Molecular Docking Study of Phytochemicals as Novel Therapeutic Leads for Alzheimer's Disease by Targeting Acetylcholinesterase

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ABSTRACT

Objectives: The molecular docking and ADMET studies of ferulic acid, vanillic acid, catechin hydrate, and chlorogenic acid were the objectives of this work. Targeting the binary complex of natural hAChE and the crystal structure of recombinant human acetylcholinesterase for anti-Alzheimer activity, the receptor affinity was evaluated employing molecular docking.

Materials and Methods: For molecular docking analysis of compounds, PDB ID 6O4W and PDB ID 4EY6 were selected for anti-Alzheimer activity, and donepezil and galantamine were selected as reference standards. For the docking study of these molecules, Schrodinger software (Glide v9.1) was used. ADMET studies of these compounds were done using SwissADME and ProTox-II software.

Result: The docking scores of chlorogenic acid with the crystal structure of recombinant human acetylcholinesterase in complex showed 12.856 kcal/mol, which is higher than the reference standard Galantamine with a -12.198 kcal/mol docking score, followed by catechin hydrate, ferulic acid, and vanillic acid. The docking score of standard Donepezil towards the binary complex of natural hAChE showed the highest docking score, which was -16.990 Kcal/mol, followed by chlorogenic acid (13.794 Kcal/mol), catechin hydrate (12.801 Kcal/mol), ferulic acid (8.000 Kcal/mol), and vanillic acid (6.016 Kcal/mol).

Conclusion: When compared to other phytochemicals and the reference standard galantamine, chlorogenic acid shows an excellent docking score with respect to the crystal structure of recombinant human acetylcholinesterase. However, donepezil surpasses chlorogenic acid in the human acetylcholinesterase binary complex. Ferulic acid, vanillic acid, and catechin hydrate follow. These findings imply that chlorogenic acid is a viable substitute for treating

Alzheimer's disease. Additionally, these phytochemicals' ADMET characteristics suggest that they don't have any negative impacts.

Keywords: anti-Alzheimer; ADMET; acetylcholinesterase; donepezil; galantamine

INTRODUCTION

A wide range of foods and drinks contain naturally occurring phytochemicals such as vanillic acid, ferulic acid, catechin hydrate, and chlorogenic acid. Green coffee beans are high in chlorogenic acid, but bran, certain whole grains, and fruits like apples and pineapples are high in ferulic acid (1,2). The flavonoid catechin hydrate is found in significant concentrations in green tea and cocoa (3). Vanillic acid is present in several fruits and vanilla beans (4). These materials serve a multitude of purposes. In the food industry, for instance, ferulic acid and chlorogenic acid may be used as nutraceuticals and preservatives (1,2). Catechin hydrate is being researched for application in cosmetics due to its antioxidant properties (3).

Vanillic acid, in addition to being applied as an ingredient in flavouring, has been studied for its anti-inflammatory effects (4). Additionally, studies imply a potential role for such substances in the condition known as Alzheimer's. Investigations demonstrate that the compounds chlorogenic acid, ferulic acid, and catechin hydrate could potentially reduce the effects of oxidative stress and inflammation in the brain's neurons, which have been linked to Alzheimer's disease (5–7). Vanillic acid could help enhance nerve cell functioning and interactions (8). But further investigation will be required to validate their effectiveness in either preventing or treating Alzheimer's disease.

The elderly are the most affected by Alzheimer's disease (AD), a progressive neurological condition (9). It is characterised by behavioural and personality abnormalities, memory loss, and cognitive deterioration (10). Amyloid-beta (Aβ) plaques and neurofibrillary tangles made of hyperphosphorylated tau protein are the pathophysiology of Alzheimer's disease; these aggregates cause neuronal death and atrophy, especially in the hippocampus and cortex (11).

Alzheimer's disease concept and understanding underlying the amyloid cascade theory (12). The breakdown of amyloid precursor protein (APP) leads to the formation of amyloid beta peptides, which ultimately results in neurodegeneration (13). Fibrils, oligomers, and extracellular amyloid plaques are developed when these peptides come along (14). The formation of intracellular neurofibrillary tangles is the result of abnormal tau hyperphosphorylation, which ultimately results in cell death and neuronal transport system

impairment (15). Also, in AD, inflammation plays a critical role in the pathogenesis (16). In response to amyloid plaques, microglia are activated, which are the immune cells that live in the brain and produce cytokines (17). Cytokines are responsible for neuronal damage caused by neuroinflammation as they promote inflammation (18). Also, advancement in Alzheimer's illnesses is caused by oxidative stress and mitochondrial dysfunction (19).

Preclinical, moderate cognitive impairment (MCI), and Alzheimer's dementia are the three stages of AD (20). In the preclinical stage, pathogenic alterations take place in the brain long before the symptoms manifest (21). In the second step of moderate cognitive impairment, they experience memory issues, but their ability to go about their everyday lives has not been affected (22). The third step of AD is the hallmark of Alzheimer's dementia, which affects day-to-day functioning (23). Cholinesterase inhibitors (such as donepezil and rivastigmine) and NMDA receptor antagonists are two examples of pharmacological treatments that control the cognitive symptoms of AD (24). There are a number of treatments that try to lessen symptoms and delay the disease's development, although there isn't a known cure for Alzheimer's. The significance of human acetylcholinesterase in cholinergic signalling, both in recombinant form (rhAChE) and naturally occurring (hAChE), is associated with Alzheimer's disease (25). To breakdown the neurotransmitter acetylcholine (ACh), acetylcholinesterase (AChE) is required, which is an enzyme in the brain, degradation of this enzyme results in memory loss and cognitive impairment (26). Administration of AChE inhibitors is the primary pharmaceutical strategy for managing the symptoms of AD (27).

The purpose of this study was to determine the vanillic acid, catechin hydrate, ferulic acid, and chlorogenic acid modulated binary complex of natural hAChE and the crystal structure of recombinant human acetylcholinesterase. The ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) properties of these phytochemicals were assessed using SwissADME and ProTox-II software. Through molecular docking studies, interactions between vanillic acid, catechin hydrate, ferulic acid, chlorogenic acid, and these two types of AChE can be revealed. Through this research, we can understand the interaction mechanisms, binding affinities, and possible efficacy of AChE inhibitors in AD. To confirm the efficacy and safety of these phytochemicals as anti-Alzheimer medicines, in-vivo experimentation is necessary.

Materials and Methods

Software

To perform the molecular docking studies Schrodinger software (Glide v9.1) software was used. ADMET studies carried out by using ProTox-II and SwissADME software. MMGB-SA were used to computed binding free energies.

Molecular docking study

For the molecular docking studies, the Schrodinger program's Glide module (Glide v9.1) was used. Next, we used MM-GBSA to compute the binding free energies. ProTox-II (https://tox-new.charite.de/protox_II/) was used to estimate the toxicity of the highest scoring hits from the MM-GBSA research, and ADMET Prediction and SwissADME were used to compute the ADME characteristics. Every single technique has been explained separately.

The protein structures that were co-crystallized were obtained from the Protein Data Bank, which is accessible to the general public at http://www.resb.org/PDB IDs 6O4W and 4EY6. For molecular docking investigations, a chosen ligand and reference molecules were employed in the interim. The protein preparation wizard was then used to process the imported protein through a number of steps, including adding the missing H atom and assigning the correct band arrangement. Protein co-factors were formalised and an internal ligand heterostate was generated. In order to maintain the relaxed state of the co-crystallized protein complex and eliminate any unphysical connections between the protein or ligand atoms, the last step involved executing OPLS 2005 forcefield energy minimization. Furthermore, any water molecules that were range between five and six were removed. The purpose of the receptor grid was to pinpoint the precise binding site of the compounds under investigation. When the default settings were used with a scale factor of 1.0, the centroid of the co-crystallized crystals was discovered inside a 10 Å site. The Protein Production Wizard has been used to do this. The reference molecule's ligands were able to generate their potential conformers, tautomers, or stereoisomers by using the OPLS2005 forcefield and the maestro's ligand preparation tool. Using the XP mode and a Van der Scaling of 0.8, these artificial ligands with energy minimization were employed in molecular docking experiments.

MM-GBSA

In computational chemistry and structural biology, the binding free energy between proteins and ligands (small molecules) is calculated using a computer method called MMGB/SA

(Molecular Mechanics/Generalized Bom Surface Area). It is widely used in drug development and protein-ligand interaction studies to forecast the binding affinity of possible therapeutic candidates to target proteins. The MMGBSA approach combines surface area calculations, molecular mechanics, which uses force fields to characterise a system's internal energy, and generalised Born solvent models.

ADMET Prediction

To forecast toxicity, ProTox-II was utilised. Using SwissADME, ADME characteristics were calculated. The result consists of several ADMET property predictions.

RESULTS

Protein preparation and receptor grid generation for anti- Alzheimer activity: Alongside the crystal structure of recombinant human acetylcholinesterase in complex with (-)-galantamine (PDB ID: 4EY6) and the binary complex of native hAChE with donepezil (PDB ID: 6O4W) were obtained. After processing the protein structure using the protein preparation wizard panel, a grid encircling the active site of the protein was created using Glide v9.1's receptor grid building panel. The protein structure is appropriate for docking and additional computer investigation.

Ligand Preparation: Ligand preparation involved first illustrating the compounds with the 2D sketcher and then optimising them by geometric minimization with the OPLS 5 force field. LigPrep was utilised to ensure that compound structures were optimised for additional research effort. Ensuring the correctness of computational studies and generating a more reliable representation of the molecular properties and interactions inside the compounds are dependent on this procedure.

Glide Ligand Docking: Launch the Glide application, then choose between Standard Precision (SP) and Extra Precision (XP) docking techniques. XP offers more precision even if it uses more computer power. Configure the scoring functions, docking parameters, position sampling, and ligand flexibility. The molecular docking scores are shown in Tables 1 and 2.

Table 1: Molecules with their dock score and Binding Free Energies for Binary complex of native hAChE.

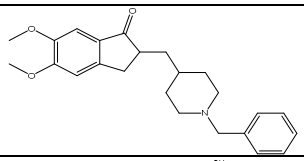
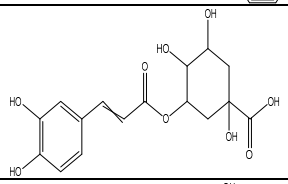
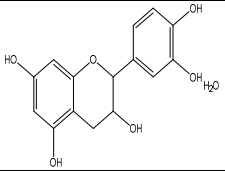
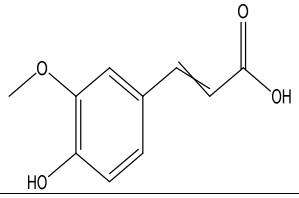
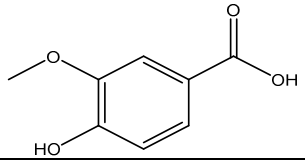
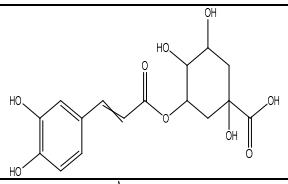
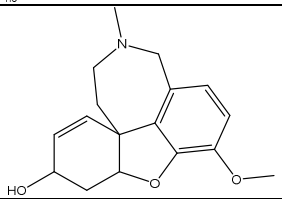
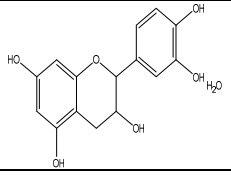
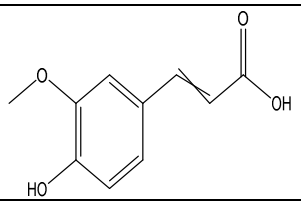
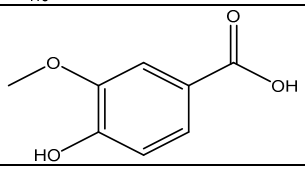
Compound name	2D structure	Dock Score Kcal/mole	dG Binding Kcal/mole
Reference Donepezil		-16.990	-83.46
Chlorogenic acid		-13.794	-47.60
Catechin hydrate		-12.801	-56.74
Ferulic acid		-8.000	-1.79
Vanillic acid		-6.016	-12.60

Table 2: Molecules with their dock score and Binding Free Energies for with Crystal Structure of Recombinant Human Acetylcholinesterase in Complex.

Compound name	2D structure	Dock Score Kcal/mole	dG Binding Kcal/mole
Chlorogenic acid		-12.856	-36.83
Reference Galantamine		-12.198	-26.38
Catechin hydrate		-9.596	-35.16

Ferulic acid		-6.116	-3.63
Vanillic acid		-5.417	9.26

MM-GBSA: The Prime/MM-GBSA process was employed to determine the relative binding energy of particular ligands. The study's input for the MM-GBSA was the pv.maegz file, which was generated via XP docking. The protein's active site was engineered to conform to the ligand within a range of 5 o' A, as seen in Tables 2 and 3. This methodology ensures a comprehensive analysis of ligand-protein interactions, taking into consideration the flexibility of the active site and providing valuable insights into the energetics of ligand binding. More research should be done on the compounds that have lower (more negative) total binding free energies as they are more likely to interact significantly with the target protein. The binary complex of native human acetylcholinesterase with donepezil (PDB ID 6O4W) and the crystal structure of recombinant human acetylcholinesterase in complex with (-)-galantamine (PDB ID 4EY6), along with the binding interactions of all molecules, are displayed in Tables 3, 4, and 5, 6.

Table 3: Binding interactions of all molecules with Binary complex of native hAChE with Donepezil (PDB ID 6O4W).

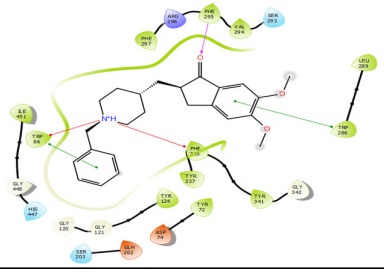
Compound Name	2D Interactions	Distance (A^0)
Reference Donepezil	H-Bond:PHE295 Pi-Cation:PHE338,TRP86 Pi-Pi Stacking:TRP86,TRP286	2.13 6.23,4.75 3.88,3.96
Chlorogenic acid	H-bond: TRY337,TYR341,TYR124,TYR124, SER125,ASP74,SER293,PHE295 Pi-Pi Stacking:TRP286	1.92,2.10,1.96,2.50, 1.94,1.89,2.73,2.20 4.36
Catechin hydrate	H-bond:SER203,GLY122,GLY121,PHE295 Pi-Pi Stacking:HIS447,TYR341	2.12,2.42,2.36,2.05 5.06,4.12

Ferulic acid	H-bond: GLY122,GLY121 Pi-Pi Stacking:HIS447	1.92,1.93 4.96
Vanillic acid	H-bond: TYR124, ASP74, PHE295 Pi-Pi Stacking:TYR124,TYR341	2.24,1.69,1.63 5.48,4.10

Table 4: Binding interactions of all molecules with Crystal Structure of Recombinant Human Acetylcholinesterase in Complex with (-)-galantamine (PDB ID 4EY6).

Compound Name	2D Interactions	Distance (A^0)
Chlorogenic acid	H-bond:TYR133,GLH202,HIS447,PHE295	2.09,1.70,1.97,2.08
Reference Galantamine	H-bond:SER125 Pi-Cation:TRP86,TRP86	2.35 4.64,4.27
Catechin hydrate	H-bond:TYR337,GLH202,TYR72 Pi-Pi Stacking:TRP86,TRP86	1.91,2.01,1.92 3.85,3.70
Ferulic acid	No Interaction	No Distance
Vanillic acid	H-bond: TYR133,GLH202 Pi-Pi Stacking:TRP86,TRP86	2.06,1.88 4.19,3.50

Table 5: Interaction images of all molecules with Binary complex of native hAChE with Donepezil (PDB ID 6O4W).

Compound Name	3D Image	2D Image
Reference Donepezil		

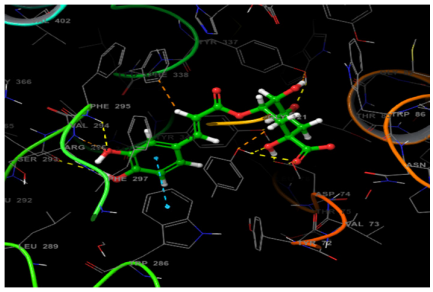
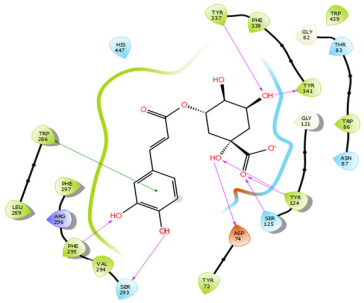
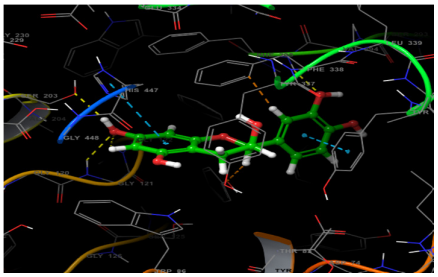
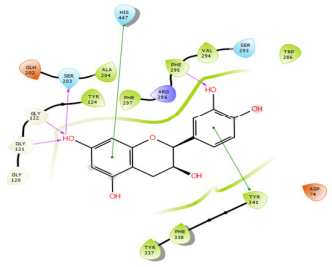
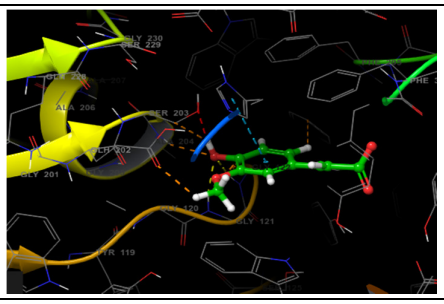
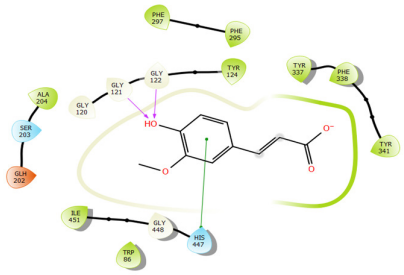
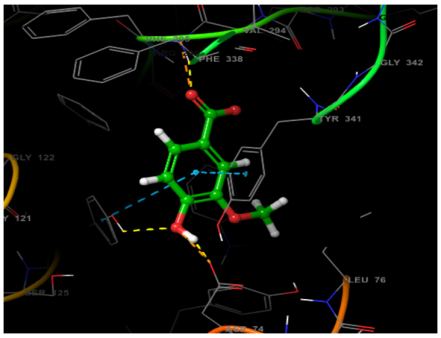
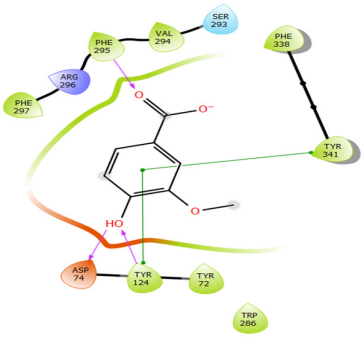
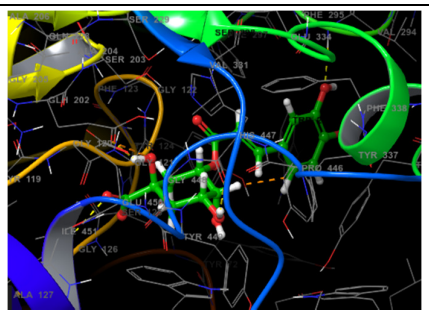
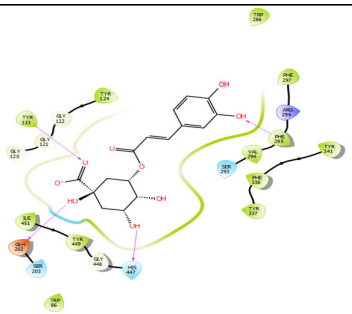
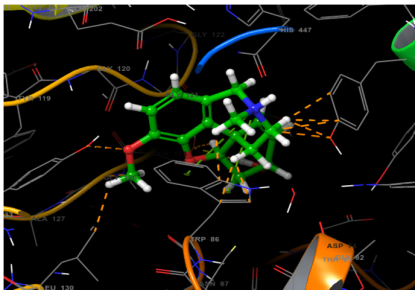
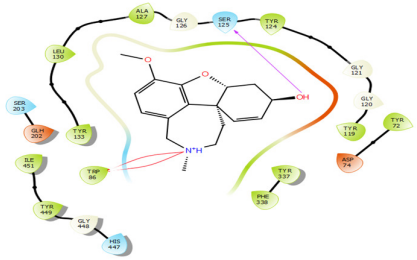
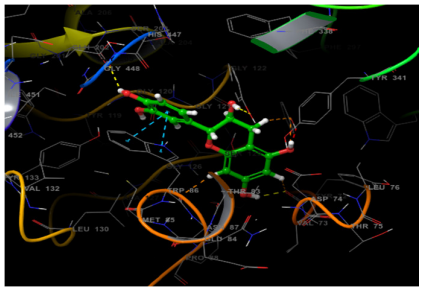
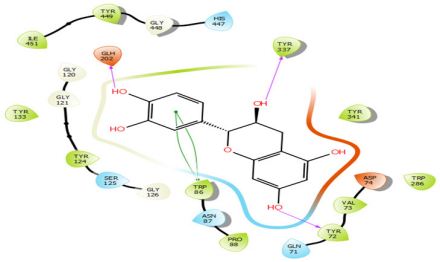
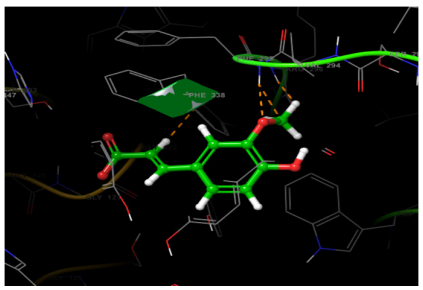
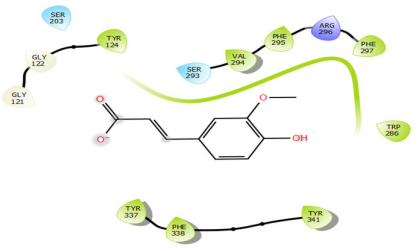
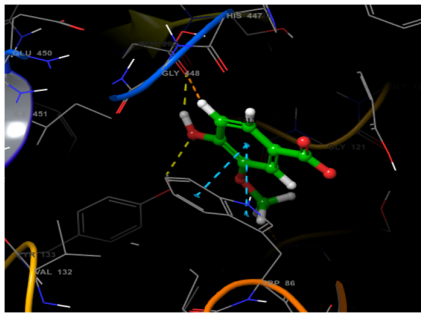
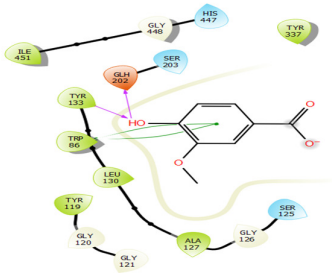
Chlorogenic acid		
Catechin hydrate		
Ferulic acid		
Vanillic acid		

Table 6: Interaction images of all molecules with Crystal Structure of Recombinant Human Acetylcholinesterase in Complex with (-)-galantamine (PDB ID 4EY6).

Compound Name	3D Image	2D Image
Chlorogenic acid		
Reference Galantamine		
Catechin hydrate		
Ferulic acid		
Vanillic acid		

ADMET Prediction: SwissADME was utilised to assess the various ADME (Absorption, Distribution, Metabolism, and Excretion) features of the substances. Using SwissADME, the compounds' diverse ADME properties were assessed. These properties cover a broad spectrum

of important variables, providing a thorough understanding of the pharmacokinetic and drug-like properties. Among the assessed features are molecular weight, the quantity of Rule of Five infractions, GI Absorption, Bioavailability Score, Synthetic accessibility, and the anticipated blood/brain partition coefficient (Log BB) and octanol/water partition coefficient (Log Po/w). The pharmacological potential of the compounds is elucidated by these comprehensive investigations, which aid in determining their suitability for medication development. Table 7 displays the ADME analysis of compounds conducted by SwissADME.

Table 7: ADME study of molecules by SwissADME.

Sr.no.	Compound Name	MW	H-bond Acceptor	H-bond Donors	Log P	GI Absorption	BBB Permeant	Bioavailability Score	Lipinskin Violation	Synthetic Accessibility
1	Chlorogenic Acid	354.31 g/mol	9	6	0.96	Low	No	0.11	1	4.16
2	Catechin Hydrate	308.28 g/mol	7	6	1.17	High	No	0.55	1	3.60
3	Vanillic Acid	168.15 g/mol	4	2	1.40	High	No	0.85	0	1.42
4	Ferulic Acid	194.18 g/mol	4	2	1.62	High	Yes	0.85	0	1.93
5	Reference Donepezil	379.49 g/mol	4	0	3.92	High	Yes	0.55	0	3.36
6	Reference Galantamine	287.35 g/mol	4	1	2.64	High	Yes	0.55	0	4.57

To predict toxicity for the optimal score obtained from the MM-GBSA study and Docking Score, ProTox-II was employed. As indicated in Table 8, the compounds were additionally subjected to toxicity prediction via the ProTox- II portal for hepatotoxicity, carcinogenicity, mutagenicity, cytotoxicity, and immunotoxicity.

Table 8: Toxicity study of molecules by ProTox-II.

S. No.	Compound Name	ProTox II Class	Hepatotoxicity	Carcinogenicity	Immunotoxicity	Mutagenicity	Cytotoxicity
1	Chlorogenic Acid	5	Inactive	Less Inactive	Active	Inactive	Inactive
2	Catechin Hydrate	6	Inactive	Inactive	Inactive	Less Active	Inactive
3	Vanillic Acid	4	Less Inactive	Less Inactive	Inactive	Inactive	Inactive
4	Ferulic Acid	4	Less Inactive	Less Inactive	Active	Inactive	Inactive

5	Reference Donepezil	4	Inactive	Active	Active	Less Inactive	Active
6	Reference Galantamine	3	Inactive	Less Inactive	Active	Inactive	Less Active

DISCUSSION

Comparing the two phytoconstituents with the other, chlorogenic acid and catechin hydrate shown superior anti-Alzheimer effects, according to a molecular docking research. The crystal structure of recombinant human acetylcholinesterase in complex with (-)-galantamine and the binary complex of native hAChE with donepezil both displayed remarkable binding affinities, according to docking studies. A larger negative value, as determined by the MM-GBSA binding free energies, denotes more favourable interactions between the ligands and target proteins. According to the ADMET analysis of all the chemicals, donepezil and galantamine belong in classes 3 and 4, respectively, while vanillic acid, ferulic acid, chlorogenic acid, and catechin hydrate appear in classes 5, 6, 4, and 5. All four phytoconstituents lacked hepatotoxicity, cytotoxicity, carcinogenicity, and mutagenicity. These phytochemicals are suitable for use in pharmaceutical applications because they are not associated with any significant adverse outcomes.

CONCLUSION

This molecular docking study directed excellent outcomes revealed by the interaction of several molecules with the crystal structure of recombinant human acetylcholinesterase in complex with (-)-galantamine. Conspicuously, chlorogenic acid shows highest docking score than the reference stranded galantamine. After galantamine, catechin hydrate ferulic acid and vanillic acid exhibit docking score. However, any binding interactions does not show by Ferulic acid. In contrast, donepezil as reference standard showed highest docking score than other four phytochemicals when molecules interaction with the binary complex of native hAChE. This was followed by chlorogenic acid, catechin hydrate, ferulic acid and vanillic acid. ADMET study of these all molecules reveals the potential effects which suggest that these phytochemicals don’t show any adverse effect. Toxicity study direct that the exception of vanillic acid and catechin hydrate other compounds showed immunotoxicity. While, donepezil was linked to carcinogenicity, immunotoxicity, and cytotoxicity. Whereas galantamine demonstrated immunotoxicity and less cytotoxicity. This study conclude that the chlorogenic acid and catechin hydrate may target the Alzheimer disease and work well together to treat to

treat this condition. However, further in vivo research is needed to evaluate the therapeutic potential of these phytochemicals.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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None

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Human participants or animal experiments were not used in this study. Since this study did not include human participants, informed consent was not applicable.

ABBREVIATIONS

hAChE = Human acetylcholinesterase

ADMET= Adsorption, degradation ,metabolism, excretion, toxicity.

OPLS2005 = Optimized potentials for liquid simulations 2005

XP = Extra precision

MM-GBSA = Molecular mechanics generalized born surface area

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