

Molecular Docking Studies of Phytoconstituents for Neuroprotection in Alzheimer's Disease

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Abstract

Alzheimer's disease (AD) is a neurodegenerative disorder marked by cognitive and behavioural impairment that causes memory loss and cognitive decline. This review uses molecular docking against the human acetylcholinesterase enzyme (PDB ID: 4EY7) to investigate the neuroprotective potential of phytochemicals, such as terpenes, alkaloids and polyphenols. Compounds with better binding affinities (up to -10.8 kcal/mol) and dual-site interactions at the Catalytic Anionic Site (CAS) and Peripheral Anionic Site (PAS) are highlighted by analysis, such as Pterygospermine and Huperzine A. This work emphasizes the function of phytochemicals as multi-target therapeutic leads that can reduce oxidative stress and neuroinflammation by assessing molecular dynamics and binding orientations.

Keywords: Alzheimer's disease, Acetylcholinesterase, Molecular docking, Phytochemicals, Neuroprotection, Binding affinity.

1. INTRODUCTION: Alzheimer's disease (AD) is the most prevalent type of dementia, which accounts for 60-80% of cases worldwide [1]. Alzheimer's disease (AD) is a neurodegenerative brain disease responsible for both mental and physical deterioration in patients, causing death. Memory loss is thought to be caused by a number of different etiological factors, including advancing age, the production of free radicals, a decrease in cholinergic firing in the brain and the abnormal builds up of hyperphosphorylated tau protein and amyloid-beta (A β) peptides. Severe cognitive decline, synapse loss and extensive brain injury are the outcomes of this cascade [2]. Beyond these clusters, a complicated network of oxidative stress, mitochondrial failure and neuroinflammation accelerates the development of AD [3]. These interrelated occurrences further impair neuronal function and cause macroscopic brain shrinkage [4].

2. MOLECULAR DOCKING : PRINCIPLES AND APPLICATIONS

Molecular docking, a vital computational method is employed to forecast a ligand's primary binding mechanism within a target protein's active site. This approach involves the modelling of non-covalent interactions between enzymes like acetylcholinesterase (AChE) and neuroprotective phytoconstituents, within the context of Alzheimer's research.

2.1. STEPS INVOLVED IN MOLECULAR DOCKING:

- 1) **Protein preparation:** The 3D structure of the protein must be taken and pre-processed from the PDB in order to form side chains, stabilise charges and removing water molecules and fill in missing residues.
- 2) **Prediction of active site:** After protein preparation, need to choose the particular active site of interest from a number of candidates, usually by removing hetero atom and water molecules because they affect binding process.
- 3) **Making of ligand:** By using tools like ChemSketch, ChemDraw or Databases like PubChem for Ligand identification. Researchers frequently use Lipinski's rule of 5 within Computer Aided Drug Design (CADD) framework to predict drug like behaviour. This greatly increases the likelihood of success by filtering for compounds that meets critical pharmacokinetic criteria.
- 4) **Docking:** By doing docking using software like Auto dock, Dock Vina, PyRx Protein and Ligand interaction are investigated.
- 5) **Visualizing:** By using software like BIOVIA Discovery Studio, visualises Protein and Ligand binding.

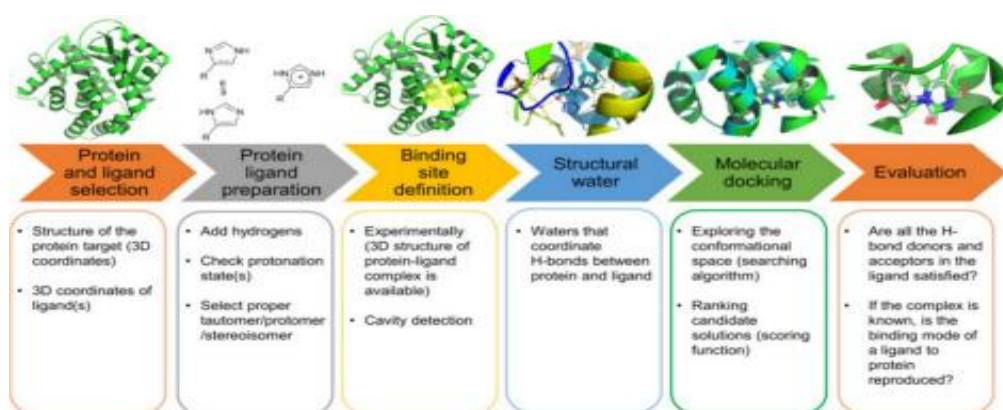


Figure 1 : Steps involved in Molecular Docking [9]

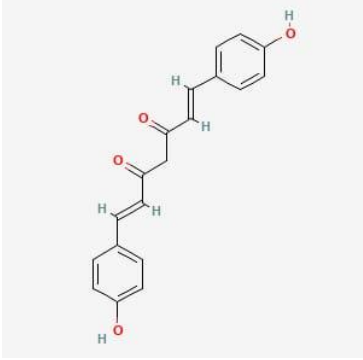
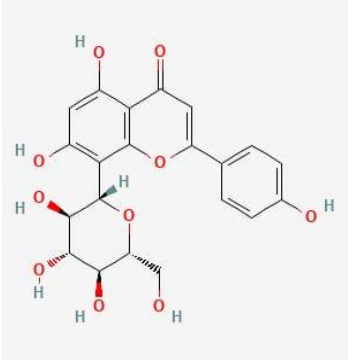
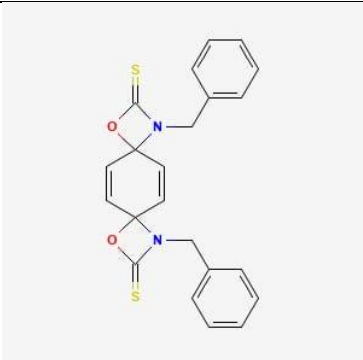
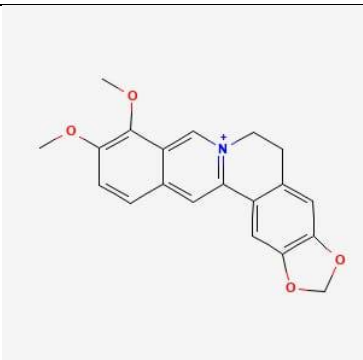
2.2. BASICS OF MOLECULAR DOCKING:

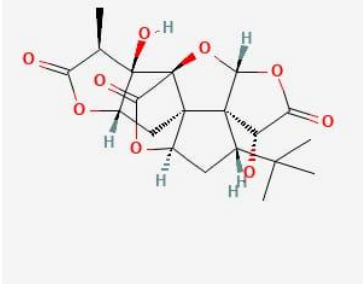
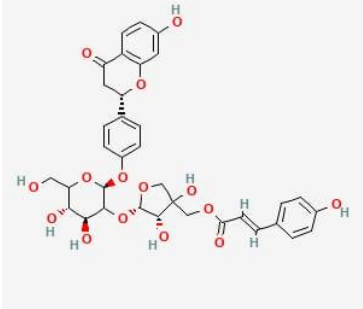
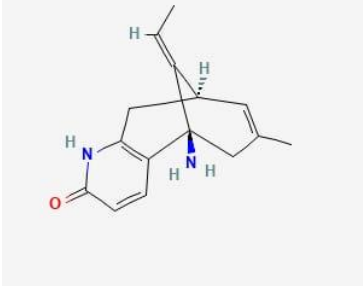
Molecular docking involves two main components: search methods and scoring functions. The search method identifies the optimal ligand position within the receptor pocket, while the scoring function predicts binding affinity as Gibbs free energy (ΔG). In AChE docking (PDB ID: 4EY7), important binding regions include the catalytic triad and peripheral anionic site (PAS) [10]. More negative ΔG values indicate stronger and more stable ligand–protein interactions, suggesting higher inhibitory potential [11]. These interactions help explain the effectiveness of plant-derived alkaloids and glycosides in managing cholinergic deficiencies [12].

3. **PHYTOCONSTITUENTS AS NEUROPROTECTIVE AGENTS:** In the complicated pathology of Alzheimer's disease (AD), where synthetic monotherapies frequently fail, phytoconstituents provide a

multi-targeted strategy to neuroprotection. These naturally occurring bioactive substances fall into different chemical classes, each of which has a special way to slow down neurodegeneration.

3.1 CLASSIFICATION AND MECHANISM OF ACTION OF NEUROPROTECTIVE PHYTOCONSTITUENTS :

Sr. No.	Phytoconstituents	Structure	Chemical Class	Primary Mechanism
1.	Bisdemethoxycurcumin		Polyphenol (curcuminoid)	Interference with BBB permeability and the development of A β plaque
2.	Vitexin		Flavone Glycoside	AChE inhibition via structure based docking and MD simulations
3.	Pterygospermine		Alkaloid (Moringa)	Human AChE (4EY7) inhibition and high binding stability
4.	Berberine		Isoquinoline Alkaloid	AChE's dual binding site affinity for its catalytic and peripheral sites

5.	Ginkgolide A		Terpene Lactone	Enhancement of NMDA neuroprotection and cerebral blood flow
6.	Liquorice Glycoside		Triterpenoid Glycoside	Modulation of the AChE environment by antioxidants and anti-inflammatory agents
7.	Huperzine A		Sesquiterpene Alkaloid	Strong and reversible inhibition of the catalytic active site of AChE

3.2 .MOECULAR DOCKING STUDIES OF PHYTOCONSTITUENTS FOR NEUROPROTECTION: A

structural base for understanding how various phytoconstituents interact with the human Acetylcholinesterase (AChE) (PDB ID: 4EY7) which is provided by molecular docking studies. From that we can evaluate the chances of amino acids interacting with each other and binding energies (ΔG) to stop the many factors that lead to Alzheimer's disease (AD).

- a. **TARGETING AMYLOID-BETA AGGREGATION:** AChE mainly controls neurotransmission, but due to its Peripheral Anionic Site (PAS), it also has a non-cholinergic function that speeds up the production of A β plaque. It has been shown that bisdemethoxycurcumin interacts well with these hydrophobic pockets, which may help to counteract the enzyme's pro-aggregation effect [13]. Isoquinoline alkaloids, like berberine, work in a similar way. They bind to both the catalytic triad and the PAS at the same time by preventing A β fibrils from aggregating due to enzyme action [14].
- b. **INHIBITION OF TAU HYPERPHOSPHORYLATION:** Cholinergic dysfunction is strongly linked to tau disease. Vitexin and other phytoconstituents have been examined using structure-based Molecular docking for determination of how stable they are in the 4EY7 pocket. Molecular Dynamics (MD)

simulations suggest that, prolonged binding to AChE may have an indirectly influence downstream kinase activities like GSK-3 β , which leads hyperphosphorylation of tau protein [15].

- c. **MODULATION OF NEUROINFLAMMATION:** When microglia are constantly activated, they release pro-inflammatory cytokines. In docking tests, pterygospermine isolated from *Moringa oleifera*, exhibited significant inhibitory ability against human AChE (4EY7), indicating a potential role in mitigating the neuroinflammatory load associated to cholinergic hyperactivity [16]. These substances help in regulating the "cholinergic anti-inflammatory pathway" by preserving acetylcholine levels.
- d. **ANTIOXIDANT PATHWAYS AND OXIDATIVE STRESS MITIGATION:** Oxidative stress is the main cause of neuronal apoptosis. Ginkgolide A and liquorice glycosides (such as Liquiritin) function as multi-target agents. In docking models, these substances show good binding scores, which means they can help keep the neural environment stable. Particularly in, ginkgolide A have ability to prevent oxidative damage by preserving mitochondrial integrity and lowering the generation of ROS [17, 18].
- e. **OTHER POTENTIAL TARGETS AND MECHANISM:** One of the most effective reversible inhibitors of AChE is still the sesquiterpene alkaloid Huperzine A. Strong hydrogen bonding with residues like Gly120, Gly121 and Ser203 characterizes its high affinity for the 4EY7 active site [19]. Its higher clinical performance over many other natural leads can be explained by this exact molecular match.

4. METHODOLOGICAL CONSIDERATION:

Reliable molecular docking studies require validation with experimental data such as crystallographic structures (e.g., 4EY7) and binding assays, especially for evaluating ligand–protein interactions in Alzheimer’s disease. Clear reporting of docking parameters, conformational sampling, and scoring functions for Gibbs free energy (ΔG) is essential for reproducibility. Additionally, RMSD values below 2.0 Å confirm accurate prediction of ligand binding poses. Standardizing these methods reduces false positives and improves identification of promising phytoconstituents, such as Huperzine A and Berberine, for further preclinical and clinical investigation.

5. STUDIED PARAMETERS :

Sr. No.	Phytoconstituents	Binding Energy ΔG (Kcal/mol)	Key Amino Acid Interactions	Interaction site
1.	Bisdemethoxycurcumin	-7.3	TRP86, TYR124, TRP286, PHE295, TYR337, PHE338	Catalytic Anionic Site(CAS), Peripheral Anionic Site(PAS)
2.	Vitexin	-8.2	TRP286, PHE295, TYR337, PHE338, TYR341, HIS447	CAS And PAS
3.	Pterygospermine	-10.28	TYR133 And GLU202	Long, narrow aromatic gorge
4.	Berberine	-7.3 to -8.5	TYR124, PHE295, TRP286, TRP86, TYR337, PHE338	Dual binding

5.	Ginkgolide A	-7.5 to -8.1	TYR72,TRP286,TYR341	Mixed / Peripheral site
6.	Liquorice Glycoside	-7.9	TYR124,SER293,PHE295,TRP86, TRP286,TYR337,TYR341	CAS And PAS
7.	Huperzine A	-9.2 to -10.8	GLY120,GLY121,SER203,TYR133 , TRP86,PHE338,TYR337	CAS

6. CHALLENGES AND FUTURE DIRECTIONS :

Despite advances in in-silico methods, identifying neuroprotective phytoconstituents remains challenging due to factors like protein flexibility, solvent effects, and difficulty in predicting binding for flexible ligands [20]. Promising compounds such as bisdemethoxycurcumin and vitexin often fail in vivo due to poor bioavailability and limited blood–brain barrier penetration. Additionally, variability in plant extracts and lack of standardization hinder clinical translation [21]. Integrating molecular docking with Molecular Dynamics (MD) simulations and AI, along with standardized extraction and formulation methods, can help overcome these limitations [22].

7. CONCLUSION: Nature offers many promising compounds for Alzheimer’s disease, but translating in-silico results into clinical success remains challenging. Multi-target phytochemicals like berberine and ginkgolide A show better potential than single-target drugs, yet issues like poor brain delivery and bioavailability persist. Combining AI, nanotechnology, and standardized testing methods can help unlock their therapeutic potential for future Alzheimer’s treatment.

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