

In Silico Screening of Bioactive Phytochemicals from *Centella asiatica* (L.) Urb. as Human Acetylcholine Esterase Inhibitors for Cognitive Enhancement.

¹Savitha S Desai, ²Savita Sajjan, ³Basavaraj S Hungund, ⁴Anant Kumar

ABSTRACT:

Background: Dementia is a neurodegenerative disease characterized by progressive impairment of cognitive abilities and has become a significant global health concern. One approach is to focus on inhibiting acetylcholinesterase. Phytochemicals from *Centella asiatica* (L.) Urb. have been associated with cognitive enhancement and neuroprotection. In the present study, in silico docking of phytochemicals against human acetylcholinesterase (AChE) was performed to identify the phytochemical with the best binding to the catalytic site. **Materials and methods:** Bioactive phytochemicals associated with *Centella asiatica* (L.) Urb. were identified using IMMPAT, and their structures were retrieved from PubChem. These were used as ligands. The crystal structure of AChE obtained from the PDB was used as the receptor/protein for docking. AutoDock Vina and PyRx were used for docking. Two-dimensional (2D) and three-dimensional (3D) structures, and the interaction between the ligand and the protein, were evaluated using Discovery Studio and LigPlot+ analysis. ADMET properties were also analysed for the ligands. **Results:** Among the four phytochemicals docked against human acetylcholinesterase, asiaticoside showed the highest negative binding energy (-9.3 Kcal/mol), followed by Asiatic acid (-8.8 Kcal/mol), madecassic acid (-8.3 Kcal/mol), madecassoside (-8.2 Kcal/mol), and standard donepezil (-7.5 kcal/mol). Molecular docking revealed favorable binding of the ligands to AChE through hydrogen-bonding and hydrophobic interactions, indicating their potential to interact with the enzyme. Drug-likeness properties indicate that Asiatic acid and madecassic acid pass Lipinski's Rule of Five, while asiaticoside and madecassoside failed due to their bulky structure. ADME analysis indicated good properties, suggesting their acceptance as computationally prioritized candidates. **Conclusion:** Asiatic acid demonstrated favorable drug-likeness and ADME properties, despite asiaticoside showing a high docking score with AChE. This indicates that phytochemicals from *Centella asiatica* (L.) Urb. have potential as drug candidates for AChE inhibition. However, further in vitro and in vivo studies are needed to validate its efficacy and safety.

KEYWORDS: ADMET analysis, *Centella asiatica* (L.) Urb., Human acetylcholine esterase, Molecular docking, Phytochemicals.

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Corresponding Author Email:

joshisavitha@gmail.com

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1. INTRODUCTION

As per the World Health Organization (WHO), dementia is more of a syndrome than a disease, which is primarily categorized by the progressive decline of memory and cognitive functions. [1] It is now termed as Major Neurocognitive Disorder (MND) since its definition has been updated as per the DSM-5 criteria. [2] It can be categorized into two: those that are "neurodegenerative" (originally called "irreversible") and non-neurodegenerative (potentially "reversible"). [3] There are various causative factors for Dementia, among them, Alzheimer's disease contributes to seventy percent of all cases. [4] Some other causes of dementia are traumatic brain injury, vascular disease, and frontotemporal lobar degeneration. [5] So far, 47 million people are affected, and this number may rise to 131 million by 2050. [6] It is observed that the number of patients with dementia doubles every five years. [7] Additionally, a global burden of disease study conducted by the WHO and according to World Bank estimation, dementia results in 4.1% of all disability-adjusted life years (DALYs). [8, 9] Dementia is associated with low levels of the neurotransmitter acetylcholine (ACh), neuroinflammation, and oxidative stress in the brain. ACh is a neurotransmitter that is involved in memory, learning, and other behaviors. [10, 11] Therefore, increased acetylcholine during neurotransmission can improve a patient's memory. [12] A key therapeutic target for dementia management is the inhibition of acetylcholinesterase (AChE). [13] Acetylcholinesterase stops impulse transmission by the rapid hydrolysis of ACh to acetic acid and choline. [14] Inhibiting AChE in the cholinergic system is a well-established way for treating neurodegenerative diseases, as it helps increase ACh levels. AChE inhibitors are classified as reversible or irreversible, with the former preferred for therapeutic applications due to their safer profile, whereas irreversible inhibitors are associated with toxicity. Individuals diagnosed with Mild Cognitive Impairment may either transition to major neurocognitive disorders such as dementia, [15] stabilize, or revert to normal cognition at

early stages. [16, 17] Due to the neuronal cell loss in dementia, there is no curative treatment available so far. [18] Donepezil, tacrine, eserine, rivastigmine, and galantamine are the major drugs used for the treatment of Alzheimer's disease, which have several side effects like tremors and nausea. [18-21] Meanwhile, natural products from medicinal plants are known for their safety and efficacy. [22] *Centella asiatica* (L.) (CA), also known as "Gotu kola," has been used for many years in Ayurvedic medicine to improve memory, enhance cognition, alleviate anxiety, and support brain function. [23] The plant is abundant in phytochemicals, including flavonoids and triterpenoids, many of which have been reported to have anti-inflammatory, antioxidant, and neuroprotective properties. [24] The biological activity is primarily due to triterpene compounds such as asiatic acid, asiaticoside, madecassic acid, and madecassoside. These compounds have been reported to enhance cognitive function through cholinesterase inhibition. [25,26,27] To investigate the interaction between *Centella asiatica* phytoconstituents and acetylcholinesterase (AChE), the crystal structure of human AChE (PDB ID: 4PQE) was selected as the molecular docking target.

We hypothesize that selected phytochemicals identified in this plant can bind effectively to the active site of human acetylcholinesterase (AChE) and exhibit binding affinities comparable to those of known AChE inhibitors. We further hypothesize that these phytoconstituents possess favorable drug-likeness, pharmacokinetic, and toxicity profiles as predicted by in silico ADMET analyses. Therefore, compounds demonstrating strong AChE interactions together with acceptable ADMET characteristics may serve as promising lead candidates for the development of novel therapeutic agents against Alzheimer's disease.

Tools such as PyRx, Discovery Studio and LigPlot+ were used for molecular docking, to analyze ligand–enzyme interactions and their hydrogen bonding and hydrophobic interaction respectively at the molecular level. [28] These analyses help us to understand the mechanism of

interaction of phytochemical with AchE. Further ADMET and Lipinski's Rule of Five criteria [29] were used, to further analyze its pharmacokinetics.

Several reports studied the neuroprotective potential of *Centella asiatica* and reported molecular docking analyses of selected phytochemicals against neurological targets, including acetylcholinesterase. However, many of these investigations did not incorporate drug-likeness, pharmacokinetic, and toxicity evaluations. Therefore, the present study was undertaken to perform an integrated in silico assessment of major *C. asiatica* phytochemicals against human acetylcholinesterase, coupled with ADMET profiling and toxicity prediction, to prioritize compounds with favorable therapeutic potential. [30] Despite its promising effects on brain health, limited long-term clinical evidence is available to support the use of *Centella asiatica* for dementia and memory-related disorders. As a result, in silico approaches are increasingly being used to investigate the molecular interactions, pharmacological properties, and cognitive-enhancing potential of bioactive compounds, thereby establishing a scientific foundation for future experimental validation and detailed investigations into disease-relevant mechanisms. [31]

2. MATERIALS AND METHODS

Molecular docking studies: Molecular docking is a computational method used to predict interactions between ligands and target proteins or receptors. It also estimates the binding affinity of molecules.

Selection and preparation of ligand: Four bioactive phytoconstituents of *Centella asiatica*— asiaticoside, madecassoside, Asiatic acid, and madecassic acid were selected for the study based on the information on the Indian Medicinal Plants, Phytochemistry and Therapeutics database (IMPPAT) database <https://cb.imsc.res.in/imppat/> accessed on December 2025 and on previous reports. [10] The structures of ligands asiaticoside ([11954171](#)), madecassoside([24825675](#)), Asiatic acid ([119034](#)), and madecassic acid ([73412](#)) were retrieved from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov>) in SDF/MOL

format ([Figure 1](#)) accessed in January 2026. Donepezil ([5741](#)) was used as a standard.

The structures of ligands were processed using the Open Babel module integrated within PyRx and converted to PDB format. The stereochemical configurations available in the source database were retained throughout the study. Polar hydrogen atoms were added, and partial atomic charges were assigned automatically during ligand preparation. The ligands were subsequently converted into PDBQT format, and rotatable bonds were automatically defined using the AutoDock protocol implemented in PyRx. The ligands were maintained in their default protonation/ionization states as provided by the source database and PyRx/Open Babel workflow. Donepezil, a clinically approved acetylcholinesterase inhibitor, was included as a positive control for comparative analysis.

Protein selection and preparation: The three-dimensional crystal structure of human acetylcholinesterase (AChE) was retrieved from the RCSB Protein Data Bank (<https://www.rcsb.org>), PDB ID: 4PQE ([Figure 1](#)), accessed during January 2026. The reason for selecting PDB ID 4PQE was mainly because it represents the human acetylcholinesterase enzyme, the principal therapeutic target in Alzheimer's disease. [32] The structure was determined by X-ray crystallography with a resolution of 2.60 Å. Chain A was selected for molecular docking studies as it contains the complete catalytic domain of the enzyme. PDB ID 4PQE was chosen because it represents the human form of AChE and provides a well-resolved structural model suitable for investigating ligand–protein interactions.

Protein preparation was performed using UCSF Chimera and PyRx. The native ligand, water molecules, and other non-essential heteroatoms were removed from the protein structure. Missing hydrogen atoms were added. The cleaned receptor structure was imported into PyRx and converted into an AutoDock macromolecule. Polar hydrogen atoms and Kollman charges were assigned, and the receptor was saved in PDBQT format. The prepared

protein was subsequently employed for molecular docking against the selected phytochemical ligands.

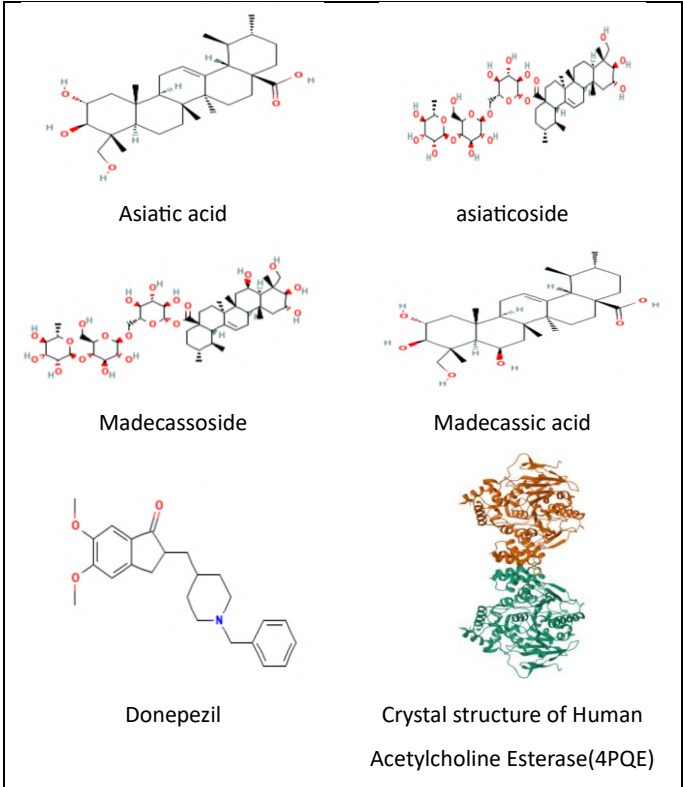


Figure 1: 2-D Structure of ligands and crystal structure of acetylcholine esterase.

Structural validation of protein: The protein structure was validated using PROCHECK. Secondary structure analysis was performed using PDBsum (<https://www.ebi.ac.uk/pdbe/>). It was accessed during February 2026. Binding pocket

Table 1: Grid box parameters used for molecular docking (PyRx 0.8; exhaustiveness = 8 for all compounds; num_modes = 9; energy_range = 3 kcal/mol; single run; default seed)

Compound	Grid Center X (Å)	Grid Center Y (Å)	Grid Center Z (Å)	Dimension X (Å)	Dimension Y (Å)	Dimension Z (Å)
Donepezil (standard)	-21.2488	20.4863	0.6650	78.6038	72.4468	25.0000
Asiatic acid	-27.0923	25.3141	0.6650	89.1998	86.8049	28.5416
asiaticoside	-23.1987	18.6613	0.6650	87.8512	88.9725	31.2876
Madecassoside	-23.8650	22.8662	0.6650	111.3172	79.6034	25.0000
Madecassic acid	-24.4945	24.5012	0.6650	77.7077	80.2974	32.3852

Prediction of drug-likeness: The pharmacokinetic, drug-likeness, and toxicity profiles of Donepezil and the selected *Centella asiatica* phytoconstituents (Asiatic acid, asiaticoside, madecassic acid, and madecassoside) were evaluated using the SwissADME, pkCSM, and ProTox-3.0 online platforms. Canonical SMILES representations of the

identification was performed using the CASTp 3.0 (Computed Atlas of Surface Topography of Proteins) server (<https://sts.bioe.uic.edu/castp/>), accessed in February 2026. The cleft and tunnel analysis was carried out using CASTp. [33]

Molecular docking: Molecular docking of the selected compounds against AChE was performed using the molecular docking software PyRx 0.8. The torsion angles of the ligands were determined during docking. The docking grid was centered on the predicted active site of AChE. Grid box dimensions were adjusted to accommodate each ligand's size while ensuring complete coverage of the binding pocket (Table 1). The final docked pose was selected based on the lowest free binding energy and used for analysis in Discovery Studio Visualizer ver21.1.0.20298, accessed during April-May 2026.

Analysis using LigPlot+: Protein–ligand interactions were analyzed using LigPlot+ (<https://www.ebi.ac.uk/>, European Bioinformatics Institute, UK), which generates two-dimensional diagrams of binding interactions and was used to identify and visualize hydrogen bonds and hydrophobic interactions between the ligand and receptor, thereby providing insights into ligand-binding affinity and interaction mechanisms. [32]

compounds were retrieved from the PubChem database, converted using Open Bable and used as input for the respective prediction servers. [34-39]

SwissADME (<https://www.swissadme.ch>, accessed during May 2026) was employed to assess physicochemical characteristics, lipophilicity, water solubility, gastrointestinal

absorption, blood–brain barrier permeability, bioavailability, and compliance with established drug-likeness criteria such as Lipinski's Rule of Five. The pkCSM platform was used to predict key pharmacokinetic parameters related to absorption, distribution, metabolism, and excretion, including intestinal absorption, volume of distribution, plasma protein binding, cytochrome P450 interactions, and total clearance. pkCSM (<https://biosig.lab.uq.edu.au/pkcsml>) and ProTox-3.0 (<https://tox.mhazau.edu.cn/protox3>), were used to evaluate the toxicological properties of the ligands, which provided prediction of toxicity endpoints, including Ames mutagenicity, hepatotoxicity, skin sensitization, hERG inhibition, and acute oral toxicity (LD₅₀), using computational tools. The predicted results were compared to assess the potential safety profiles of the investigated compounds. This integrated in silico approach aided in identifying phytochemicals with favorable drug-like characteristics and acceptable toxicity profiles for further investigation as potential acetylcholinesterase inhibitors.

3. RESULTS AND DISCUSSION

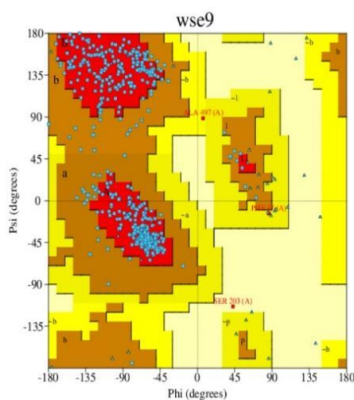


Figure 2: Ramachandran plot

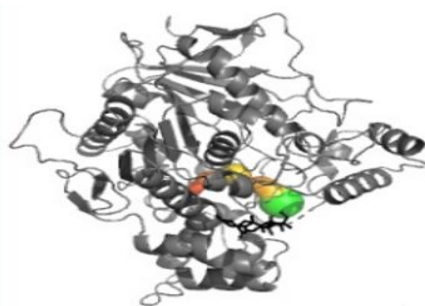


Figure 4: Tunnel analysis of the protein structure

The results of docking and ADME properties of the ligands are presented in this section.

Structural validation of the protein: The protein structure validation was done using PROCHECK. The Ramachandran plot (Figure 2) analysis indicates that 85.3% of residues are in the most favored regions; 14.0% are in the allowed regions, indicating suitability of protein structure for docking. The secondary structure of protein is depicted in Figure 3 which shows the presence of alpha-helices (purple color), which are involved in conferring structural stability. It also contains beta-sheet (yellow) and coils and turns. All these contribute to stability and ligand interaction during docking.

The presence of tunnels in the protein was analyzed using Mole 2.0 server (Figure 4). These tunnels facilitate substrate entry, product exit, and ligand transportation. The cleft analysis (Figure 5) indicates the presence of Cleft 1, having a large volume (4946.91 Å³), implying that it is the active site for the binding of ligands. Amino acids in the active site of the enzyme include Ser 203, His 447, and Glu 334.

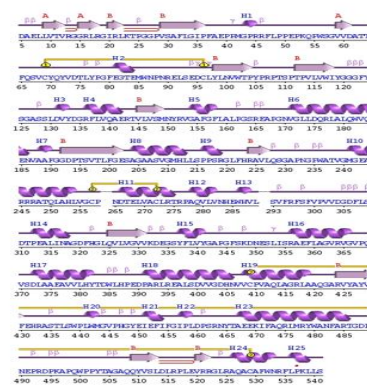


Figure 3: Secondary structure organization of protein

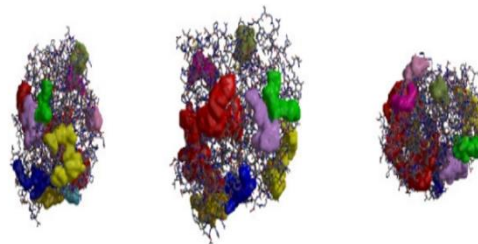


Figure 5: Cleft/Binding pocket analysis of the protein

Molecular docking: acetylcholinesterase is an important target in Alzheimer's disease because it is involved in aggregation of A β fibrils. An approach to slow down disease progression is by modifying the enzyme activity. The present study aimed to evaluate the interaction of metabolites of *centella asiatica* and AChE. Metabolites included for study were asiaticoside, Asiatic acid, madecassoside, madecassic acid, and the reference drug donepezil. It was observed that asiaticoside exhibited the most favorable docking score (-9.3 Kcal/mol), followed by Asiatic acid (-8.8 Kcal/mol), madecassic acid (-8.3 Kcal/mol), and madecassoside (-8.2 Kcal/mol). These values exceed those of donepezil (-7.5 kcal/mol), suggesting stronger interactions and potentially superior inhibitory activity, as indicated by the binding energy analysis (Table 2). While the more negative docking score for Asiatic acid indicates a thermodynamically favorable predicted pose, the in-silico binding energy is not directly equivalent to experimental inhibitory potency; this finding should be interpreted as supportive rather than confirmatory evidence and warrants kinetic validation. These results support the use of phytochemicals from *Centella asiatica* and provide a strong rationale for further in vitro and in vivo validation in AD therapy development. These results are similar to those obtained by Ekka *et al.* [32]

The interaction of protein and ligand, the close-up view, amino acid interaction and surface interaction, hydrogen bond, hydrophobicity, ionization, and SAS for each ligand-protein interaction and standard are shown in Figures 6 - Figure 10. The ligand-protein docked structures were visualized with LigPlot+ to assess amino acid interactions. The docked best pose structure of Asiatic acid and enzyme and its close up view has been shown in Figure 6a and Figure 6b. The docked structure was visualized in Discovery Studio to assess the interactions. The LigPlot+ analysis of Asiatic acid (Figure 6c) and the enzyme reveals the presence of hydrogen- and hydrophobic interactions. The amino acids involved in hydrogen bonding are Gln 413 (2.98 Å), Asn 533 (3.01 Å), and Glu 313 (3.19 Å), and the hydrophobic

interactions include Val 370, Pro 410, and Leu 540. The Glutamate 313 residue is near the enzyme's active site, indicating a predicted interaction with the binding site. The close-up view of the interaction is shown in Figure 6d. The hydrogen-bond surface reflects that ligand is surrounded by hydrogen-bond donor and acceptor regions which suggest that there exist stable polar interactions contributing to binding affinity (Figure 6e). While the hydrophobicity map shows that much of the ligand occupies hydrophobic regions of the pocket, promoting van der Waals and hydrophobic interactions essential for complex stabilization (Figure 6f). The ionizability surface highlights that near the binding site acidic and basic regions, indicating the potential for electrostatic interactions with the ligand's ionizable functional groups (Figure 6g). Additionally, the solvent-accessible surface (SAS) analysis demonstrates that large part of the ligand is buried within the protein cavity, with minimum solvent exposure, reflecting strong ligand encapsulation and favorable binding (Figure 6h). Collectively, these interaction profiles support the formation of a stable protein-ligand complex with significant contributions from hydrogen bonding, hydrophobic contacts, electrostatic complementarity, and optimal surface accessibility.

The docked complex of asiaticoside and human acetylcholinesterase is shown in Figure 7a and close up view is depicted in Figure 7b. The LigPlot+ analysis revealed various interactions like hydrogen bond Thr 238 (1.93 $^{\circ}$ A), Arg 269 (2.06 $^{\circ}$ A), Gln 369 (2.34 $^{\circ}$ A), His 405 (2.64 $^{\circ}$ A), Gln 413 (2.76 $^{\circ}$ A), Asn 533(2.76 $^{\circ}$ A), and hydrophobic interaction involves Pro235 and Pro 537 amino acid residue (Figure 7c). The surface interaction is shown in Figure 7d. The close-up view and the presence of hydrogen donors and acceptors in the vicinity of the ligand suggest hydrogen-bond interactions (Figure 7d-7e). The ligand also interacts with hydrophobic amino acids (Figure 7f), thereby stabilizing the complex. The presence of acidic and basic amino acids results in electrostatic interaction (Figure 7g). The SAS (Fig 7h) indicates the solvent interaction is limited.

[Figure 8a](#) illustrates the docked complex of madecassoside and enzyme and [Figure 8b](#) provides a close-up view of the interaction. Interaction analysis using LigPlot+ revealed the hydrogen bonding between His 405 (2.96°A,2.24°A), Asn233 (2.95°A), Glu 313 (3.17°A, 3.02°A), Thr 238 (3.10°A, 2.99°A, 2.93°A), Gly240 (2.99°A), Pro 235(2.59°A,2.32°A), Arg296 (2.81°A) and hydrophobic interaction with amino acids like Pro 410, Thr 311, Pro 312, Gly234, Val 303, Val 239, Glu 243, Gln369, Leu 540, Pro537 ([Figure 8c](#)) as depicted by LigPlot+ analysis. The hydrophobic, electrostatic, and solvent interactions ([Figure 8d - Figure 8h](#)) indicate that they help stabilize the ligand in the catalytic pocket and, hence, catalysis. The predicted pose of binding of madecassic acid to enzyme is shown in [Figure 9a](#) and close up view is shown in [Figure 9b](#). Interaction analysis revealed the hydrogen bonding between Gly 240(2.99°A), Glu 313(2.71°A), Gln 413 (3.1°A), and hydrophobic interaction includes Val 303, Thr 238, Pro 235, Gly 234, Asn 233, Trp 532, Asn 533, Leu 540, Leu 536, Val 239, Thr 311 ([Figure 9c](#)). The hydrophobic,

electrostatic, and solvent interactions at the surface ([Figure 9d- Figure 9h](#)) reveal the presence of various amino acids in the region, which help stabilize the ligand in the pocket. The predicted docking confirmation of standard donepezil and enzyme is shown in [Figure 10a](#) and its close-up view is shown in [Figure 10b](#). Interaction analysis revealed the hydrogen bonding between Arg 247(2.86, 3.02 Å) and hydrophobic interaction, including Arg 296, Pro 368, Val 370, His 405, Trp 532, Asn 533, Pro 537, Leu 536, Leu 540, Pro 235, and Glu 369 ([Figure 10c](#)). The hydrophobic, electrostatic, and solvent interactions at the surface ([Figure 10d- Figure10h](#)) reveal the presence of various amino acids in the region, which help stabilize the ligand in the pocket. As reported previously, the enzyme has four interaction sites: the active site, anionic site, acyl pocket, and choline-binding site. The amino acids in the active site include His, Ser, and Glu. [40, 41] The ligand is stabilized by hydrophobic alkyl, hydrophobic π -alkyl, and hydrophobic π -sigma interactions with the enzyme. [42]

Table 2: Binding energy of the phytochemicals and standard donepezil.

Sl. No	Ligand	Binding energy (Kcal/mol)	Ligand Efficiency (kcal/mol/atom)	H-bond Residues	Hydrophobic Contacts
1	asiaticoside	-9.3	0.139	Thr 238 (1.93°A), Arg 269 (2.06°A), Gln 369 (2.34°A), His 405 (2.64°A), Gln 413 (2.76°A), Asn 533(2.76°A)	Pro235 Pro 537
2	Asiatic acid	-8.8	0.251	Gln 413 Asn 533 Glu 313	Val 370 Pro 410 Leu 540
3	Madecassoside	-8.2	0.121	His 405 (2.96°A,2.24°A), Asn233 (2.95°A), Glu 313 (3.17°A, 3.02°A), Thr 238 (3.10°A, 2.99°A, 2.93°A), Gly240 (2.99°A), Pro 235(2.59°A,2.32°A),Arg296 (2.81°A)	Pro 410, Thr 311, Pro 312,Gly234, Val 303, Val 239, Glu 243, Gln369, Leu 540, Pro537
4	Madecassic Acid	-8.3	0.231	Gly 240(2.99°A), Glu 313(2.71°A), Gln 413 (3.1°A)	Val 303, Thr 238, Pro 235, Gly 234,Asn 233, Trp 532, Asn 533,Leu 540, Leu 536, Val 239, Thr 311(9c
5	Donepezil	-7.5	0.268	Arg 247(2.86,3.02°A)	Arg 296, Pro 368, Val 370, His 405, Trp 532, Asn 533, Pro 537, Leu 536, Leu 540, Pro 235, Glu 369

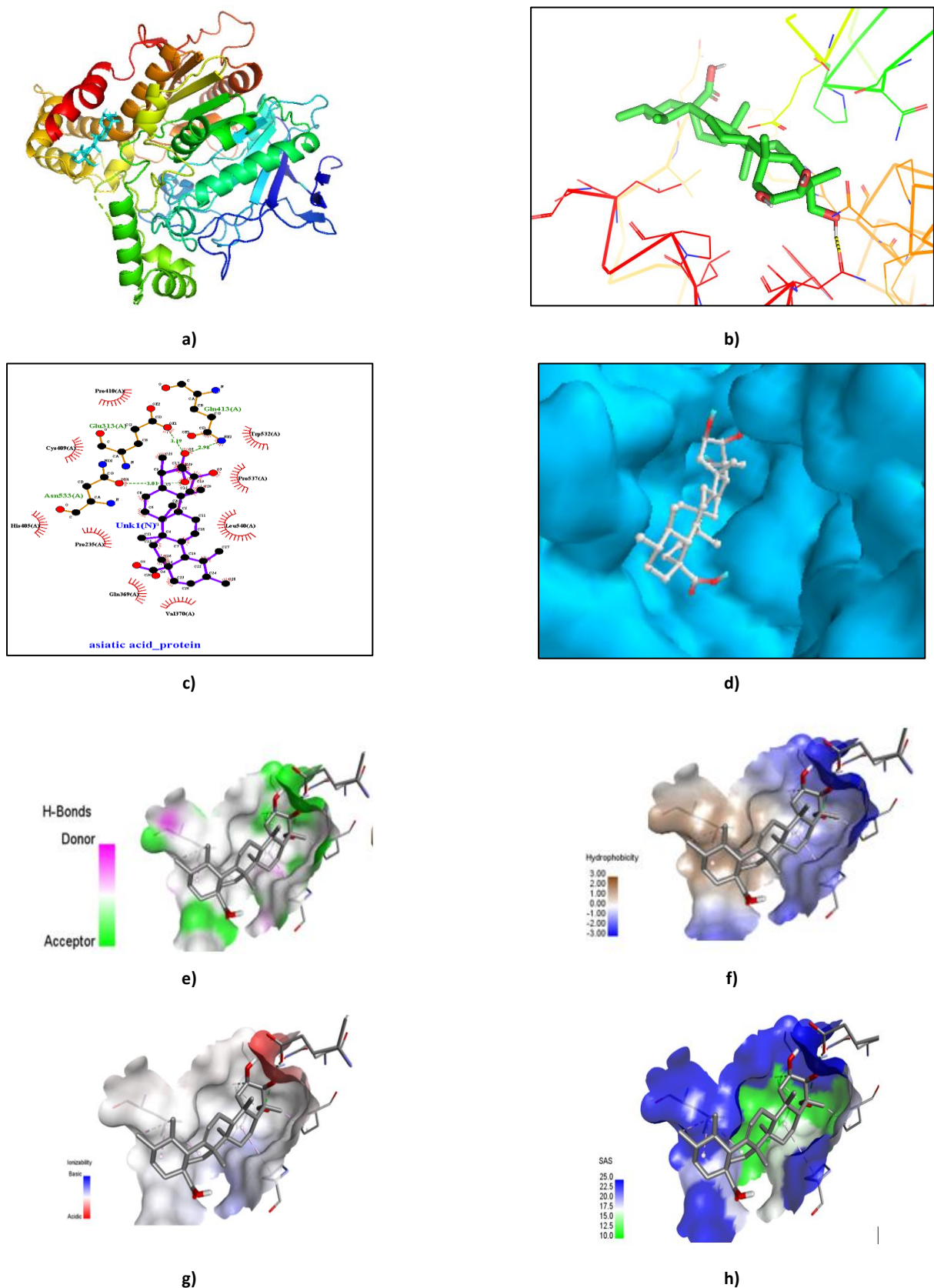


Figure 6: a) Docked ligand Asiatic acid with enzyme b) Close up view of the interaction c) Amino acid interaction d) Surface interaction view e) Hydrogen bond interaction f) Hydrophobicity g) Ionizability h) SAS

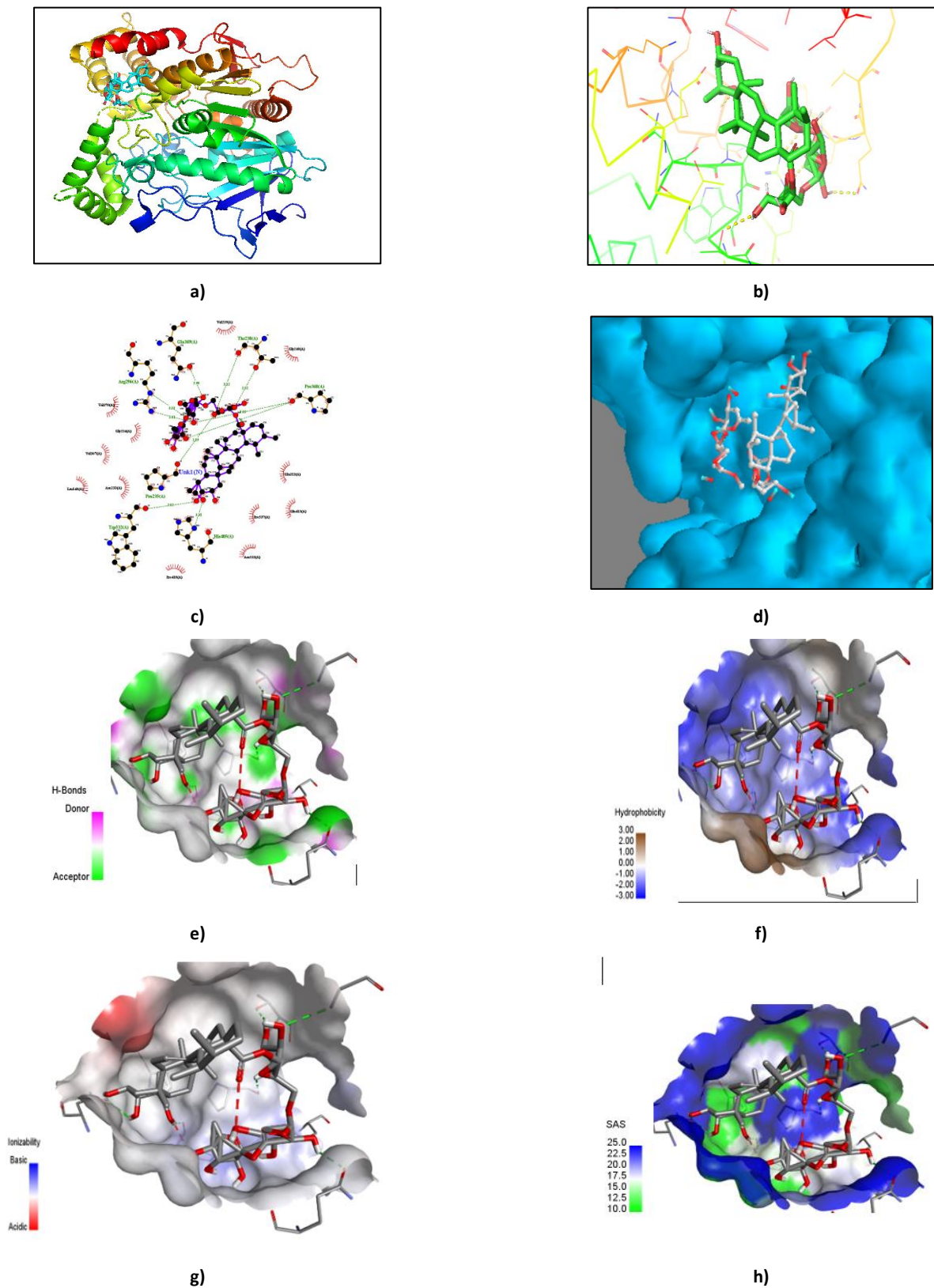


Figure 7: a) Docked ligand asiaticoside with enzyme b) Close up view of the interaction c) Amino acid interaction d) Surface interaction view e) Hydrogen bond interaction f) Hydrophobicity g) Ionizability h)SAS

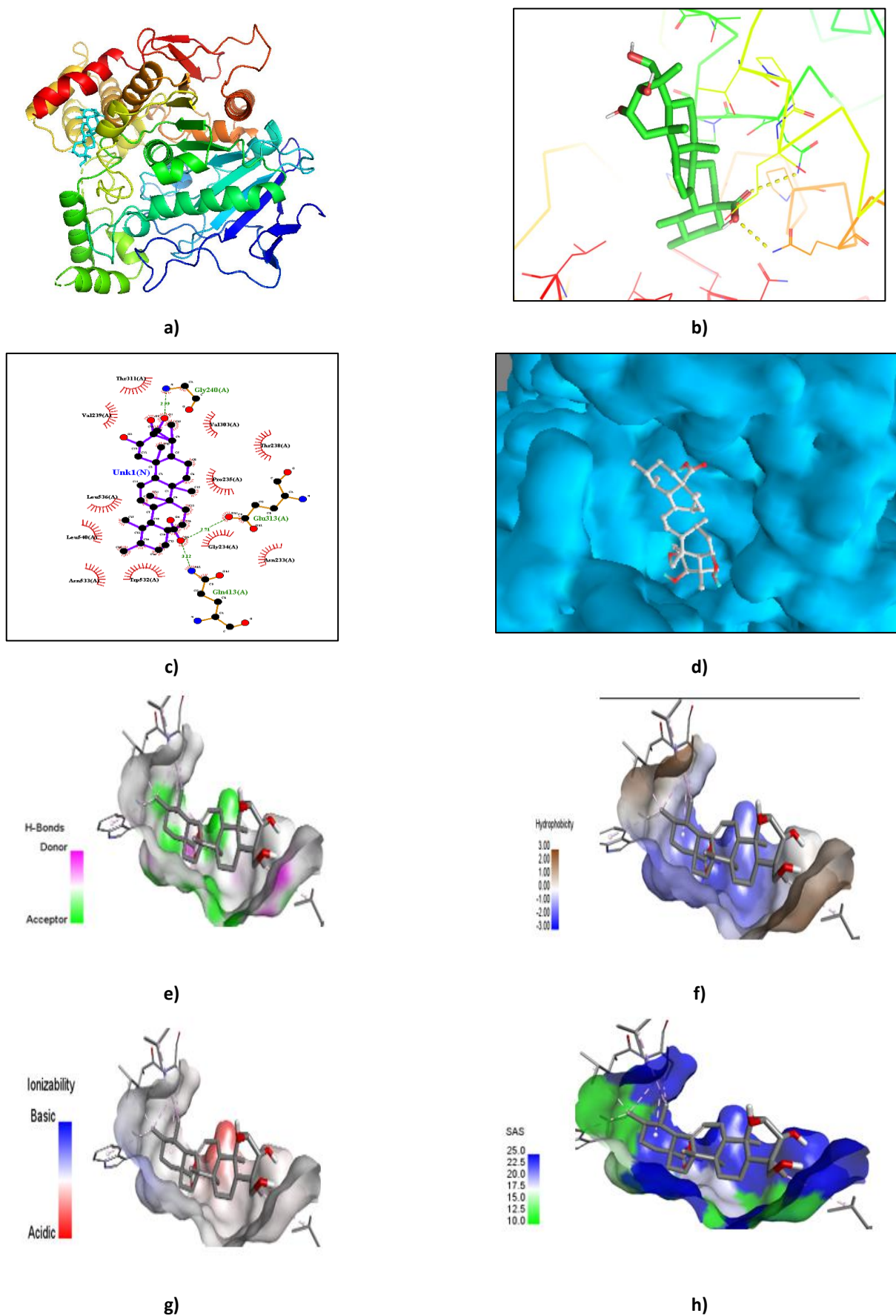


Figure 9: a) Docked ligand madecassic acid with enzyme b) Close up view of the interaction c) Amino acid interaction d) Surface interaction view e) Hydrogen bond interaction f) Hydrophobicity g) Ionizability h) SAS

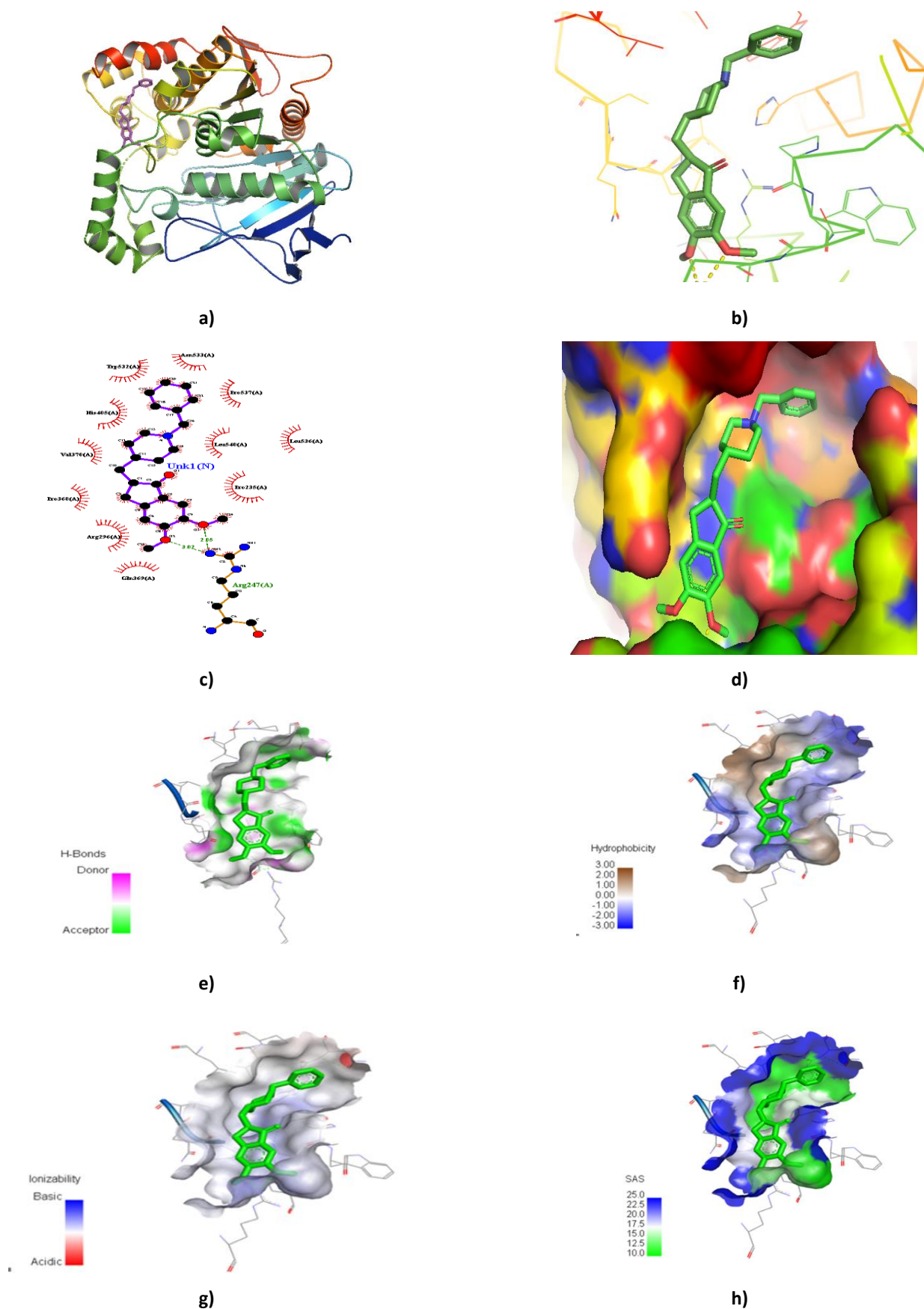


Figure 10: a) Docked ligand donepezil with enzyme b) Close up view of the interaction c) Amino acid interaction d) Surface interaction view e) Hydrogen bond interaction f) Hydrophobicity g) Ionizability h) SAS

Asiatic acid, a pentacyclic triterpenoid, binds within the catalytic domain of AChE through various interactions and contributes to the inhibition of AChE activity. Madecassic acid, a triterpenoid containing an additional hydroxyl group, may form more hydrogen-bond interactions than Asiatic acid, resulting in enhanced stabilization within the active site and potentially stronger inhibitory activity against AChE. asiaticoside is a glycosylated derivative of Asiatic acid. It contains a sugar moiety that provides multiple interactions, but its large structure limits its use as drug. Madecassoside, the glycosylated form of Madecassic acid, contains hydroxyl rich sugar residues and triterpenoid core which contributes to the highest number of hydrogen interaction. It has the capacity of interacting simultaneously with both catalytic-site and peripheral-site residues, potentially conferring strong binding affinity and effectively modulating AChE activity, as predicted by in silico analysis. Its bulky group affects its penetration and limits its use as drug.

Overall, molecular docking suggests that these phytochemicals possess potential inhibitory activity against acetylcholinesterase and interact with key amino acid residues. However, in vivo studies are required before they can be considered active drug molecules.

Drug-likeness and ADMET analysis using in silico tools: The drug likeness of the ligand is shown in [Table 3](#). Only Asiatic acid and Madecassic acid pass the Lipinski Rule; hence,

Table 3: Lipinski/Drug-likeness properties

Property	Asiatic acid	asiaticoside	Madecassic Acid	Madecassoside
Molecular Weight (Da)	488.35	958.51	504.35	974.51
H-bond Donors	4	12	5	13
H-bond Acceptors	5	15	6	20
Rotatable Bonds	2	10	2	10
TPSA (Å²)	97.99	315.21	118.22	335.44
GI Absorption	Low	Low	Low	Low
BBB Permeability	Low (+)	No (--)	Low (+)	No (--)
Lipinski Rule	Accepted	Rejected	Accepted	Rejected
Drug-likeness (QED)	0.409	0.097	0.363	0.087

these two are used for ADMET analysis. All the analyses are done in silico.

Asiatic acid is a pentacyclic triterpenoid with a molecular weight of 488.35 Da that satisfies both Lipinski and Pfizer drug-likeness rules, making it the most drug-like aglycone in this series. Its QED score of 0.409 is the highest among the four compounds, and its moderate logP of 2.942 suggests a reasonable balance between aqueous solubility and membrane permeability ([Table 3](#)). GI absorption is lower, and it shows marginal potential for BBB penetration. The relatively high values of carcinogenicity, skin sensitization, and nephrotoxicity require attention and in vivo validation. Nevertheless, it is accepted by two major drugs- likeness and no CYP enzyme inhibition makes it computationally prioritized candidates requiring in-vitro, in-vivo, and pharmacokinetic validation.

Madecassic acid: The structural feature indicates that it is the hydroxylated analog of Asiatic acid. Its molecular weight is 504.35 Da. It passes the Lipinski rule and has a QED of 0.363, making it the second most drug-like compound. It has a slightly lower logP value than Asiatic acid, indicating it has marginally better aqueous solubility. Like Asiatic acid, it also has low nephrotoxicity and skin sensitization. However, the DILI score is lower, suggesting improved hepatic safety. The carcinogenicity probability is also lower than that of Asiatic acid ([Table 4](#)).

Table 4: Pharmacokinetic and toxicological properties of donepezil, Asiatic acid and madecassic acid.

Property	Model Name	Unit	Donepezil	Asiatic acid	Madecassic acid
Absorption	Water solubility	log mol/L	-4.992	-3.008	-3.037
Absorption	Caco2 permeability	log Papp in 10 ⁻⁶ cm/s	1.234	0.479	0.455
Absorption	Intestinal absorption (human)	% Absorbed	97.374	62.855	57.113
Absorption	Skin Permeability	log Kp	-3.100	-2.735	-2.735
Absorption	P-glycoprotein substrate	Categorical (Yes/No)	Yes	No	Yes
Absorption	P-glycoprotein I inhibitor	Categorical (Yes/No)	Yes	No	No
Absorption	P-glycoprotein II inhibitor	Categorical (Yes/No)	Yes	No	No
Distribution	VDss (human)	log L/kg	0.738	-1.600	-1.555
Distribution	Fraction unbound (human)	Fu	0.121	0.119	0.181
Distribution	BBB permeability	log BB	0.244 (good)	-0.646 (Moderate)	-0.737 (Moderate)
Distribution	CNS permeability	log PS	-1.396	-1.984	-2.944
Metabolism	CYP2D6 substrate	Categorical (Yes/No)	No	No	No
Metabolism	CYP3A4 substrate	Categorical	Yes	Yes	Yes
Metabolism	CYP1A2 inhibitor	Categorical	Yes	No	No
Metabolism	CYP2C19 inhibitor	Categorical	No	No	No
Metabolism	CYP2C9 inhibitor	Categorical	No	No	No
Metabolism	CYP2D6 inhibitor	Categorical	Yes	No	No
Metabolism	CYP3A4 inhibitor	Categorical	Yes	No	No
Excretion	Total Clearance	log ml/min/kg	1.005	0.202	0.212
Excretion	Renal OCT2 substrate	Categorical	No	No	No
Toxicity	AMES toxicity	Categorical	No	No	No
Toxicity	Max. tolerated dose (human)	log mg/kg/day	0.632	0.078	0.085
Toxicity	hERG I inhibitor	Categorical	No	No	No
Toxicity	hERG II inhibitor	Categorical	Yes	No	No
Toxicity	Oral Rat Acute Toxicity (LD50)	mol/kg	2.465	2.592	2.619
Toxicity	Oral Rat Chronic Toxicity (LOAEL)	log mg/kg_bw/day	1.737	0.575	2.672
Toxicity	Hepatotoxicity	Categorical	Yes	No	No
Toxicity	Skin Sensitization	Categorical	No	No	No
Toxicity	T. Pyriformis toxicity	log µg/L	1.217	0.285	0.285
Toxicity	Minnow toxicity	log mM	-0.369	1.106	2.123

Reports establish that glycosidic compounds exhibit neuroprotective activity, improving microglial activity and behavioral dysfunction [43]. It has been demonstrated that Asiatic acid and madecassic acid are potential AChE-targeting compounds, more effective than other triterpenes, [44] supporting the traditional use of *Centella asiatica* as an herbal remedy for memory improvement and cognitive enhancement. It has been reported independently that components of *Centella asiatica*, has

potential inhibitory activity against acetylcholinesterase [45] and to cross the blood-brain barrier [46] as evidenced by experimentation using raw extract. Among different flavonoids and terpenoids, luteolin and quercetin could inhibit AChE inhibition in-vitro. [47] Mechanistically, the predicted interaction of Asiatic acid with the catalytic gorge of acetylcholinesterase (AChE) suggests that it may restrict substrate access to the catalytic site ([Figure 11](#)). Such interaction may result in reduction of acetylcholine

hydrolysis and prolong its availability at the synapse. This would support cholinergic signaling associated with memory formation. However, this proposed mechanism requires experimental validation. Among the four *Centella asiatica* metabolites screened, Asiatic acid exhibited the most favorable docking score and predicted pharmacokinetic profile, identifying it as a computationally promising candidate for further investigation of potential AChE inhibitory activity. Nevertheless, molecular docking and ADMET predictions cannot establish actual enzyme inhibition, neuroprotective activity, pharmacological efficacy, safety, or cognitive-enhancing effects. Therefore, these findings should be considered preliminary and hypothesis-generating. Further validation through in vitro AChE inhibition assays, cell-based neuroprotection studies, and appropriate in vivo pharmacological and toxicological investigations is necessary to assess the biological activity and therapeutic potential of Asiatic acid.

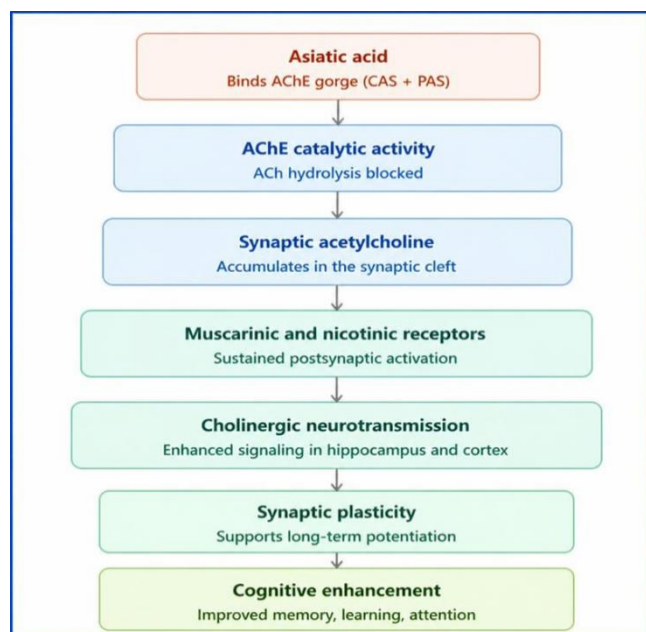


Figure 11: Proposed mechanism of Asiatic acid–AChE interaction.

Limitations of the study: This study is completely based on molecular docking and in silico pharmacokinetic analysis, which needs to be validated experimentally. Since 4PQE lacks a co-crystallized ligand, redocking-based validation could not be performed. However, a standard donepezil was included as a reference inhibitor for comparison, but

this does not substitute for standard docking validation. Molecular dynamics simulations were also not carried out to assess complex stability. Docking score gives an estimate of binding affinity. Future computational and experimental confirmation is needed to validate the results.

4. CONCLUSION

Among the four metabolites selected from *Centella asiatica*, Asiatic acid was identified as a well-balanced metabolite with favorable binding energy, acceptable drug-likeness and pharmacokinetic characteristics. Although the glycosidic derivatives showed comparable or stronger predicted binding affinities, their poor physicochemical and pharmacokinetic properties may limit permeability and oral bioavailability. These findings provide preliminary computational evidence to support the prioritization of Asiatic acid for further investigation. However, molecular docking and ADMET predictions alone cannot confirm AChE inhibition, efficacy, safety, or cognitive-enhancing effects. In vitro AChE assays and in vivo studies are required to validate the biological relevance of the present predictions and to determine the therapeutic potential of Asiatic acid.

Authors Details:

^{1*}P.G. Department of Studies and Research in Biotechnology, KLE'S P.C Jabin science college, Vidyanagar, Hubballi, 580031. Karnataka, India.

ORCID link: <https://orcid.org/0000-0001-8030-0588>

²Professor, Post Graduate and Ph. D Studies, Department of Samhita Siddhant, Ayurveda Mahavidyalaya and Hospital, Hubballi, Karnataka, India. **ORCID link:** <https://orcid.org/0000-0002-2299-1031>

³Professor and Head, Bioresource Development Laboratory, Department of Biotechnology, KLE Technological University, Hubballi–580031, Karnataka, India. **ORCID link:** <https://orcid.org/0000-0002-0971-3715>

⁴3rd Professional BAMS, Ayurveda Mahavidyalaya and Hospital, Hubballi, Karnataka, India. **ORCID link:** <https://orcid.org/0009-0003-2357-9027>

Authors Contribution:

Conceptualization: SS, BSH, AK

Data collection and literature search: AK, SS, SSD

Writing – original draft preparation: SSD, BSH, AK

Reviewing & editing: SSD, BSH, SS

Approval of final manuscript: All authors

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