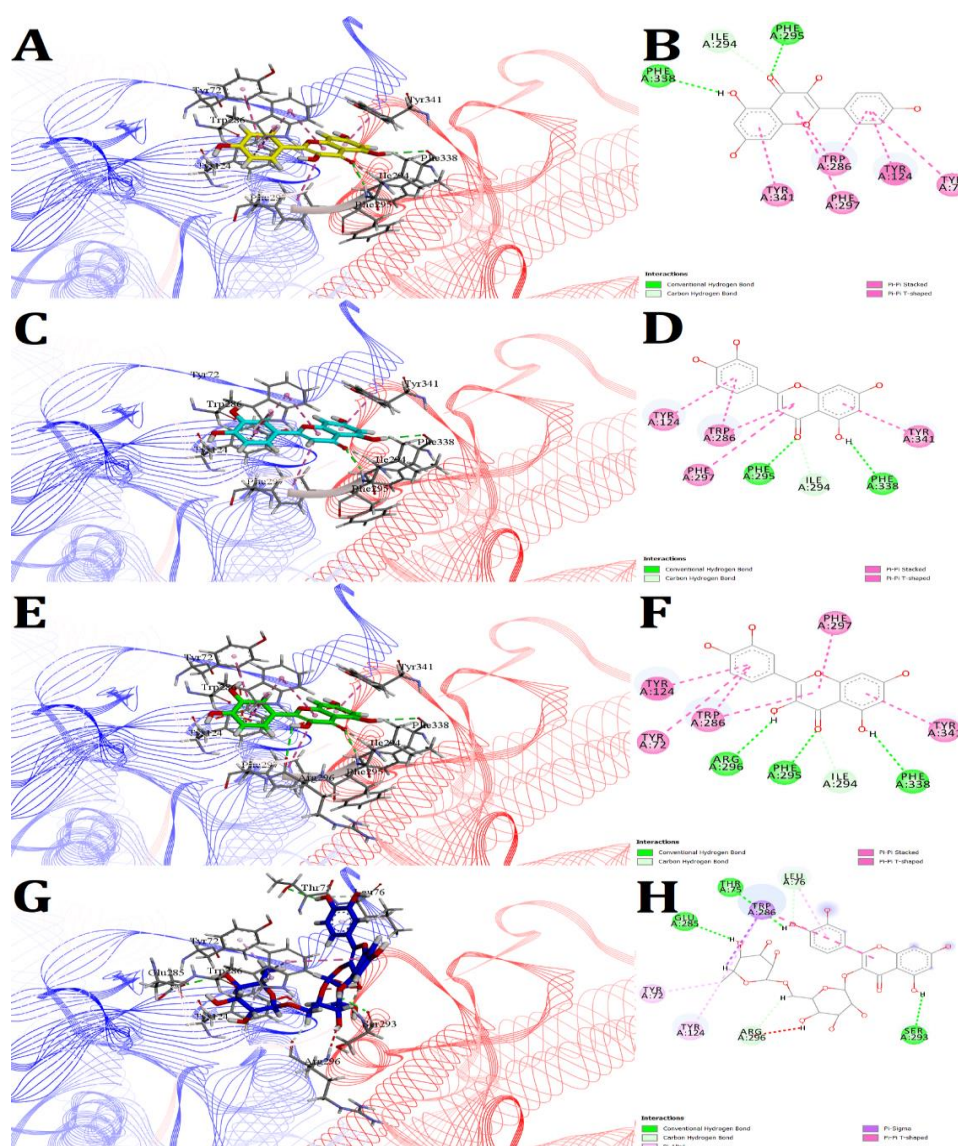
The molecular docking analysis of the four selected natural compounds (rutin, quercetin, kaempferol and luteolin) from the Tt plant was conducted using the flexible or blind docking method. PyRx virtual screening tool (Auto Dock Vina) was employed to perform molecular docking studies on the chosen protein. The kaempferol, luteolin, quercetin and rutin with AChE revealed a negative value for binding energies. The details of docking energies are given in Table 1. Docking results indicated a high affinity for the active site and had high negative binding energies were rutin, quercetin, luteolin, and kaempferol. The H-bond interaction with hydrophobic residues is presented in Figure 1, which contain 2D and 3D interactions. The docked scores of 04 compounds against AChE ranged from  $-6.64$  to  $-7.50$  kcal/mol. The order of highest docking scores was as follows: rutin > quercetin > hecogenin > luteoline > kaempferol.

The majority of the selected compounds exhibited favorable interactions with essential amino acid residues such as Tyr72, Tyr 124, Tyr286, Asp74, Trp86, Tyr124, Ser293, Phe295, Phe297, Phe338, Tyr341, and Arg293. Interestingly, rutin, with a docking score of  $-7.50$  kcal/mol, demonstrated 08 conventional hydrogen bond interactions with the essential amino acids of the PAS and CAS regions: Tyr72, Tyr 124, Tyr286, Ser293, Thr75, Glu285, Leu76, Arg296. Additionally, rutin formed two carbon-hydrogen bonds, three conventional hydrogen bonds, three PI, one PI-sigma and one P-PI T-Shaped.

**Table 1.** Docking results of selected compounds with AChE

Structure	Compounds	Docking Score (kcal/mol)	RMSD
	Rutin	-7.50	2.46
	Quercetin	-6.90	1.29
	Kaempferol	-6.64	1.55
	Luteolin	-6.87	0.54

Our findings align with those reported by (Bouabdallah et al., 2024), where the docking scores of 15 compounds against AChE ranged from -11.22 to -24.68 kcal/mol, in contrast to -14.62 kcal/mol for donepezil (co-crystal ligand). Rutin exhibited the highest and most stable docking score (-24.68). According to Bouabdallah et al., docking energies lower than -24 kcal/mol indicated that terrestrosin C, protodioscin, rutin, and saponin C were the most stable docked compounds. The order of highest docking scores was as follows: rutin > saponin C > protodioscin > terrestrosin C > trillarin > epigallocatechin > terreside B > cynaroside > disogluside, apigetrin > kaempferol > quercetin > hecogenin > luteoline > donepezil > caffeic acid. If we focus on the molecules belonging to flavonoids, we derive the following ranking: rutin > cynaroside > disogluside > apigetrin > kaempferol > quercetin > hecogenin > luteoline > donepezil. However, when considering the ranking of docking scores for the other molecules, there is a discrepancy between the current data (rutin > quercetin > luteoline > kaempferol) and the previous findings (rutin > kaempferol > quercetin > luteoline). The differences in the results can be attributed to the variation in the docking methods used in the two studies. According to Bouabdallah et al., the Molecular Operating Environment (MOE)-Dock 2019.09 program was employed for the docking analysis. They observed that the majority of the selected compounds exhibited favorable interactions with essential amino acid residues such as Tyr72, Asp74, Trp86, Tyr124, Ser293, Phe295, Phe297, Tyr337, Phe338, Tyr341, and Trp286. Interestingly, rutin, with a docking score of -24.68 kcal/mol, demonstrated 11 conventional hydrogen bond interactions with the essential amino acids of the PAS and CAS regions: Tyr124 (1.64 Å), Phe295 (2.93 Å), Tyr341 (2.45 Å), Tyr341 (3.04 Å), His447 (2.85 Å), Asp74 (2.71 Å), Thr83 (2.79 Å), Asn87 (2.50 Å), Trp86 (3.12 Å), and Glu202 (3.05 Å and 3.22 Å). Additionally, rutin formed six carbon-hydrogen bonds, two  $\pi$ -donor hydrogen bonds, and eight  $\pi$ - $\pi$ -stacked bonds.



**Figure 1.** Docking results (A) 3D interactions of kaempferol, (B) 2D interactions of kaempferol with AChE, (C) 3D interactions of luteolin with AChE (D) 2D interactions of luteolin with AChE, (E) 3D interactions of quercetin with AChE (F) 2D interactions of quercetin with AChE, (G) 3D interactions of rutin with AChE (H) 2D interactions of rutin with AChE.

### 3.3. Pharmacokinetics of investigated compounds

All four selected molecules (rutin, quercetin, kaempferol and luteolin) exhibit favorable ADME properties, suggesting their potential as drug candidates. A summary of the ADME properties and drug likeness analysis of the screened compounds is provided in Table 2. In essence, three out of the four selected molecules (quercetin, kaempferol and luteolin) adhere to Lipinski's Rule of Five, with criteria met for molecular weight ( $MW < 500$ ), hydrogen bond donors ( $HBD \leq 5$ ), hydrogen bond acceptors ( $HBA \leq 10$ ), octanol-water partition coefficient ( $iLOGP$ ), and molar refractivity ( $MR$  40-130). Additionally, the bioavailability score of all molecules was assessed as shown in Table 2.

Swiss ADME provides additional information and tools relevant to medicinal chemistry and drug discovery. Within this section, Swiss ADME offers analysis related to potential drug candidates and their chemical properties. Pan-Assay INterference compoundS (PAINS) are substructures commonly found in screening libraries that are known to interfere with many biological assays, often leading to false-positive results. Identifying and filtering out compounds containing PAINS substructures is important in drug discovery to avoid wasting resources on false leads. As for "Brenk," it's likely a reference to the work of Dr Ruth Brenk, a medicinal chemist known for her research in fragment-based drug design and structure-based drug discovery. It's possible that Swiss ADME includes tools or references related to her work or methodologies in medicinal chemistry. Overall, the Medicinal Chemistry section of Swiss ADME likely provides valuable resources and tools for medicinal chemists to assess and optimize potential drug candidates.

In terms of PAINS analysis, rutin, quercetin, and luteolin exhibit 1 alert for catechol\_A, suggesting the presence of a catechol substructure, whereas kaempferol shows no alerts in this analysis. Moving to the Brenk analysis, Three of the four chosen molecules, rutin, quercetin and luteolin, demonstrate 1 alert for catechol. Synthetic accessibility scores reveal that rutin has the highest score of 6.52, followed by quercetin with a score of 3.23, kaempferol with 3.14, and luteolin with 3.02. Lastly, bioavailability scores indicate that rutin has the lowest score of 0.17, while quercetin, kaempferol, and luteolin share a higher score of 0.55.

The presence of alerts for catechol in both PAINS and Brenk analyses indicates that rutin, quercetin and luteolin, contain a catechol substructure, potentially affecting their behavior in biological assays or drug development processes. In terms of synthetic accessibility, rutin emerges with the highest score, suggesting potential challenges in synthesis compared to quercetin, kaempferol, and luteolin. Moreover, rutin exhibits the lowest bioavailability score among the molecules analysed, implying reduced absorption and availability for pharmacological effects compared to quercetin, kaempferol, and luteolin. In summary, rutin stands out with its lower bioavailability score and higher synthetic accessibility score, while quercetin, kaempferol, and luteolin share similar bioavailability and synthetic accessibility scores but differ slightly in their PAINS and Brenk analyses.

Catechol is a chemical compound with a benzene ring containing two adjacent hydroxyl (-OH) groups. In the context of drug discovery and medicinal chemistry, the presence of catechol substructures can be considered as an alert or a potential risk factor for toxicity (Kim, and Kwon, 2013). Catechol substructures can undergo metabolic transformations, such as oxidation or conjugation, leading to the formation of reactive metabolites.

**Table 2.** Compilation of physicochemical properties and drug likeness assessments for the four selected ligand molecules (pharmacokinetics).

Ligand	MW	NHA	NAHA	NRB	NHbA	NHbD	MR	TPSA	iLOGP	PAINS	Brenk	BS	SA
Rutin	610.52	43	16	6	16	10	141.38	269.43	0.46	1 Alert: catechol A	1 Alert: catechol	0.17	6.52
Quercetin	302.24	22	16	1	7	5	78.03	131.36	1.63	1 Alert: catechol A	1Alert catechol	0.55	3.23
Kaempferol	286.24	21	16	1	6	4	76.01	111.13	1.7	0	0	0.55	3.14
Luteolin	286.24	21	16	1	6	4	76.01	111.13	1.86	1 Alert: catechol A	1 Alert: catechol	0.55	3.02

**MW:** Molecular weight, **NHA:** Number of heavy atoms, **NAHA:** Number of aromatic heavy atoms, **NRB:** Number of Rotatable bonds **NHbA:** Number of H-Bond acceptors, **NHbD:** Number of H-bond Donors, **MR:** Molar refractivity, **TPSA:** Topological Polar Surface Area, **iLOGP:** Octanol/water partition coefficient, **PAINS:** Pan-Assay INterference compoundS, **Brenk:** Reference to Dr. Ruth Brenk, **BS:** Bioavailability score, **SA:** Synthetic accessibility.

These reactive intermediates may covalently bind to cellular macromolecules, such as proteins or DNA, potentially causing cellular damage or toxicity. Redox cycling catechol-containing compounds are prone to undergo redox cycling reactions, where they can be oxidized to semiquinone radicals and further to quinones. Quinones are highly reactive species that can participate in redox reactions and generate reactive oxygen species (ROS), leading to oxidative stress and cellular damage. Catechol-containing compounds may also exhibit inhibitory effects on enzymes involved in various metabolic pathways. For example, they can inhibit enzymes such as catechol-O-methyltransferase (COMT) or monoamine oxidase (MAO), which are involved in the metabolism of neurotransmitters like dopamine and serotonin. Dysregulation of these pathways can lead to neurotoxicity or other adverse effects (Lin et al., 2018). Catechol-containing compounds may interact with specific receptors or transporters in the body, leading to pharmacological effects or adverse reactions. Depending on the target and the downstream signaling pathways involved, these interactions can result in therapeutic effects or toxicity. Overall, while catechol substructures are not inherently toxic, their presence in drug candidates can raise concerns due to their potential to undergo metabolic activation, generate reactive species, or interfere with biological processes (Asha and Devaraj, 2011). Therefore, identifying and evaluating compounds containing catechol substructures is important in drug discovery to assess and mitigate potential risks of toxicity (Bajorath, 2002; Goodnow et al., 2008). Synthetic accessibility refers to the ease or difficulty of synthesizing a compound in the laboratory. Compounds with higher synthetic accessibility scores may be more challenging or costly to synthesize compared to those with lower scores (Reymond et al., 2012). This can have implications for toxicity, as compounds that are difficult or expensive to synthesize may be less likely to undergo thorough preclinical toxicity testing or may be less extensively studied for their toxicological profiles.

Bioavailability scores represent an assessment of the likelihood of a drug or compound being absorbed into the bloodstream and being available for pharmacological effects. Compounds with higher bioavailability scores are more likely to exert their intended pharmacological effects, including potential toxic effects, compared to those with lower scores. However, high bioavailability may also increase the risk of toxicity due to increased systemic exposure to the compound (Gleeson et al., 2008; Lipinski et al., 2004; Veber et al., 2002).

This analysis provides insights into how synthetic accessibility and bioavailability scores can influence the toxicity of molecules, indirectly through factors such as ease of synthesis and systemic exposure, which are important considerations in drug discovery and development.

#### 4. Conclusion

This research employs computational molecular docking and drug-likeness assessment to evaluate ligands for effectiveness. The four phytoconstituents chosen exhibited favorable binding affinities ranging from -6.64 to -7.50 kcal/mol during the docking simulation. According to the findings, these molecules have shown potential in generating anti-acetylcholinesterase activity, exhibiting reduced toxicity. Additionally, ADME analysis indicated their high absorbability into the bloodstream.

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