

Computational Investigation of Flavonoids as Acetylcholinesterase Inhibitors using Molecular Docking, ADMET and Regression Analysis

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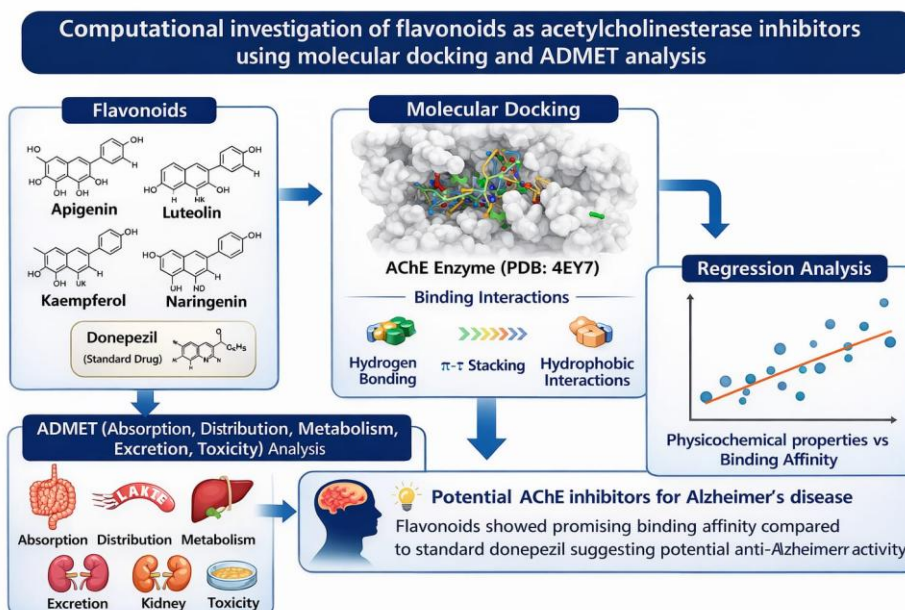
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ABSTRACT

Alzheimer's disease is a progressive neurodegenerative disorder characterized by gradual loss of memory and cognitive impairment. Acetylcholinesterase (AChE) is responsible for the breakdown of acetylcholine and its inhibition is considered an effective therapeutic strategy for the management of Alzheimer's disease. The present study aimed to evaluate selected flavonoids as potential acetylcholinesterase inhibitors using computational approaches. Molecular docking analysis was performed using AChE protein (PDB ID: 4EY7) to evaluate the binding affinity of selected compounds. Donepezil was used as a standard drug for comparison. Docking results

showed that the selected flavonoids exhibited good binding affinity towards the active site of acetylcholinesterase. Interaction analysis revealed important contacts with key catalytic residues including TRP86, GLU202, SER203, TYR337 and HIS447. ADMET prediction using SwissADME indicated favorable pharmacokinetic properties and good drug likeness for the studied compounds. Furthermore, regression analysis was performed to evaluate the relationship between physicochemical properties and binding affinity. The results suggest that selected flavonoids may serve as promising candidates for acetylcholinesterase inhibition and could be further explored for Alzheimer's disease management.



KEYWORDS

Alzheimer's disease, Acetylcholinesterase, Molecular docking, Flavonoids, ADMET, Regression analysis.

I. INTRODUCTION

Alzheimer's disease is a progressive neurodegenerative disorder characterized by gradual loss of memory, cognitive impairment and behavioral disturbances. It is one of the most common causes of dementia worldwide and represents a major healthcare challenge. The cholinergic hypothesis suggests that cognitive decline in Alzheimer's disease is mainly due to reduced levels of the neurotransmitter acetylcholine. Acetylcholinesterase (AChE) is the key enzyme responsible for the hydrolysis of acetylcholine and therefore inhibition of this enzyme is considered an important therapeutic strategy for the management of Alzheimer's disease^{1,2,3,4,5}.

Natural phytoconstituents, particularly flavonoids, have gained significant attention due to their antioxidant, anti-inflammatory and neuroprotective properties. Several studies suggest that flavonoids may act as potential acetylcholinesterase inhibitors and could serve as promising candidates for drug development^{6,7,8,16}.

In the present study, selected phytoconstituents were evaluated for their inhibitory potential against acetylcholinesterase using molecular docking analysis. The binding interactions between ligand and receptor were analyzed to understand the stability of complexes. Furthermore, ADMET prediction was carried out to evaluate pharmacokinetic properties and drug-likeness characteristics. Statistical regression analysis was also performed to understand the relationship between molecular descriptors and binding affinity^{11,12,27,29}. This integrated computational approach may help in identifying promising lead molecules for further drug development studies.

The present study aims to investigate the potential acetylcholinesterase inhibitory activity of selected flavonoids using molecular docking analysis. The study further evaluates pharmacokinetic properties through ADMET prediction and analyzes the relationship between physicochemical parameters and binding affinity using regression analysis^{12,28,30}. This computational approach may help identify potential lead compounds for further development of therapeutic agents for Alzheimer's disease.

II. Materials and Methods

2.1 Target Protein Selection

The three-dimensional crystal structure of **acetylcholinesterase (AChE)** enzyme was obtained from the **Protein Data Bank (PDB)**. The structure with **PDB ID: 4EY7** was selected for the present study due to its high resolution and availability of structural information suitable for molecular docking analysis^{11,10}. The protein structure was downloaded in PDB format and used as the receptor molecule for docking studies.

2.2 Protein Preparation

The downloaded protein structure was **prepared prior to docking** to ensure **structural accuracy**. The preparation involved **removal of co-crystallized ligands and water molecules** from the protein structure. **Unnecessary heteroatoms** were eliminated and the cleaned protein structure was used as the **receptor for docking simulations**. The prepared protein structure was then used for **cavity detection and molecular docking analysis**¹².

2.3 Ligand Selection

Four flavonoid compounds were selected for the study based on their reported **antioxidant, neuroprotective**, and potential **acetylcholinesterase inhibitory activities**³⁴:

- **Apigenin**
- **Luteolin**
- **Kaempferol**
- **Naringenin**

These phytoconstituents were chosen to evaluate their **interaction with acetylcholinesterase** and compare their **binding affinity** with the standard drug⁵.

2.4 Ligand Preparation

The chemical structures of the selected flavonoids and the standard drug **Donepezil** were obtained from the **PubChem database**⁶. The **3D structures** of the compounds were downloaded in **SDF format** and converted into **PDB format** when required. The ligand structures were checked for **proper geometry** and used for **molecular docking studies**⁷.

2.5 Standard Drug Selection

Donepezil, a clinically approved **acetylcholinesterase inhibitor** used in the treatment of **Alzheimer's disease**, was selected as the **standard reference drug**⁸. The docking results of the selected flavonoids were compared with

Donepezil to evaluate their **potential inhibitory activity** against acetylcholinesterase.

2.6 Molecular Docking Study

Molecular docking was performed using the **online docking server CB-Dock2**⁹. CB-Dock2 is a **cavity-detection guided docking approach** that automatically identifies **potential binding sites** on the protein surface and performs docking using **AutoDock Vina**¹⁰.

The prepared protein structure was uploaded to the CB-Dock2 server and the **ligand molecules** were submitted individually. The server automatically detected **possible binding cavities** within the protein and performed docking simulations for each ligand within these cavities. The docking results provided information regarding **binding energy, docking pose, best binding cavity, and cavity volume**. The **docking pose with the lowest binding energy** was selected as the most **favorable binding conformation** for further analysis¹¹.

2.7 Docking Result Analysis

The docking results obtained from **CB-Dock2** were analyzed to evaluate the **binding affinity** of the selected compounds with **acetylcholinesterase**¹. The parameters analyzed included **binding energy values (kcal/mol), best docking cavity, and cavity volume**. **Lower binding energy values** were considered to indicate **stronger and more stable ligand-protein interactions**².

2.8 Protein-Ligand Interaction Analysis

Protein-ligand interactions were analyzed to identify **important amino acid residues** involved in binding³. The interaction analysis focused on identifying **hydrogen bonding interactions, hydrophobic interactions, and active site residues** involved in ligand binding. Particular attention was given to important **catalytic residues** of acetylcholinesterase including **TRP86, GLU202, SER203, TYR337, and HIS447**, which are known to play a critical role in **enzyme inhibition**⁴.

2.9 ADMET Prediction

The **pharmacokinetic properties** of the selected compounds were predicted using the **online tool SwissADME**⁵. The **SMILES structures** of the compounds were obtained from **PubChem** and submitted to the SwissADME server. The predicted parameters included:

- **Molecular weight**
- **Lipophilicity (LogP)**
- **Gastrointestinal absorption**

- **Blood-brain barrier (BBB) permeability**
- **Lipinski rule of five**

These parameters were used to evaluate the **drug-likeness** and **pharmacokinetic behavior** of the selected compounds⁶.

2.10 Statistical Analysis

Statistical analysis was performed using **Microsoft Excel**. **Regression analysis** was carried out to evaluate the relationship between **physicochemical properties** such as **molecular weight** and **LogP** with **binding energy**⁷. **Scatter plots** and **regression plots** were generated to visualize the correlation between these parameters. The **correlation coefficient (R²)** was calculated to determine the strength of the relationship between **molecular descriptors** and **docking affinity**⁸.

III. RESULTS AND DISCUSSION

3.1 Molecular Docking Analysis of Standard Drug

Molecular docking analysis was performed to evaluate the binding interaction of the standard drug Donepezil with acetylcholinesterase enzyme (PDB ID: 4EY7). The docking result revealed that Donepezil showed strong binding affinity with binding energy of **-11.8 kcal/mol** indicating stable interaction with the target protein (Figure 1).

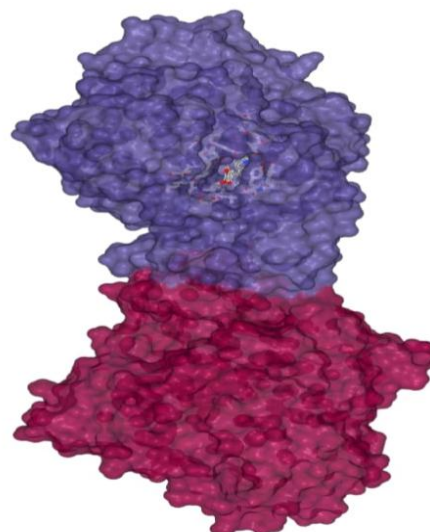


Figure 1: Docking pose of Donepezil with acetylcholinesterase active site.

3.2 Interaction Analysis of Donepezil

The docking results indicated that Donepezil binds effectively within the active site cavity of

acetylcholinesterase. The ligand showed interaction with important amino acid residues including TYR72, ASP74, TRP86, GLY120, GLY121, TYR124, TYR133, GLU202, SER203, TRP286, LEU289, SER293, VAL294, PHE295, ARG296, PHE297, TYR337, PHE338, TYR341, HIS447, GLY448 and ILE451 (Figure 2).

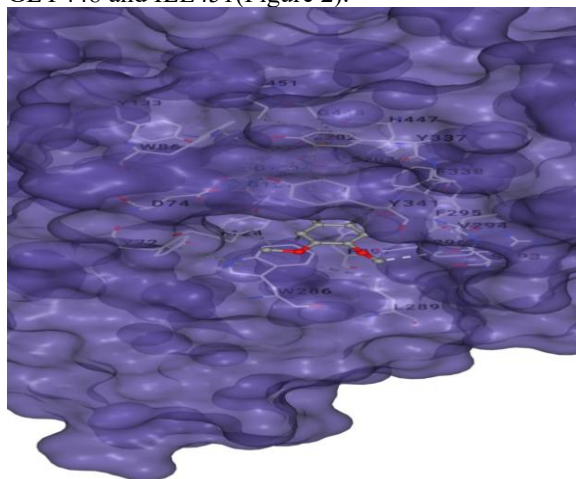


Figure 2: Protein–ligand interaction diagram showing binding interactions of Donepezil with active site residues.

3.3 Docking Analysis of Selected Flavonoids

Apigenin showed good binding affinity with acetylcholinesterase with binding energy of -10.2 kcal/mol. Luteolin showed binding energy of -10.3 kcal/mol indicating strong interaction with the enzyme (Figure 3). Kaempferol showed binding energy of -9.9 kcal/mol suggesting stable interaction within the active site. Naringenin showed

binding energy of -10.3 kcal/mol indicating favorable binding affinity.

Interaction analysis revealed that these compounds interact with important catalytic residues including TRP86, GLU202, SER203, TYR337 and HIS447 which play a significant role in acetylcholinesterase inhibition.

Among the selected flavonoids, Luteolin and Naringenin showed the highest binding affinity (-10.3 kcal/mol) indicating strong interaction with the active site residues of acetylcholinesterase.

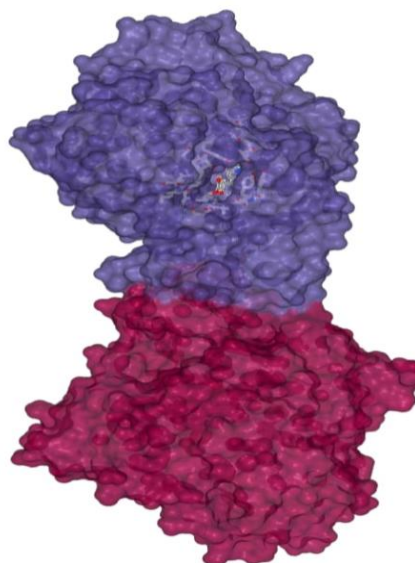


Figure 3: Docking pose of Luteolin within the active site of acetylcholinesterase.

Table 1. Molecular docking results of selected compounds with acetylcholinesterase (PDB ID: 4EY7).

Compound	Binding Energy (Kcal/mol)	Best cavity	Volume (Å ³)	Key Residue
Donepezil	-11.8	C2	1302	TYR86, SER203, HIS447
Apigenin	-10.2	C2	1302	TYR86, SER203, TRP337
Luteolin	-10.3	C4	856	TYR86, SER203, HIS447
Kaempferol	-9.9	C2	1302	TYR86, SER203, HIS447
Naringenin	-10.3	C4	856	TYR86, SER203, HIS447

3.4 ADMET Analysis

ADMET prediction was performed using SwissADME. All compounds showed high gastrointestinal absorption indicating good oral bioavailability. The flavonoids showed no BBB

permeability while Donepezil showed BBB permeation as expected for a CNS drug. All compounds satisfied Lipinski rule suggesting good drug likeness.

Table 2. ADMET properties of selected compounds predicted using SwissADME.

Compound	Mol.wt (g/mol)	Log P	Gastrointestinal Absorption	Lipinski Rule	BBB Permeability
Donepezil	379	3.8	High	Yes	Yes
Apigenin	270	2.58	High	Yes	No
Luteolin	286	2.2	High	Yes	No
Kaempferol	286	2.3	High	Yes	No
Naringenin	272	2.19	High	Yes	No

3.5 Regression Analysis

demonstrating the relationship between molecular descriptors and docking affinity. The 3D Regression analysis was performed to evaluate the relationship between physicochemical properties and binding affinity. A good correlation was observed between molecular weight, LogP and binding energy

suggesting that physicochemical properties influence ligand binding interactions. The regression analysis indicated a correlation coefficient ($R^2 \approx 0.88$) surface plot further illustrated the influence of molecular weight and lipophilicity on binding energy.

Table 3. Physicochemical properties and binding energy used for regression analysis.

Compound	Mol.wt (g/mol)	Log P	Binding Energy (Kcal/mol)
Donepezil	379	3.8	-11.8
Apigenin	270	2.58	-10.2
Luteolin	286	2.2	-10.3
Kaempferol	286	2.3	-9.9
Naringenin	272	2.19	-10.3

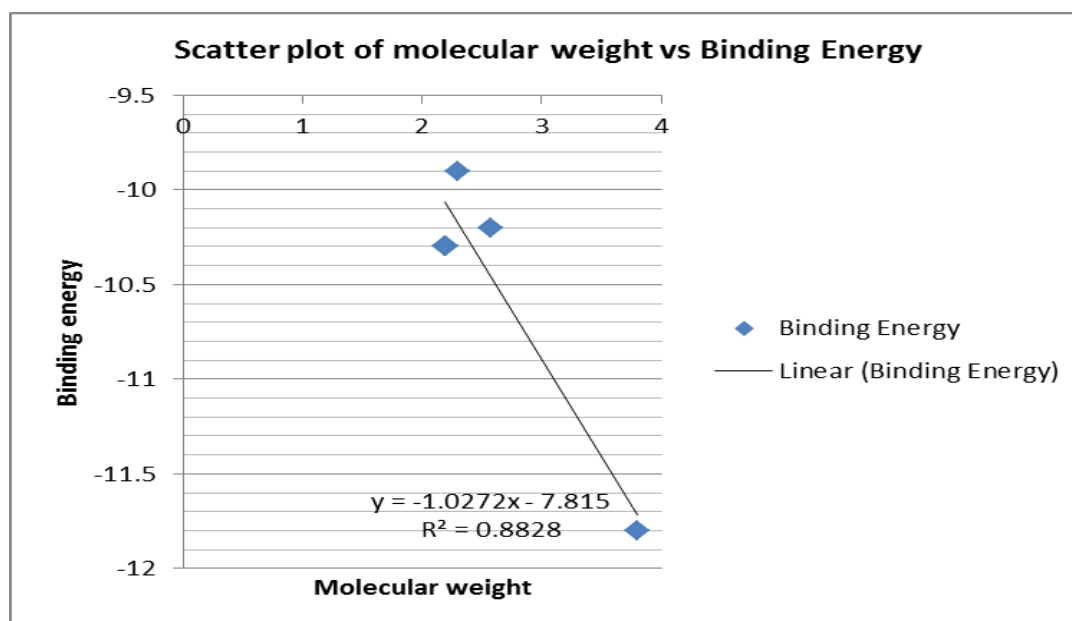


Figure 4: Regression plot showing correlation between molecular weight and binding energy of selected compounds.

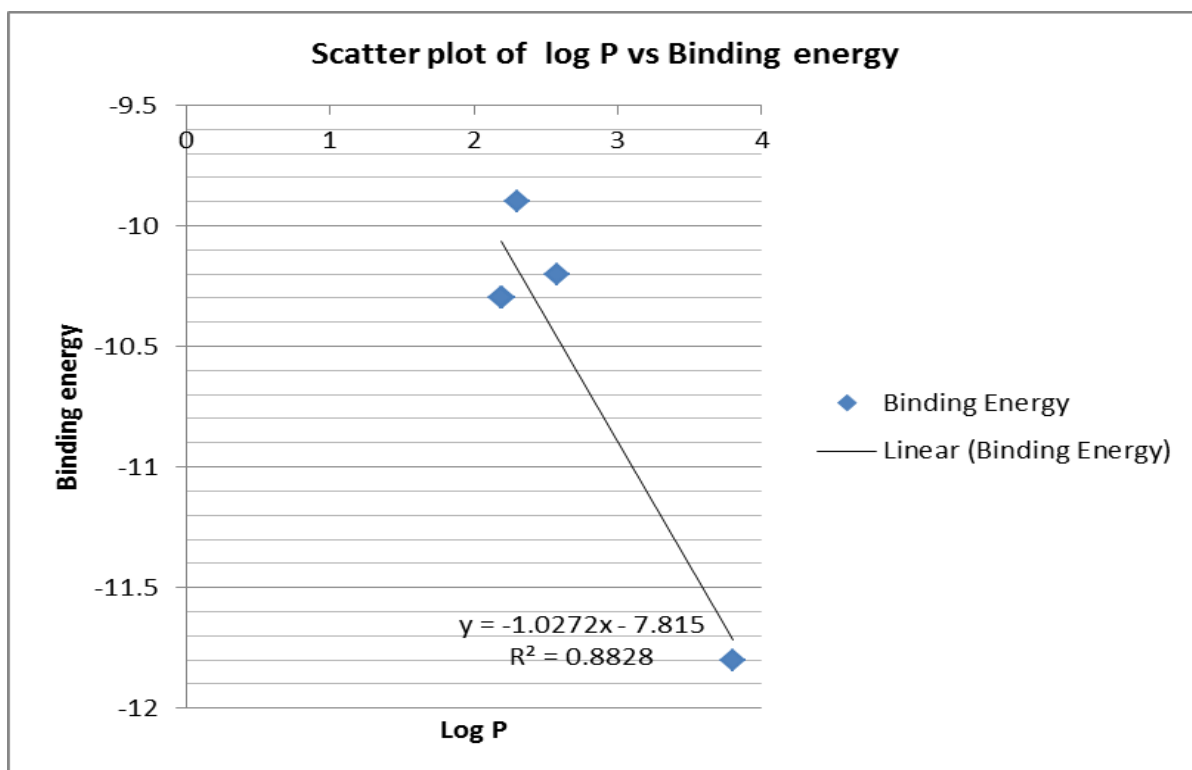


Figure 5: Regression plot showing correlation between LogP and binding energy of selected compounds.

3D Surface Plot of Docking Results

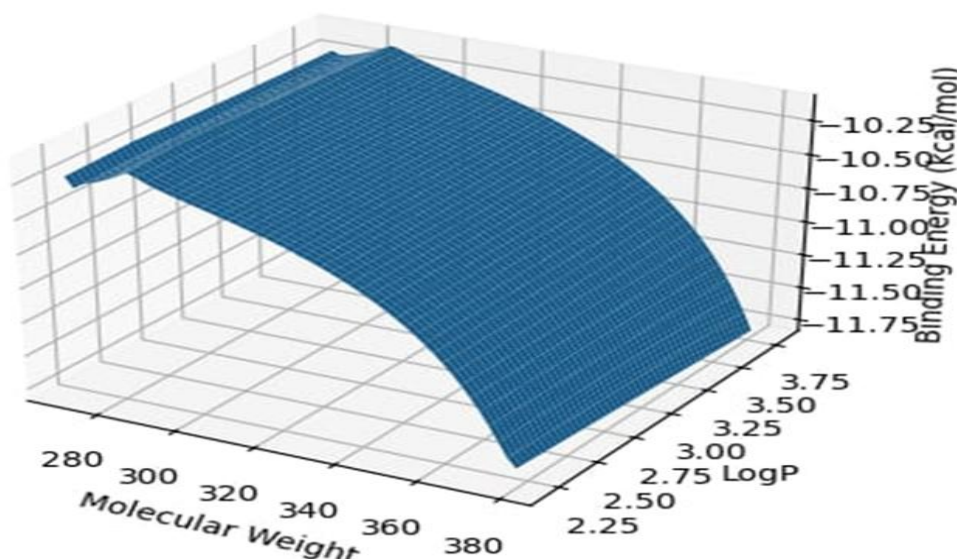


Figure 6: 3D surface plot illustrating relationship between molecular weight, LogP, and binding energy.

IV. CONCLUSION

The present computational study evaluated selected flavonoids for their potential acetylcholinesterase inhibitory activity using

molecular docking and ADMET analysis. The results indicated that the selected compounds exhibited favorable binding affinity towards acetylcholinesterase and interacted with important

catalytic residues. ADMET prediction suggested good pharmacokinetic properties and drug likeness. Regression analysis further supported the influence of physicochemical properties on binding affinity. Overall, the findings suggest that selected flavonoids may serve as promising candidates for acetylcholinesterase inhibition and could be further explored for the development of therapeutic agents for Alzheimer's disease.

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