Journal of Biomolecular Structure and Dynamics

ISSN: 0739-1102 (Print) 1538-0254 (Online) Journal homepage: www.tandfonline.com/journals/tbsd20

Networkodynamic approach to perceive the key phytoconstituents of *E. officinalis* (Amla) as natural BACE1 inhibitors: an *in-silico* study

Dhairiya Agarwal, Jatin Malik, Neeru Bhanwala, Ramesh Ambatwar, Sumit Kumar, Lokesh Chandrakar, Ashok Kumar Datusalia & Gopal L. Khatik

To cite this article: Dhairiya Agarwal, Jatin Malik, Neeru Bhanwala, Ramesh Ambatwar, Sumit Kumar, Lokesh Chandrakar, Ashok Kumar Datusalia & Gopal L. Khatik (2024) Networkodynamic approach to perceive the key phytoconstituents of *E. officinalis* (Amla) as natural BACE1 inhibitors: an *in-silico* study, Journal of Biomolecular Structure and Dynamics, 42:22, 12304-12316, DOI: 10.1080/07391102.2023.2269260

To link to this article: https://doi.org/10.1080/07391102.2023.2269260

JOURNAL OF BIOMOLECULAR STRUCTURE AND DYNAMICS
2024, VOL. 42, NO. 22, 12304–12316
https://doi.org/10.1080/07391102.2023.2269260

Networkodynamic approach to perceive the key phytoconstituents of *E. officinalis* (Amla) as natural BACE1 inhibitors: an *in-silico* study

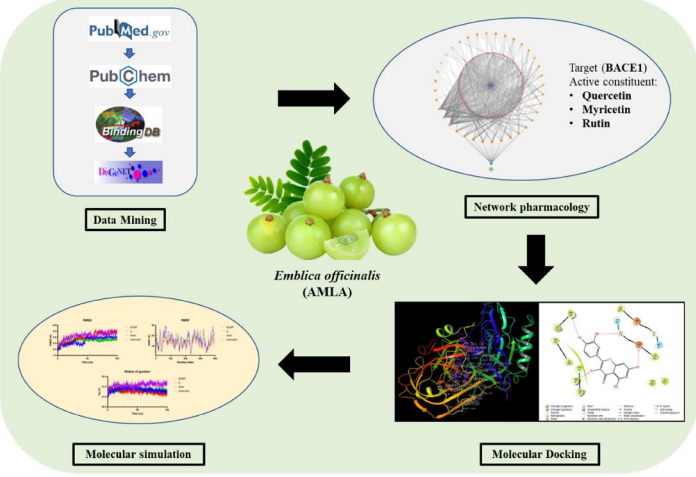
Dhairiya Agarwal[a]*, Jatin Malik[a]*, Neeru Bhanwala[a], Ramesh Ambatwar[a], Sumit Kumar[a], Lokesh Chandrakar[a], Ashok Kumar Datusalia[b] and Gopal L. Khatik[a]

[a]Department of Medicinal Chemistry, National Institute of Pharmaceutical Education and Research, Raebareli, Lucknow, Uttar Pradesh, India; [b]Department of Pharmacology and Toxicology, National Institute of Pharmaceutical Education and Research, Raebareli, Lucknow, Uttar Pradesh, India

Communicated by Ramaswamy H. Sarma

ABSTRACT

Alzheimer's disease (AD) is a deteriorating neural disorder, and currently, available drugs are ineffective in its treatment. *Emblica officinalis* (Amla) is widely recognised in the Indian medicinal system for ameliorative effects in managing diabetes, hyperlipidaemia and neurological diseases. Thus, we aimed to identify the active phytoconstituents of *E. officinalis* and their role in inhibiting the potential targets for the possible treatment of AD. The network pharmacology approach, gene ontology, molecular docking and molecular dynamics simulation (MDS) studies were performed. A total of 36 bioactive components in *E. officinalis*, 95 predicted anti-AD targets, and 3398 AD-related targets were identified from different databases. The network analysis showed that BACE1, ABCB1 and AChE, CA2 are the most potential AD targets. Based on gene ontology and topology analysis results, BACE1 was a significant target related to AD pathways, and quercetin, kaempferol and myricetin showed the highest interaction with target genes. The molecular docking results found that rutin and quercetin displayed better binding affinities −7.5, −5.67 kcal/mol than the BACE1 bound internal ligand. Furthermore, MDS results suggested that quercetin and rutin could be potential inhibitors against BACE-1 protein and may have therapeutic effects in treating AD. Such promising results could be further helpful in new drug discovery against AD.



ARTICLE HISTORY
Received 24 May 2023
Accepted 4 October 2023

KEYWORDS
Network pharmacology; data mining; molecular docking; molecular dynamics simulation (MDS)

CONTACT Gopal L. Khatik gopal.khatik@niperraebareli.edu.in, gopal_niper@rediffmail.com Department of Medicinal Chemistry, National Institute of Pharmaceutical Education and Research, Raebareli, New Transit Campus, Bijnor-Sisendi Road, Near CRPF Base Camp, Sarojini Nagar, Lucknow - 226301, Uttar Pradesh, India
*These authors contributed equally to this work.

Supplemental data for this article can be accessed online at https://doi.org/10.1080/07391102.2023.2269260.

1. Introduction

Neurodegenerative disease, such as Alzheimer's disease (AD), is the furthermost prevalent type of dementia. In the world, about 50 million persons are already suffering from AD. If no therapies or preventative measures are discovered soon. In that case, that may significantly rise to 150 million by 2050 ("2022 Alzheimer's Disease Facts and Figures," 2022). Several variables, including acetylcholine (AChE) insufficiency, aberrant amyloid-β (Aβ) build-up, tau hyperphosphorylation and oxidative stress, are believed to play a crucial part in the complicated aetiology of AD. The build-up of internal neurofibrillary tangles containing tau protein and extracellular amyloid plaques carrying Aβ-peptide. Aβ is the neuropathological hallmark of AD. Therefore, several researchers choose treatment techniques that target the β-sheet formation and aggregation of Aβ since these processes are thought to be crucial ones that enable these peptides to become neurotoxic. Aβ is a compact protein consisting of 39–43 amino acids in different biophysical forms. It can be categorised into two main isoforms: soluble $A\beta_{40}$ and insoluble $A\beta_{42}$. Among these, $A\beta_{42}$, found in higher concentrations in individuals with AD, has a greater tendency to form aggregates (Burdick et al., 1992). Aβ is produced through the proteolytic cleavage of a transmembrane protein called amyloid precursor protein (APP), which is highly conserved throughout evolution. This cleavage process involves the actions of β-secretase and γ-secretase. The mutations in the APP gene are the primary causes of familial AD (De Strooper, 2003). The production of Aβ occurs through the amyloidogenic pathway, as illustrated in (Figure 1).

β-secretase, specifically beta-site APP cleaving enzyme 1 (BACE-1) and BACE2, are responsible for generating Aβ, while γ-secretase is a complex composed of presenilins (PSEN1 or PSEN2), Nicastrin and Presenilin enhancer 2 (PEN2). Numerous studies have demonstrated that manipulating these secretase can influence Aβ production. For instance, when α-cleavage is abolished in cells lacking ADAM17, Aβ generation is affected. A complete depletion of neuronal Aβ secretion occurs when BACE-1 is knocked out in mice. The mutations in PSEN1 and PSEN2 impacted APP cleavage, leading to alterations in Aβ production (Cai et al., 2001).

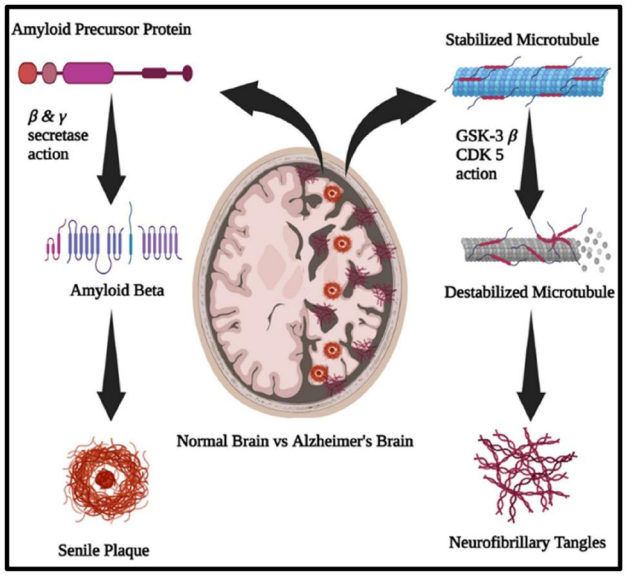


Figure 1. Pathophysiology of AD. GSK-3β: glycogen synthase kinase-3 beta; CDK 5: cyclin-dependent kinase 5.

Human chromosome 21 is known to harbour the gene that codes for the Aβ-precursor protein. Less than 1% of AD cases are due to dominantly inherited genetic mutations in APP, PSEN1 and PSEN2. An early-onset familial AD is described as AD that develops before the age of 60 years due to inheriting any of these genetic mutations that speed up AD (Gaudreault & Mousseau, 2019). Its symptoms are cumulative and permanent, beginning with recurrent short-term memory loss and progressing to the decline of higher cognitive abilities, including language and decision-making (Takeda, 2019). Because of the severity of these symptoms, the disease has a significant financial and societal cost. This neurodegeneration is the product of numerous interconnected pathways, supported by hereditary and environmental variables, rather than a single cause or process (Van der Schyf, 2011). The medications currently on the market only offer symptomatic relief due to the complex pathophysiology, making therapy challenging. The oxidative stress, toxic Aβ plaques and cholinergic hypothesis are three common theories that have gained traction in understanding the pathology and pathophysiology of AD and guiding treatment options (Hangya et al., 2015).

The Siddha, Unani and Ayurvedic systems of conventional Indian medicine suggested antioxidant properties of *E. officinalis* (Amla). It is a member of the Euphorbiaceous family and has been used in traditional medicine as a nutritious tonic with important vitamins, amino acids and different phytoconstituents (Figure 2; Husain et al., 2019). It is extensively dispersed in various countries/regions like India, Indonesia, China and Southeast Asia. The plant has long been used in diseases including diabetes, cancer, hyperlipidaemia, neurological problems, ophthalmic ailments, etc. Due to its combined antioxidant, cholesterol-lowering and anti-inflammatory characteristics, *E. officinalis* is known to have a probable beneficial property in neurodegenerative conditions. One of the key elements of the pathophysiology of AD is believed to be cholinergic dysfunction. Methanolic extract of *E. officinalis* fruit has been shown in *in vitro* experiments to have the potential to inhibit AChE and BuChE and offer additional free radical scavenging activity (Mathew & Subramanian, 2014).

Two BACE-1 inhibitors were identified from the pomegranate husk (Punica granatum) during the screening process for anti-dementia medicines from natural products. With IC_{50} values of 3.9×10^{-6} and 4.1×10^{-7} M and *Ki* values of 2.4×10^{-6} and 5.9×10^{-7} M, respectively, they were recognised as punicalagin and ellagic acid (Kwak et al., 2005). The inhibitory potential of *Sophora flavescens* lipophilic alkylated (C10-C5) flavanones against β-secretase was investigated. In transfected human embryonic kidney (HEK-293) cells, lavandulyl flavanones inhibited Aβ secretion dose-dependently. They exhibited significant beta-secretase inhibitory actions with IC_{50} values of 3–8 μm (Hwang et al., 2008). Exploration of the active constituents of *E. officinalis* displayed that it is

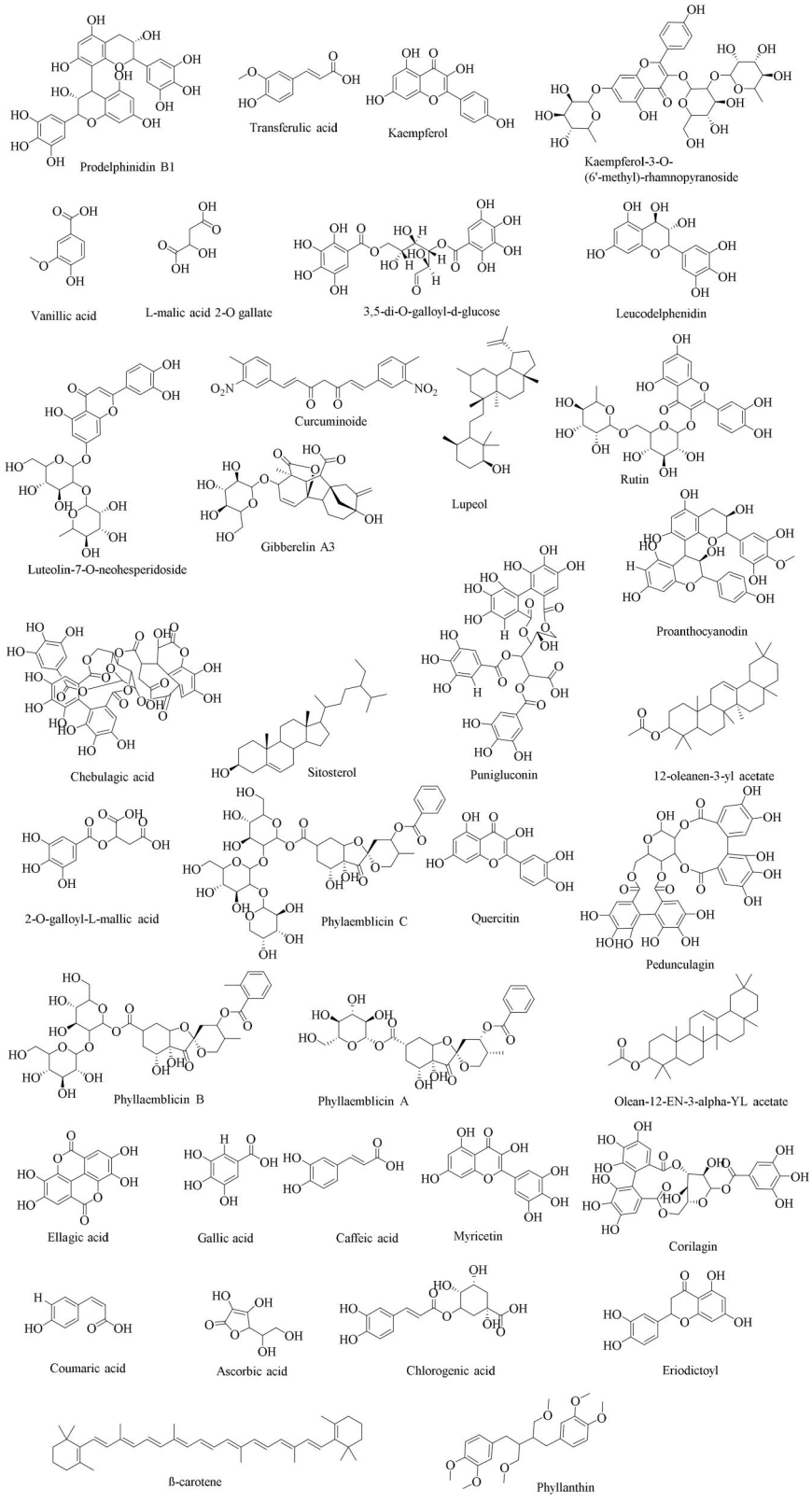


Figure 2. Phytoconstituents present in *E. officinalis*.

enriched with flavonoids, tannins and polyphenols, which could contribute to its anti-amyloidogenic activity in AD.

Quercetin (28%), rutin (3.89%), polyphenols, carbohydrates and others were found as bioactive/chemical constituents present in *E. officinalis* (Figure 2; Singh et al., 2012). A significant protective effect against H_2O_2-induced neuronal oxidative DNA damage was seen using an aqueous or methanolic extract of *E. officinalis* (concentration 0.1–1.0 mg/ml).(Dehaene & Naccache, 2001; Mani & Parle, 2007; Yokozawa et al., 2007). An alcohol (20%)-induced brain mitochondrial damage in male Wistar rats was reversed via impaired NO, protein carbonyl and antioxidant system and

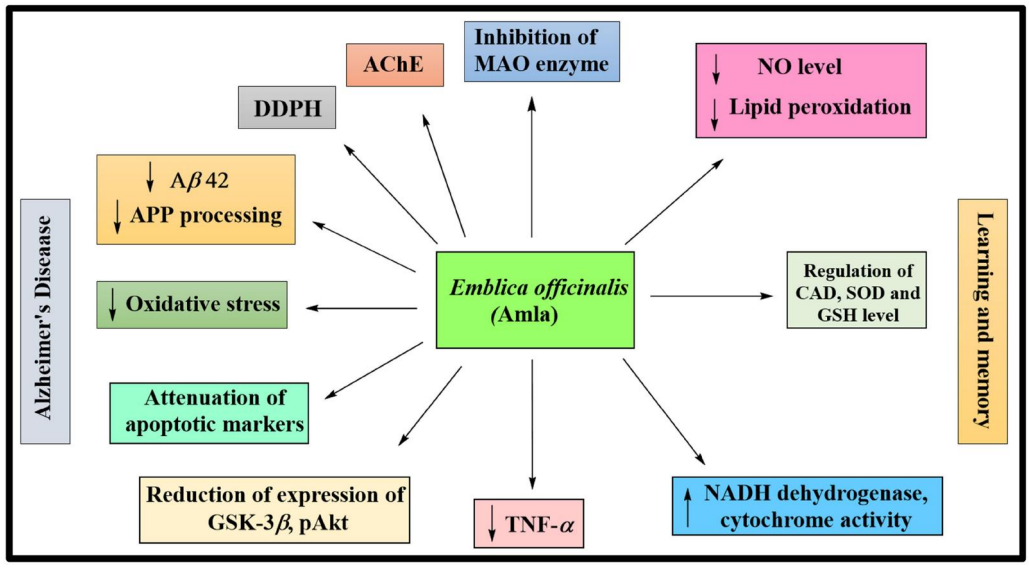


Figure 3. Diagrammatic application of *E. officinalis*.

cytochrome C oxidase activity of fruit extract of *E. officinalis* also many pathways were shown to understand its key bioactivities (Figure 3; Akhtar et al., 2011; Bansal et al., 2015; Bhattacharya et al., 2000; Firuz Fatema Pria, 2019; H. J. Kim et al., 2014).

A detailed understanding of the key phytochemicals of *E. officinalis* and their mechanistic roles is still unclear; therefore, we explored the computational tool to identify and investigate the mechanistic role.

2. Materials and methodology

2.1. Identification and retrieval of active components of E. officinalis

The biologically active phytochemicals of *E. officinalis* were identified through a literature review and downloaded in Structure Data File (SDF) format from the PubChem database. These phytochemicals were further subjected to network pharmacology (NP) followed by molecular docking and dynamics studies (Chugh et al., 2023; Kaur et al., 2022; S. Kim et al., 2016).

2.2. Prediction of E. officinalis targets and ND targets

All the potential reported targets of *E. officinalis* were retrieved from the BindingDB database by inserting the SMILE code of each phytoconstituent and assigning a similarity index 0.85 (T. Liu et al., 2007).

2.3. Gene ontology (GO) and pathway enrichment analysis of targets

GO is a way to depict detailed information about genes and their products in terms of molecular function (MF), biological process (BP) and cellular component (CC; Thomas, 2017). The PANTHER (Protein Analysis Through Evolutionary Relationships) tool was used for GO analysis of selected target genes with their role in different KEGG pathways (Mi et al., 2017).

2.4. Target network construction and topological analysis

One of the most well-established methods for fusing biology and bioinformatics to understand the intricate connections between medications, targets and diseases is NP-based drug design (Hopkins, 2007). In NP, scientific findings or statistical measurements can be used to build connections (also known as edges) between various biological ideas (also known as nodes). A network is a diagram that shows how different nodes interact with one another. Among the nodes are bioactivities, targets, tissues, diseases, disease types and pathways. These nodes were displayed using the Cytoscape programme. They are joined by lines known as edges, which describe their relationship. Bioactive elements, molecular targets or diseases linked to a molecular target can all operate as nodes in a network. NP uses computational power to create a network of molecular targets for all bioactive substances in a multicomponent mixture, such as a plant extract. A network of interactions between identified protein targets is built, and their relationships with various diseases are also investigated. In-depth knowledge of pharmacological mechanisms and interactions with targets is contained in these newly established networks, and it can be used for the therapeutic use of intricate plant extracts. The primary active ingredients in plant extracts and their related targets, the mechanisms of action of complex plant extracts, and probable side effects can all be predicted by network analysis. NP can also repurpose medications to treat different ailments by discovering newly undiscovered molecular targets for bioactive substances (Chandran et al., 2017; Kumar et al., 2022). The selected target genes were imported into the Cytoscape plugin, MCODE (Molecular Complex Detection Algorithm) was used to construct their network based on their functional relations (Smoot et al., 2011). The resulting network of genes was then analysed based on their co-expression, genetic interaction, physical interaction, pathway, co-localization, predicted and shared protein domains. Furthermore, the CytoNCA plugin was used to study the topological

parameters such as betweenness centrality (BC), degree centrality (DC) and closeness centrality (CC) of the resulting network (Tang et al., 2015).

2.5. Molecular docking

Docking is a programme for developing drugs based on the receptor and ligand. It is used to forecast the receptor and ligand with binding energy as complex. The sampling and scoring components comprise a protein–ligand docking algorithm (Jakhmola et al., 2021). A sampling is a process of creating potential ligand binding orientations close to a protein's binding site. In scoring, a physical or empirical energy function calculates the binding energy. The ligprep, protein prepwizard, grid construction and docking calculations were carried out for the molecular docking experiment using the Schrödinger Maestro programme. The grid box dimensions were found to be X, Y and Z coordinates ($X = -15.239$; $Y = -18.749$; $Z = 0.445$), and the area was $40 \times 40 \times 40$ Å (in chain A; Supplementary Figure S4). This programme uses the Glide SP (standard precision) module. All docking calculations were performed in SP mode with the OPLS3e force field. The aforementioned docking process used a flexible docking mode, automatically generating conformations for each input ligand. The produced was subjected to a number of hierarchical filters that evaluated the interactions, including the hydrophobic, hydrogen-bonding and metal–ligation interactions. The scoring function of Glide was then used to rescore the shorter postures.(Bicak et al., 2019; Halperin et al., 2002) For further correlation, we used Autodock Vina to obtain binding energies (Trott & Olson, 2010).

2.5.1. Preparation of protein

The protein preparation wizard from the Schrödinger suite was used to prepare the PDB file for the BACE1 structure. The integrity of the protein structure was changed by removing ions, cofactors and water molecules and inserting hydrogen atoms. The tautomeric and protonation states were brought to pH 3.0. A 1.29 Å resolution protein (PDB ID: 6UWP) was collected to model the protein structure for this investigation. The protein structural modifications often focus on bond order, formal charges, missing hydrogen atoms, topologies and incomplete terminal amide groups. To realign side-chain hydroxyl groups and also to prevent any steric conflicts, a limited reduction of the protein structure was performed using the OPLS3e force field. The root mean square deviation (RMSD) has limits of 0.3 Å (Kalirajan et al., 2012).

2.5.2. Preparation of ligand

The ligands were prepared with the help of the LigPrep module of the Schrödinger Suite 2022. OPLS3e served as the force field for the energy reduction process. By including hydrogens, considering the lengths and angles of the bonds and selecting the conformer structure with the lowest conformational energy, which in turn depends on precise tautomers, stereochemistry and ring conformations. LigPrep transforms 2D structures into 3D structures. The EPIK 2.1 ionisation tool set the ionisation state at a pH range of 3.0–1.0. All possible ring conformations, tautomer, stereochemistry, protonation and ionisation states were produced. A maximum of 32 stereoisomers per ligand were considered while creating stereoisomers with unassigned stereogenic centres. For each ligand, only the conformation with the lowest energy was retained. The ligand structures downloaded in .sdf format were imported into the workspace, and these ligands were subsequently subjected to the LigPrep module of Maestro in the Schrödinger Suite 2022. Considering stereochemistry, ionisation, tautomeric changes, energy minimisation, geometry optimisation, desalting, chirality correction and the absence of hydrogen atoms. Later, they underwent a 3D structural change from 2D. The EPIK module created the ionisation and tautomeric states between pH 6.8 and 7.2. The conjugate gradient approach was utilised after first using the steepest descent technique for minimisation (Kalirajan et al., 2012).

2.6. Molecular dynamics

Computational molecular dynamics simulation (MDS) research profoundly impacts the characterisation of protein–ligand complex interactions. Additionally, it aids in analysing the pattern of conformational configurations of biomolecules and ligands concerning the intensity of their interactions in a specific setting (Ji et al., 2020). Desmond's various modules were the subject of extensive research (Schrödinger 2022). Before MD simulation, both complexes were cleaned using the Schrödinger suite's protein preparation wizard and Prime module to eliminate flaws, including missing side chains, incorrectly assigned bonds, erroneous charge states and missing hydrogen atoms. Restrained energy minimisation was used to eliminate the steric collisions and strained bonds/angles while permitting heavy atom movement up to 0.3 Å. An extensive 100 ns MD simulation was performed for both complexes to assess the binding stability of rutin and quercetin concerning internal ligands in the complex with BACE1. The TIP3P water model and 0.15 M NaCl simulated a physiological ionic concentration in both complex systems. The protein–ligand complex system was made inert by bringing the system's net charge into equilibrium by adding Na^+ or Cl^- counter ions. Using the Martyna–Tobias–Klein dynamic algorithm and Nose–Hoover chain, the systems were maintained at 300 K in temperature and 1.01325 bar in pressure, respectively. A hybrid energy minimisation approach, consisting of 1000 steps of the steepest descent, was developed using conjugate gradient techniques. We examined the ligand RMSD, radius of gyration (Rg), molecular surface area (MolSA), solvent-accessible surface area (SASA) and polar surface area during MD simulation (Supplementary Figure S2; Ivanova et al., 2018; Salo-Ahen et al., 2020).

3. Results

3.1. Retrieval of active phytoconstituents and targets prediction results of E. officinalis

After going through the literature review, 36 active constituents of *E. officinalis* were identified, and compounds and

their PubChem ID were downloaded in .sdf format. This study retrieved two categories of targets (targets related to AD and those related to *E. officinalis* from different public databases. According to BindingDB database, a total of 436 targets from 36 bioactive constituents of *E. officinalis* were retrieved, and 3398 targets were found related to AD from the Disgenet database. Refer to Supplementary material 2 for detailed information on these targets.

3.2. Gene–gene interaction analysis

A Cytoscape plugin, StringDB was used to construct the gene–gene interaction network of the targets. Gene–gene interaction networks enable to comprehend the functional relationships between genes and proteins. Circular nodes denoted genes and coloured edges represented their different correlations (Figure 4).

3.3. Gene ontology and pathway enrichment analysis

Several kinds of enriched BPs and pathways were observed for all 95 targets (Figure 5). In the case of BP, targets were found to be enriched in different processes such as carbohydrate homeostasis, extrinsic apoptotic signalling, glucose homeostasis. Pathways analysis of targets were found to be enriched in CCKR signalling map, AD amyloid secretase pathway, AD-presenilin pathway, angiogenesis, muscarinic acetylcholine receptors 1 and 3, for the detailed analysis of enzymes involved in AD refer to Supplementary material 1.

3.4. Drug-bioactivities-target-disease network

The 95 contender genes were linked to begin an initial PPI network using Cytoscape 3.8.1 that involved 136 nodes and 521 edges, as shown in Figure 6. The nodes in the PPI network denote the interrelationship between AD, targets and chemical constituents (Kola-Mustapha et al., 2023). Analyse tool in Cytoscape was applied to analyse the PPI diagram, and BACE1 (12), CA2 (12), F2 (10), ABCB1 (10) and AChE (10) showed a higher degree of relation (F. Liu et al., 2021).

3.5. Disease-targets-bioactive-drug network

Figure 7 shows the drug-components-targets-pathways-disease network, which includes 39 nodes. The orange octagon nodes are the active constituents, pink V-shape nodes represent the targets, a round rectangle for the disease (AD), a green circle for the drug (Amla), biological source (*E. officinalis*) is displayed by a blue hexagon. In contrast, lines depict their interactions (Wang et al., 2019). The topological analysis of active constituents revealed that several active constituents of *E. officinalis* interact with at least one target gene,

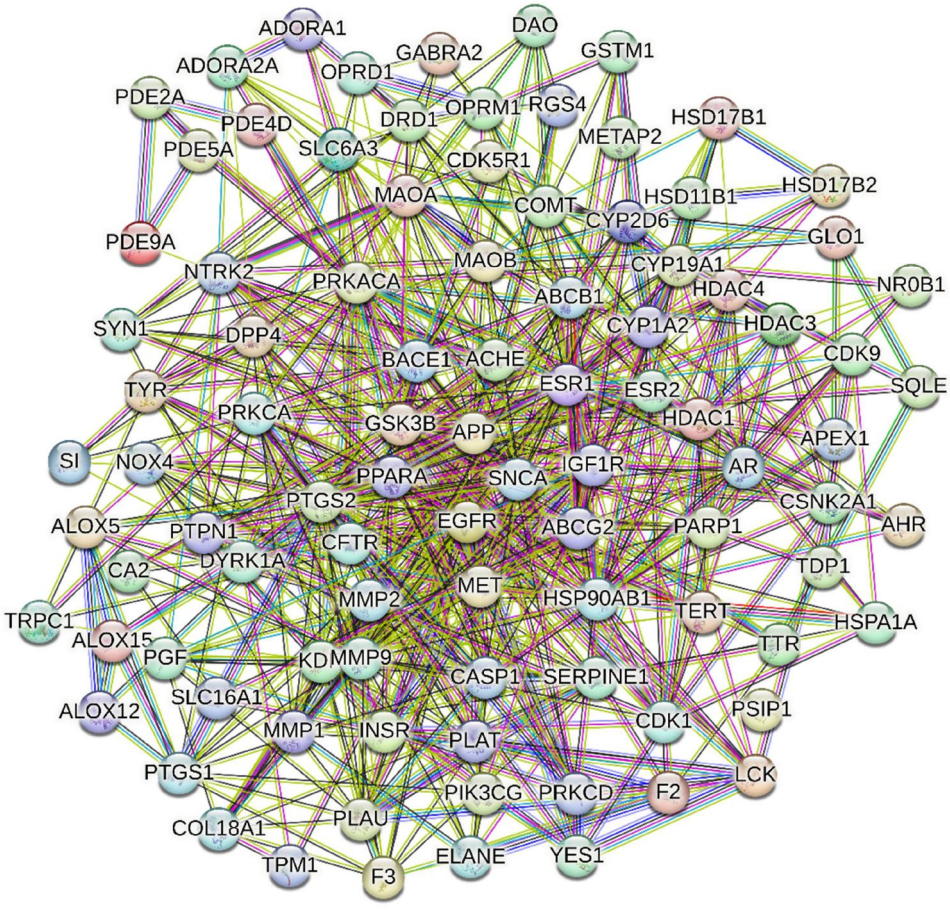


Figure 4. Gene interaction network of targets. Network displays the strength of interaction (edge thickness), type of interaction (colours) and many edges in between nodes.

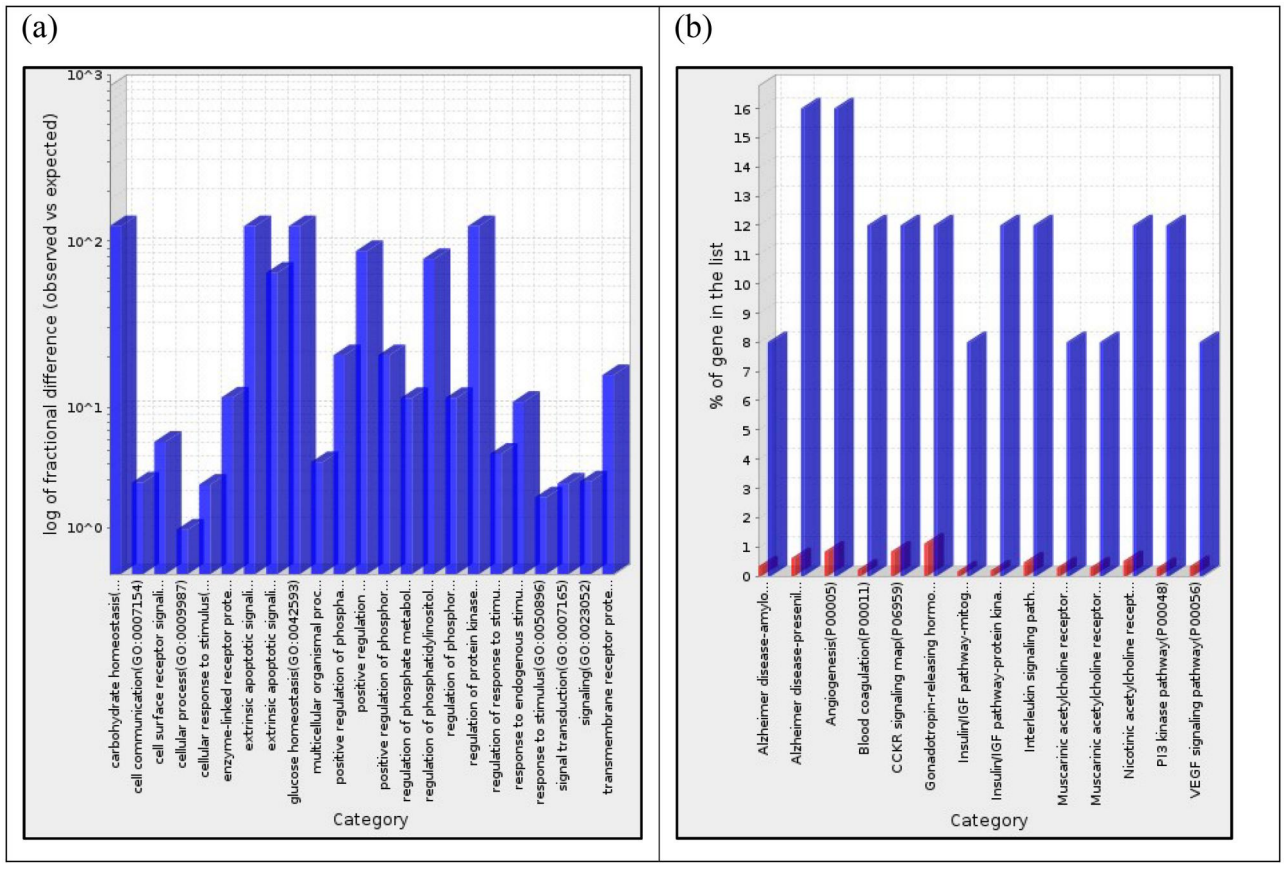


Figure 5. Classification of 95 target protein corresponding to AD according to (a) biological process and (b) pathways.

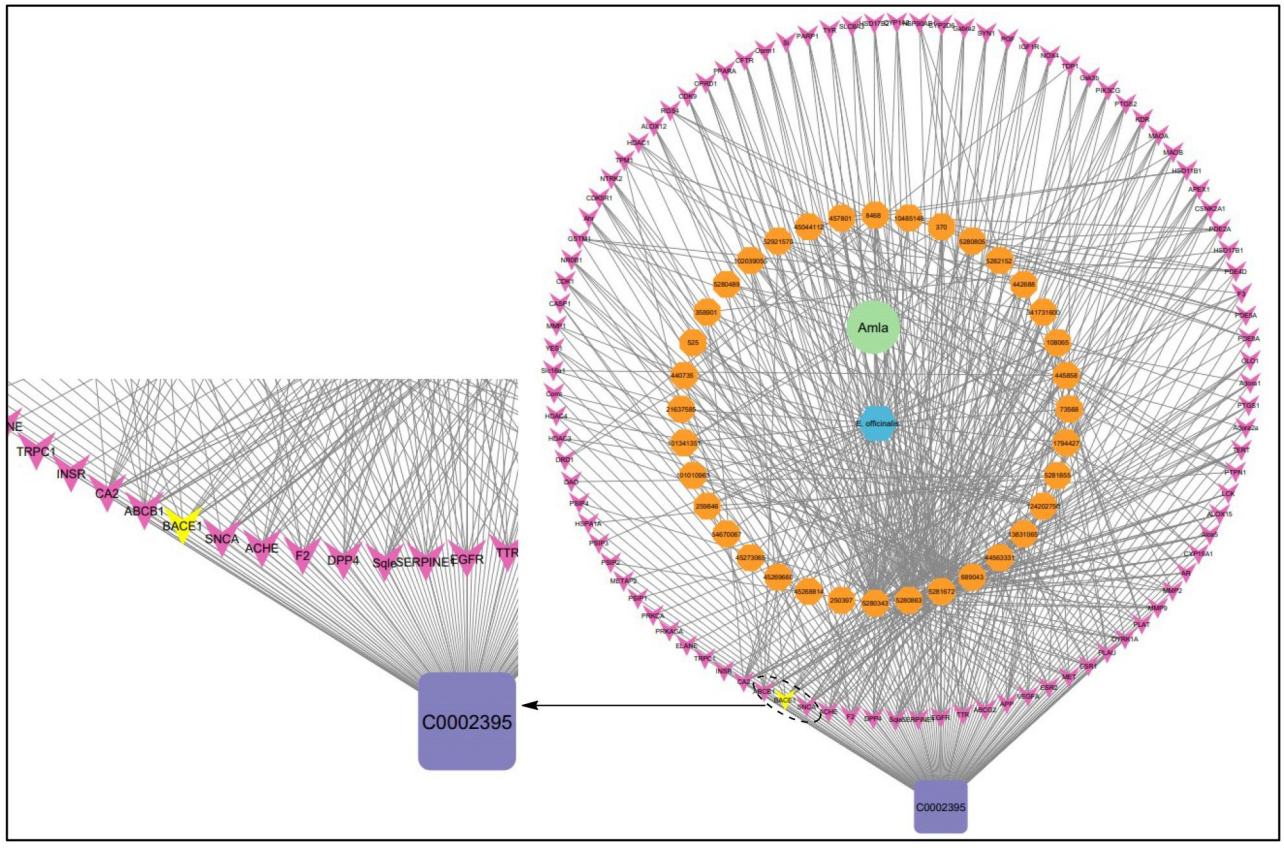


Figure 6. Interaction of targets with phytoconstituents to optimise the target (BACE1).

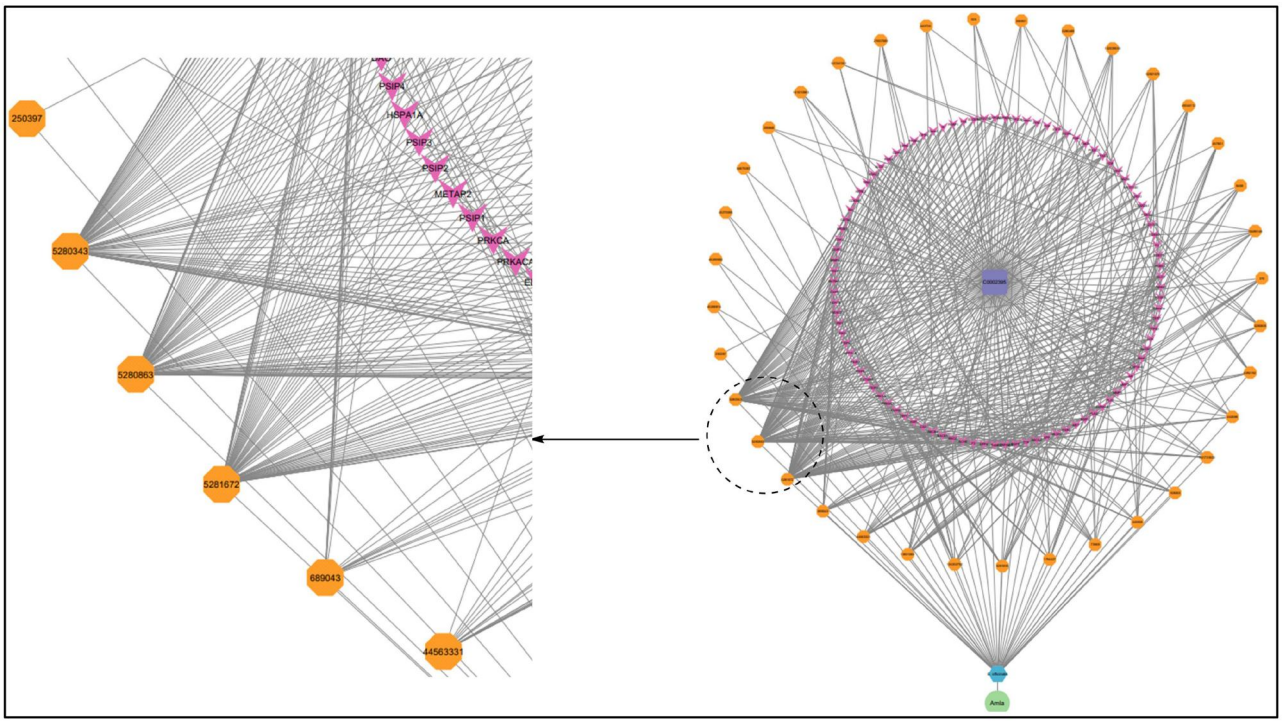


Figure 7. Interaction of phytoconstituents with their respective targets (genes) for optimising ligands (quercetin, myricetin and kaempferol).

with quercetin being the phytoconstituent with the highest degree score of 61 target genes, followed by kaempferol and myricetin with 60 target genes. Furthermore, each pathway linked to colon cancer was estimated to involve at least 10 genes, and at least two active components controlled many target genes. This network research identified the characteristics of several *E. officinalis* constituents and multiple AD treatment possibilities; refer to Supplementary material 2 for topological analysis information.

3.6. Molecular docking

Using Glide of the Schrödinger Suite 2022, the binding affinities of the ligands (bioactive phytoconstituents from *E. officinalis*) against the chosen receptor were ascertained by molecular docking against the BACE1 receptor (PDB ID: 6UWP), which was co-crystallized (Figure 8(a)) with its internal ligand. A lower Glide score indicates better affinity when determining the binding affinities of the ligands to receptors (Table 1 includes the top 10 compounds; Román et al., 2018). Further validation of results was done with clinically tested BACE1 inhibitors, i.e. atabecestat (JNJ-54861911), lanabecestat (AZD3293), which displayed G-score of −5.7 and −3.2 kcal/mol, respectively. That was lower than rutin and comparable to internal ligand (Table 1). We analysed these phytoconstituents using Autodock Vina using 6UWP protein and got similar trends in the binding energy (Supplementary Table S1). The bioactive phytoconstituent 5280805 (rutin), according to the results of the molecular docking study, had the highest binding affinity for BACE1 with a G-score of −7.5 kcal/mol. The compounds 341731600 and 442688, with G-scores of −7.2 kcal/mol and −7.1 kcal/mol, respectively, also demonstrated higher binding affinity against BACE1. An internal ligand displayed H-bond interaction with Asp32, Asp228, Trp76 and pi–pi stacking with Trp71 (Figure 8(b)). The docked pose of rutin showed H-bond interaction with Asp32, Asp228, Gly30, Ile126, Trp76 and one pi–pi stacking with Arg128 (Figure 8(c)). Quercetin (5280343), found to have the highest degree of connection established through NP, showed a G-score of −5.67 kcal/mol, which is comparatively higher than the internal ligand, and it displayed H-bond interactions with Trp76, Gly230, Phe108 and Asp228 (Figure 8(d); Ramachandran et al., 2016). For detailed binding energy of all the bioactive phytoconstituents and 2D template binding interaction of atabecestat and lanabecestat, refer to Supplementary Table S1 and Supplementary Figure S6.

3.7. Molecular dynamics

An automated Desmond simulation includes highly intricate free energy perturbation calculations, multistage MD simulations with built-in simulation protocols, equation of states prediction at various temperatures and dynamic response prediction in non-equilibrium states. Visualising and analysing computed results in the Maestro modelling environment were done (Bowers et al., 2006). Rutin displayed the highest glide score in molecular docking studies, and quercetin, the active component of *E. officinalis*, showed the highest node connection in NP and was chosen for MDS using internal ligand as a control for the protein BACE1. The BACE1 bound to rutin, quercetin and internal ligand were simulated for 100 ns in the explicit solvent model at physiological salt concentration. Unbound protein (BACE1), IL-BACE1, rutin-BACE1 and the quercetin-BACE1 docked complex were subjected to MD simulation for 100 ns. The average potential energy of the

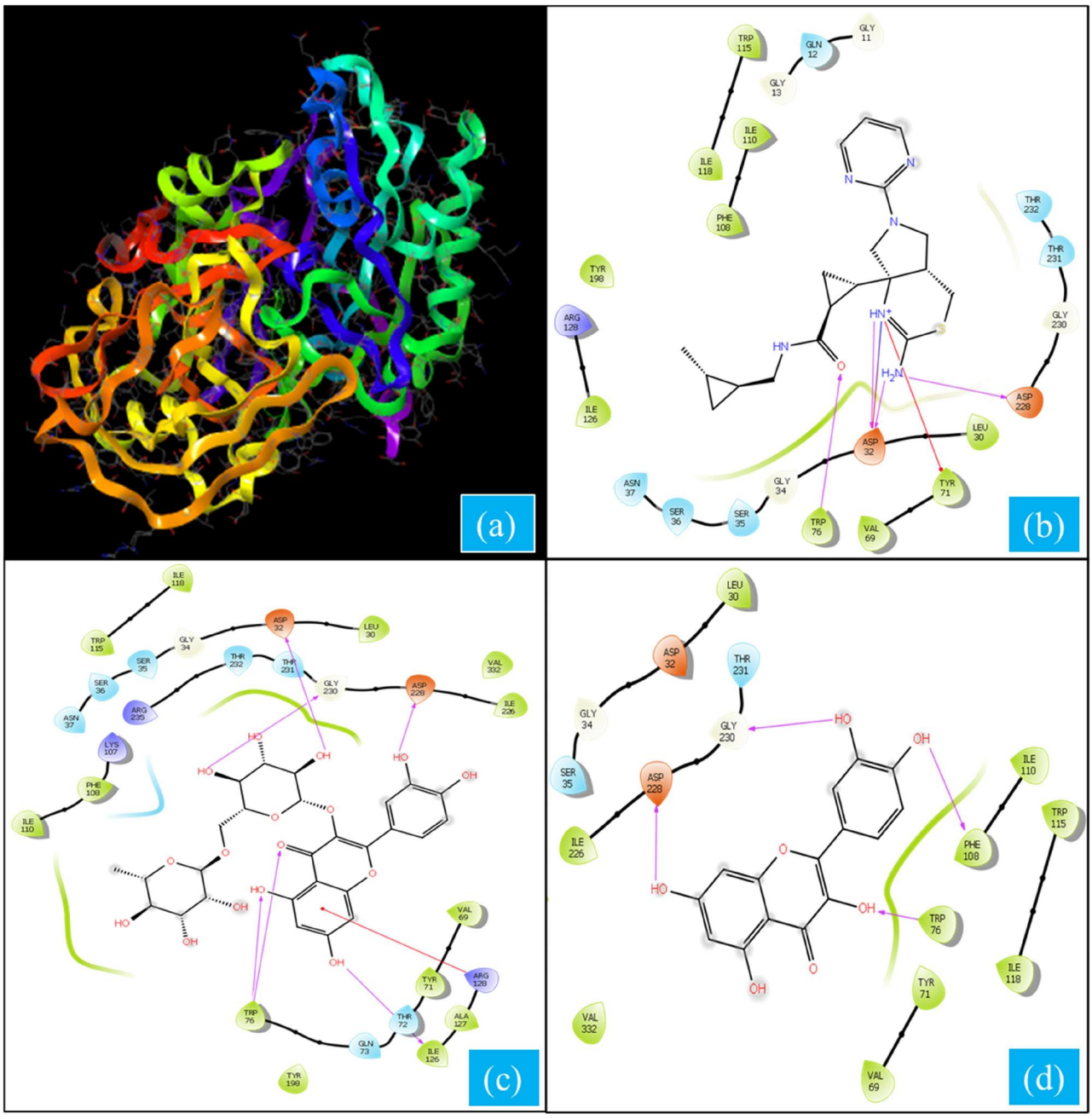


Figure 8. (a) 3D representation of BACE1 receptor (PDB ID: 6UWP), 2D template representing binding interaction of (b) internal ligand (QKA, PubChem CID145704719), (c) rutin, (d) quercetin.

Table 1. Docking results of the top 10 bioactive molecules and one internal ligand with their respective interacting amino acids.

S. No.	PubChem ID	Compound	Glide score	Interacting amino acid
1.	5280805	Rutin	−7.5	Arg128, **Asp32**, **Asp228**, Gly30, Ile126, **Trp76**
2.	341731600	3,6-Di-*O*-galloyl-d-glucose	−7.2	**Asp32**, Ile126, Lys107, Phe108, **Tyr71**, **Trp76**
3.	442688	Pedunculagin	−7.1	**Asp32**, Arg235, Thr231, Thr232
4.	250397	Chebulagic acid	−6.9	Arg235, Gly11, Thr232, Thr329, **Tyr71**, Tyr198
5.	45269660	Phyllaemblicin B	−6.9	Arg128, Asp238, Gly230, Thr231, **Tyr71**, Tyr198
6.	21637585	Punigluconin	−6.7	Arg235, Asp238, Gly34, Gly230, **Tyr71**, Thr329
7.	45268814	Phyllaemblicin A	−6.7	Arg128, Arg235, Asn37, Asp32, Ile126, Ser36, Tyr198
8.	5282152	Luteolin 7-*O*-neohesperidoside	−6.6	Arg235, **Asp32**, Gln73, Gly34, Thr231, **Trp76**, Tyr198
9.	45273065	Phyllaemblicin C	−6.4	Arg235, Gln73, Gly11, Thr231
10.	5280343	Quercetin	−5.67	**Trp76**, Gly230, Phe108, **Asp228**
11.	68254185	Atabecestat	−5.7	Gln73, Gly230, Phe108, Lys107, **Trp76**, **Tyr71**
12.	67979346	Lanabecestat	−3.2	**Tyr71**, Thr72
13.	145704719	QKA (internal ligand)	−5.5	**Asp32**, **Asp228**, **Trp76**, **Tyr71**

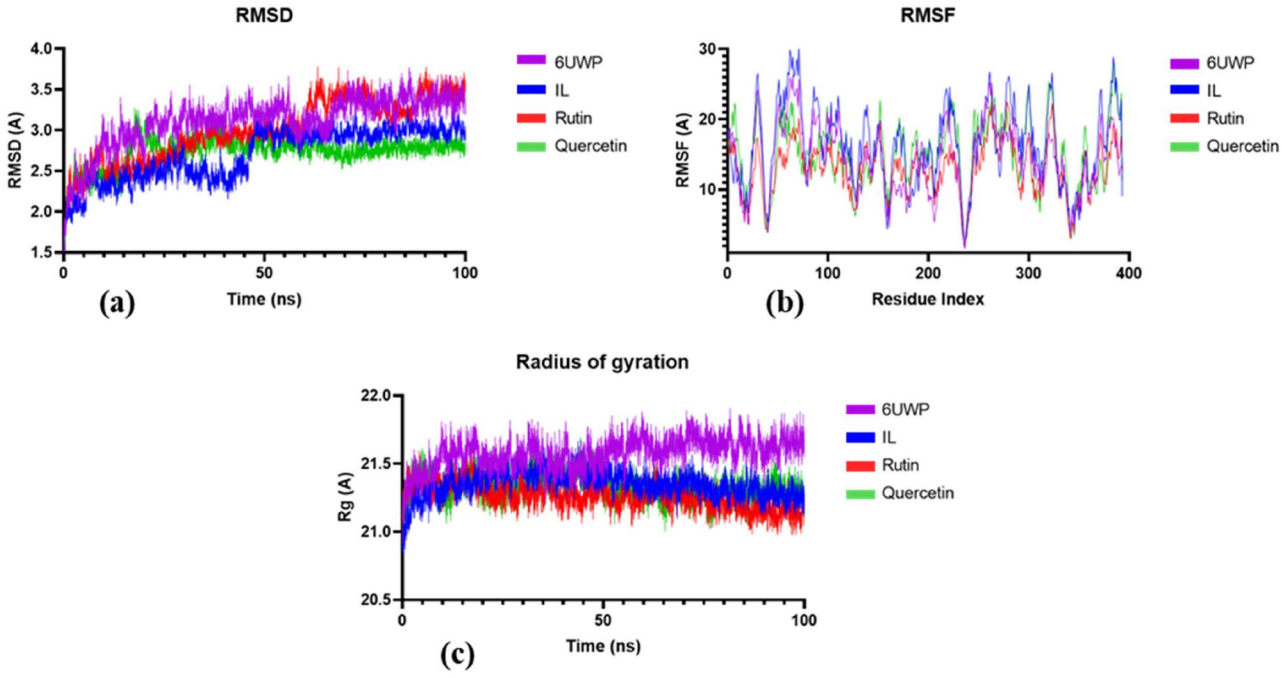


Figure 9. (a) RMSD of internal ligand, rutin and quercetin with protein as a function of 100 ns simulation, (b) RMSF of internal ligand, rutin and quercetin with protein, Radius of gyration of internal ligand, rutin and quercetin with protein, (c) radius of gyration of internal ligand, rutin and quercetin with protein (PDB ID: 6UWP). Purple, blue, red and green colours represent different molecules with BACE1 targets (unbound BACE1 protein, internal ligand, rutin and quercetin.

unbound BACE1, IL-BACE1, rutin-BACE1 and the quercetin-BACE1 complex was calculated to their equilibration and stability during the simulations. The initial frames of unbound BACE1, IL-BACE1, rutin-BACE1 and the quercetin-BACE1 docked complex were set as a reference, and the variability in RMSD of C_a atoms was monitored. It was observed that the RMSD values of unbound BACE1, IL-BACE1, rutin-BACE1 and the quercetin-BACE1 complex varied between 1.0036–3.7688, 1.0825–3.3782, 1.0577–3.7790 and 0.9827–3.2724 Å, respectively (Figure 9(a)). The average RMSD values of free BACE1, IL-BACE1, rutin-BACE1 and the quercetin-BACE1 complex were 3.1078, 2.7024, 2.9932 and 2.7319 Å, respectively. RMSD values indicate that the complex of rutin and quercetin with BACE1 has RMSD values lower than that of unbound protein, signifying the increased stability of the protein (PDB ID: 6UWP). Similarly, the RMSF values of unbound BACE1, IL-BACE1, rutin-BACE1 and the quercetin-BACE1 docked complex varied between 1.7238–26.1927, 1.7556–30.3363, 1.5650–22.5258 and 1.8436–28.1013 Å, respectively (Figure 9(b)). The average RMSF values of unbound BACE1, IL-BACE1, rutin-BACE1 and the quercetin-BACE1complex were 14.4124, 16.1981, 12.8154 and 14.9510 Å, respectively. The RMSF data suggest that the fluctuations compared with the amino acid residues of quercetin-BACE1 complex were significantly overlapping. Therefore, quercetin complex is more stable than others. The degree of compactness shown by the graph of radius of gyration (Rg) with the values of unbound BACE1, IL-BACE1, rutin-BACE1 and the quercetin-BACE1 docked complex varied between 20.9134–21.9072, 20.8413–21.6804, 20.8582–21.6463 and 20.9553–21.6902 Å, respectively (Figure 9(c)). The average Rg values of unbound BACE1, IL-BACE1, rutin-BACE1 and the quercetin-BACE1 complex were 21.5757, 21.3485, 21.2604 and 21.3280 Å, respectively. The plot indicated that the unbound BACE1 protein complex had a higher Rg value and showed huge fluctuations during the simulation. In contrast, the quercetin complex had low Rg values and showed very few fluctuations during the simulation period (Supplementary Figure S3). Therefore, the quercetin-BACE1 complex had less rigidity than other complexes (Jabir et al., 2021).

4. Discussion

AD is a neurological disorder that causes behavioural abnormalities, progressive memory loss and a deterioration in cognitive function. Age, genetics and environmental variables like stress are thought to be the root causes of many diseases. However, due to their complicated pathophysiology, it is still unclear exactly what causes them at a genetic level. AD is a progressive neurodegenerative disorder characterised by a decline in cognitive functions resulting from the extracellular deposition of amyloid beta peptides (senile plaques) and intracellular neurofibrillary tangles. *E. officinalis* has been considered to exert potential therapeutic effects in neurodegenerative disorders due to high concentrations of flavonoids, tannins and antioxidants. A dose of 200 mg/kg of *E. officinalis* for 2 months of treatment was found to significantly decrease acetylcholinesterase activity, APP, $A\beta_{42}$ and gamma-secretase activity in the brain's hippocampus and cortical area in $AlCl_3$-intoxicated male Wistar rats. Despite affecting amyloid protein, *E. officinalis* therapy restored $AlCl_3$-impaired learning, memory and locomotor activity (Thenmozhi et al., 2016). We performed in-silico studies to uncover the therapeutic benefits of phytoconstituents of *E. officinalis* related to AD using the NP approach. In the

current study, 36 active components, 436 targets of *E. officinalis* and 3398 AD-specific targets were identified from different public databases. A total of 95 targets were found to be common in both categories. Gene–gene interaction network was constructed for these targets. Topological analysis of the network revealed the top five significant targets, such as BACE1, carbonic anhydrase-2 (CA2), (F2) gene, ATP-binding cassette sub-family B member-1 (ABCB1) and AChE. BACE1 was found to be having the highest degree, betweenness and closeness scores. GO and pathway enrichment analysis of these key targets speculated the involvement in different BPs, BFs and cellular pathways. The topmost key target, BACE1, was enriched in AD-amyloid secretase and AD-presenilin pathways. Pathways analysis elucidated that the cleavage of APP sequentially by beta-secretase and presenilin gamma-secretase generates amyloid beta fragments. These fragments, when aggregating into senile plaques that led to the death of neuron cells, led to disease pathogenesis. The result suggested that BACE1 target could be used as a possible target for treating AD. The NP analysis demonstrated the involvement of 36 bioactive phytoconstituents in target prediction as BACE1 inhibition that could play a significant role in preventing the formation of Aβ oligomer. BACE1 is a promising focal point for therapeutic interventions aimed for AD (Roberds et al., 2001). The catalytic binding site of BACE1 contains critical components for its catalytic function, including the amino acids Asp32 and Asp228, along with two water molecules. Among these, one water molecule contributes to enzymatic proteolysis, while the second one plays a vital role in stabilising a tetrahedral intermediate crucial for protein cleavage. Targeting BACE1 for inhibition is a potential strategy in AD treatment due to its role in reducing Aβ accumulation. While various peptides are recognised as BACE1 inhibitors, clinically tested drugs like atabecestat (JNJ-54861911) and lanabecestat (AZD3293) but associated with serious side effects (Ghosh & Osswald, 2014). The binding energies of these clinically tested drugs (atabecestat −5.7 kcal/mol and lanabecestat −3.2 kcal/mol) revealed that rutin (−7.5 kcal/mol) and various other phytoconstituents had a superior binding affinity with the active site of BACE1. This study proposes a plausible mechanism involving polyphenolic compounds such as rutin and quercetin for inducing BACE1 inhibition. This inhibition occurs through the displacement of a water molecule, which subsequently engages in a hydrogen-bonding network with Asp32 and Asp228. This interaction is critical for BACE1's proteolytic activity. The docking studies revealed that rutin and quercetin have binding energy −7.5, −5.67 kcal/mol, respectively, which is comparatively greater than the internal ligand. We studied diversity in the structure of clinical candidates and rutin/quercetin using structural similarity with a freely available online tool, i.e. ChemMine Tools.(Backman et al., 2011). We found a minor similarity between rutin/quercetin, atabecestat (JNJ-54861911) and lanabecestat (Supplementary Figure S7). MDS confirmed the promising potential of quercetin and rutin to inhibit the BACE1 receptor. Average RMSD values of rutin and quercetin complexed with BACE1 were 2.9932, 2.7319 Å, which is lower as compared to unbound BACE1, overlapping fluctuations of quercetin with an average RMSF value of 14.9510 Å accounts for its higher stability at receptor/protein. Treatment with Quercetin was associated with a correlation to the reversal of β-amyloidosis and tauopathy levels in the brain. Additionally, it led to the improvement of astroglial and microglial reactivity in the CA1 area, the subiculum, the EC and the amygdala (Sabogal-Guáqueta et al., 2015).The concurrent administration of $AlCl_3$ and Quercetin at a dosage of 50 mg/kg of body weight to rats induced with AD resulted in a notable reduction in the fold change of BACE-1 by (0.74 ± 0.13) when compared to the rat group induced with AD alone (Elreedy et al., 2023).

5. Conclusion

The NP-based approach advances knowledge of the probable mechanisms of action and the complicated interactions between drugs and their targets. The present research work studied 36 phytoconstituents from *E. officinalis* for BACE1 inhibition using NP and *in-silico* approaches. The NP analysis demonstrated the involvement of 36 bioactive phytoconstituents in target prediction as BACE-0.1 inhibition that could play a significant role in preventing the formation of Aβ oligomer. Furthermore, these findings can suggest a promising therapeutic role of rutin or quercetin, as a BACE-1 inhibitor in treating one of the most devastating neurological disorders.

Acknowledgements

G.L.K. is grateful to the Science and Engineering Research Board, Department of Science & Technology (DST), Govt. of India for research funding.

Patient consent

Not applicable.

Disclosure statement

The authors confirm that this article's content has no conflict of interest.

Supplementary material

Related data are available online in supplementary materials 1–5.

Funding

The author(s) reported there is no funding associated with the work featured in this article.

References

Akhtar, M. S., Ramzan, A., Ali, A., & Ahmad, M. (2011). Effect of Amla fruit (*Emblica officinalis* Gaertn.) on blood glucose and lipid profile of normal subjects and type 2 diabetic patients. *International Journal of Food Sciences and Nutrition*, *62*(6), 609–616. https://doi.org/10.3109/09637486.2011.560565

2022 Alzheimer's disease facts and figures. (2022). *Alzheimer's and Dementia*, *18*(4), 700–789. https://doi.org/10.1002/alz.12068

Backman, T. W. H., Cao, Y., & Girke, T. (2011). ChemMine tools: An online service for analyzing and clustering small molecules. *Nucleic Acids Research*, *39*(Web Server issue), W486–W491. https://doi.org/10.1093/nar/gkr320

Bansal, V., Sharma, A., Ghanshyam, C., & Singla, M. L. (2015). Rapid HPLC method for determination of vitamin C, phenolic acids, hydroxycinnamic acid, and flavonoids in seasonal samples of *Emblica officinalis* juice. *Journal of Liquid Chromatography & Related Technologies*, *38*(5), 619–624. https://doi.org/10.1080/10826076.2014.936608

Bhattacharya, A., Ghosal, S., & Bhattacharya, S. K. (2000). Antioxidant activity of tannoid principles of *Emblica officinalis* (Amla) in chronic stress induced changes in rat brain. *Indian Journal of Experimental Biology*, *38*(9), 877–880.

Bicak, B., Kecel Gunduz, S., Kökcü, Y., Ozel, A., & Akyuz, S. (2019). Molecular docking and molecular dynamics studies of L-glycyl-L-glutamic acid dipeptide. *Bilge International Journal of Science and Technology Research*, *3*(1), 1–9. https://doi.org/10.30516/bilgesci.476841

Bowers, K., Chow, E., Xu, H., Dror, R., Eastwood, M., Gregersen, B., Klepeis, J., Kolossváry, I., Moraes, M., Sacerdoti, F., Salmon, J., Shan, Y., & Shaw, D. (2006, November 11–17). *Molecular dynamics – Scalable algorithms for molecular dynamics simulations on commodity clusters* [Paper presentation]. SC '06: International Conference for High Performance Computing, Networking, Storage, and Analysis, Tampa, FL, United States, p. 84. https://doi.org/10.1145/1188455.1188544

Burdick, D., Soreghan, B., Kwon, M., Kosmoski, J., Knauer, M., Henschen, A., Yates, J., Cotman, C., & Glabe, C. (1992). Assembly and aggregation properties of synthetic Alzheimer's A4/β-amyloid peptide analogs. *The Journal of Biological Chemistry*, *267*(1), 546–554. https://doi.org/10.1016/S0021-9258(18)48529-8

Cai, H., Wang, Y., McCarthy, D., Wen, H., Borchelt, D. R., Price, D. L., & Wong, P. C. (2001). BACE1 is the major β-secretase for generation of Aβ peptides by neurons. *Nature Neuroscience*, *4*(3), 233–234. https://doi.org/10.1038/85064

Chandran, U., Mehendale, N., Patil, S., Chaguturu, R., & Patwardhan, B. (2017). Chapter 5 – Network pharmacology. In B. Patwardhan & R. Chaguturu (Eds.), *Innovative Approaches in Drug Discovery* (pp. 127–164). Academic Press. https://doi.org/10.1016/B978-0-12-801814-9.00005-2

Chugh, A., Sehgal, I., Khurana, N., Verma, K., Rolta, R., Vats, P., Salaria, D., Fadare, O. A., Awofisayo, O., Verma, A., Phartyal, R., & Verma, M. (2023). Comparative docking studies of drugs and phytocompounds for emerging variants of SARS-CoV-2. *3 Biotech*, *13*(1), 36. https://doi.org/10.1007/s13205-022-03450-6

De Strooper, B. (2003). Aph-1, Pen-2, and nicastrin with presenilin generate an active γ-secretase complex. *Neuron*, *38*(1), 9–12. https://doi.org/10.1016/s0896-6273(03)00205-8

Dehaene, S., & Naccache, L. (2001). Towards a cognitive neuroscience of consciousness: Basic evidence and a workspace framework. *Cognition*, *79*(1–2), 1–37. https://doi.org/10.1016/S0010-0277(00)00123-2

Elreedy, H. A., Elfiky, A. M., Mahmoud, A. A., Ibrahim, K. S., & Ghazy, M. A. (2023). Neuroprotective effect of quercetin through targeting key genes involved in aluminum chloride induced Alzheimer's disease in rats. *Egyptian Journal of Basic and Applied Sciences*, *10*(1), 174–184. https://doi.org/10.1080/2314808X.2022.2164136

Firuz Fatema Pria, M. S. I. (2019). *Phyllanthus emblica* Linn. (Amla) – A natural gift to humans: An overview. *Journal of Diseases and Medicinal Plants*, *5*(1), 1–9. https://doi.org/10.11648/j.jdmp.20190501.11

Gaudreault, R., & Mousseau, N. (2019). Mitigating Alzheimer's disease with natural polyphenols: A review. *Current Alzheimer Research*, *16*(6), 529–543. https://doi.org/10.2174/1567205016666190315093520

Ghosh, A. K., & Osswald, H. L. (2014). BACE1 (β-secretase) inhibitors for the treatment of Alzheimer's disease. *Chemical Society Reviews*, *43*(19), 6765–6813. https://doi.org/10.1039/c3cs60460h

Halperin, I., Ma, B., Wolfson, H., & Nussinov, R. (2002). Principles of docking: An overview of search algorithms and a guide to scoring functions. *Proteins*, *47*(4), 409–443. https://doi.org/10.1002/prot.10115

Hangya, B., Ranade, S. P., Lorenc, M., & Kepecs, A. (2015). Central cholinergic neurons are rapidly recruited by reinforcement feedback. *Cell*, *162*(5), 1155–1168. https://doi.org/10.1016/j.cell.2015.07.057

Hopkins, A. L. (2007). Network pharmacology. *Nature Biotechnology*, *25*(10), 1110–1111. https://doi.org/10.1038/nbt1007-1110

Husain, I., Zameer, S., Madaan, T., Minhaj, A., Ahmad, W., Iqubaal, A., Ali, A., & Najmi, A. K. (2019). Exploring the multifaceted neuroprotective actions of *Emblica officinalis* (Amla): A review. *Metabolic Brain Disease*, *34*(4), 957–965. https://doi.org/10.1007/s11011-019-00400-9

Hwang, E. M., Ryu, Y. B., Kim, H. Y., Kim, D.-G., Hong, S.-G., Lee, J. H., Curtis-Long, M. J., Jeong, S. H., Park, J.-Y., & Park, K. H. (2008). BACE1 inhibitory effects of lavandulyl flavanones from *Sophora flavescens*. *Bioorganic & Medicinal Chemistry*, *16*(14), 6669–6674. https://doi.org/10.1016/j.bmc.2008.05.080

Ivanova, L., Tammiku-Taul, J., García-Sosa, A. T., Sidorova, Y., Saarma, M., & Karelson, M. (2018). Molecular dynamics simulations of the interactions between glial cell line-derived neurotrophic factor family receptor GFRα1 and small-molecule ligands. *ACS Omega*, *3*(9), 11407–11414. https://doi.org/10.1021/acsomega.8b01524

Jabir, N. R., Rehman, M. T., Alsolami, K., Shakil, S., Zughaibi, T. A., Alserihi, R. F., Khan, M. S., AlAjmi, M. F., & Tabrez, S. (2021). Concatenation of molecular docking and molecular simulation of BACE-1, γ-secretase targeted ligands: In pursuit of Alzheimer's treatment. *Annals of Medicine*, *53*(1), 2332–2344. https://doi.org/10.1080/07853890.2021.2009124

Jakhmola, S., Jonniya, N. A., Sk, M. F., Rani, A., Kar, P., & Jha, H. C. (2021). Identification of potential inhibitors against Epstein–Barr virus nuclear antigen 1 (EBNA1): An insight from docking and molecular dynamic simulations. *ACS Chemical Neuroscience*, *12*(16), 3060–3072. https://doi.org/10.1021/acschemneuro.1c00350

Ji, B., Liu, S., He, X., Man, V. H., Xie, X.-Q., & Wang, J. (2020). Prediction of the binding affinities and selectivity for CB1 and CB2 ligands using homology modeling, molecular docking, molecular dynamics simulations, and MM-PBSA binding free energy calculations. *ACS Chemical Neuroscience*, *11*(8), 1139–1158. https://doi.org/10.1021/acschemneuro.9b00696

Kalirajan, R., Kulshrestha, V., Sankar, S., & Jubie, S. (2012). Docking studies, synthesis, characterization of some novel oxazine substituted 9-anilinoacridine derivatives and evaluation for their antioxidant and anticancer activities as topoisomerase II inhibitors. *European Journal of Medicinal Chemistry*, *56*, 217–224. https://doi.org/10.1016/j.ejmech.2012.08.025

Kaur, B., Rolta, R., Salaria, D., Kumar, B., Fadare, O. A., da Costa, R. A., Ahmad, A., Al-Rawi, M. B. A., Raish, M., & Rather, I. A. (2022). An in silico investigation to explore anti-cancer potential of *Foeniculum vulgare* Mill. phytoconstituents for the management of human breast cancer. *Molecules*, *27*(13), 4077. https://doi.org/10.3390/molecules27134077

Kim, H. J., Kim, J., Kang, K. S., Lee, K. T., & Yang, H. O. (2014). Neuroprotective effect of chebulagic acid via autophagy induction in SH-SY5Y cells. *Biomolecules & Therapeutics*, *22*(4), 275–281. https://doi.org/10.4062/biomolther.2014.068

Kim, S., Thiessen, P. A., Bolton, E. E., Chen, J., Fu, G., Gindulyte, A., Han, L., He, J., He, S., Shoemaker, B. A., Wang, J., Yu, B., Zhang, J., & Bryant, S. H. (2016). PubChem substance and compound databases. *Nucleic Acids Research*, *44*(D1), D1202–13. https://doi.org/10.1093/nar/gkv951

Kola-Mustapha, A. T., Raji, M. A., Adedeji, O., & Ambrose, G. O. (2023). Network pharmacology and molecular modeling to elucidate the potential mechanism of neem oil against acne vulgaris. *Molecules (Basel, Switzerland)*, *28*(6), 2849. https://doi.org/10.3390/molecules28062849

Kumar, B., Thakur, A., Dwivedi, A. R., Kumar, R., & Kumar, V. (2022). Multi-target-directed ligands as an effective strategy for the treatment of Alzheimer's disease. *Current Medicinal Chemistry*, *29*(10), 1757–1803. https://doi.org/10.2174/0929867328666210512005508

Kwak, H.-M., Jeon, S.-Y., Sohng, B.-H., Kim, J.-G., Lee, J.-M., Lee, K.-B., Jeong, H.-H., Hur, J.-M., Kang, Y.-H., & Song, K.-S. (2005). β-Secretase (BACE1) inhibitors from pomegranate (*Punica granatum*) husk. *Archives of Pharmacal Research*, *28*(12), 1328–1332. https://doi.org/10.1007/BF02977896

Liu, F., Li, L., Chen, J., Wu, Y., Cao, Y., & Zhong, P. (2021). A network pharmacology to explore the mechanism of *Calculus bovis* in the

treatment of ischemic stroke. *BioMed Research International*, *2021*, 6611018. https://doi.org/10.1155/2021/6611018

Liu, T., Lin, Y., Wen, X., Jorissen, R. N., & Gilson, M. K. (2007). BindingDB: A web-accessible database of experimentally determined protein-ligand binding affinities. *Nucleic Acids Research*, *35*(Database issue), D198–201. https://doi.org/10.1093/nar/gkl999

Mani, V., & Parle, M. (2007). Memory enhancing activity of Anwala churna (*Emblica officinalis* Gaertn.): An Ayurvedic preparation. *Physiology & Behavior*, *91*(1), 46–54. https://doi.org/10.1016/j.physbeh.2007.01.016

Mathew, M., & Subramanian, S. (2014). In vitro screening for anti-cholinesterase and antioxidant activity of methanolic extracts of ayurvedic medicinal plants used for cognitive disorders. *PLoS One*, *9*(1), e86804. https://doi.org/10.1371/journal.pone.0086804

Mi, H., Huang, X., Muruganujan, A., Tang, H., Mills, C., Kang, D., & Thomas, P. D. (2017). PANTHER version 11: Expanded annotation data from Gene Ontology and Reactome pathways, and data analysis tool enhancements. *Nucleic Acids Research*, *45*(D1), D183–D189. https://doi.org/10.1093/nar/gkw1138

Ramachandran, B., Kesavan, S., & Rajkumar, T. (2016). Molecular modeling and docking of small molecule inhibitors against NEK2. *Bioinformation*, *12*(2), 62–68. https://doi.org/10.6026/97320630012062

Roberds, S. L., Anderson, J., Basi, G., Bienkowski, M. J., Branstetter, D. G., Chen, K. S., Freedman, S. B., Frigon, N. L., Games, D., Hu, K., Johnson-Wood, K., Kappenman, K. E., Kawabe, T. T., Kola, I., Kuehn, R., Lee, M., Liu, W., Motter, R., Nichols, N. F., … McConlogue, L. (2001). BACE knockout mice are healthy despite lacking the primary β-secretase activity in brain: Implications for Alzheimer's disease therapeutics. *Human Molecular Genetics*, *10*(12), 1317–1324. https://doi.org/10.1093/hmg/10.12.1317

Román, J., Castillo, A., & Mahn, A. (2018). Molecular docking of potential inhibitors of broccoli myrosinase. *Molecules*, *23*(6), 1313. https://doi.org/10.3390/molecules23061313

Sabogal-Guáqueta, A. M., Muñoz-Manco, J. I., Ramírez-Pineda, J. R., Lamprea-Rodriguez, M., Osorio, E., & Cardona-Gómez, G. P. (2015). The flavonoid quercetin ameliorates Alzheimer's disease pathology and protects cognitive and emotional function in aged triple transgenic Alzheimer's disease model mice. *Neuropharmacology*, *93*, 134–145. https://doi.org/10.1016/j.neuropharm.2015.01.027

Salo-Ahen, O. M. H., Alanko, I., Bhadane, R., Bonvin, A. M. J. J., Honorato, R. V., Hossain, S., Juffer, A. H., Kabedev, A., Lahtela-Kakkonen, M., Larsen, A. S., Lescrinier, E., Marimuthu, P., Mirza, M. U., Mustafa, G., Nunes-Alves, A., Pantsar, T., Saadabadi, A., Singaravelu, K., & Vanmeert, M. (2020). Molecular dynamics simulations in drug discovery and pharmaceutical development. *Processes*, *9*(1), 71. https://doi.org/10.3390/pr9010071

Singh, E., Sharma, S., Pareek, A., Dwivedi, J., & Yadav, S. (2012). Phytochemistry, traditional uses and cancer chemopreventive activity of Amla (*Phyllanthus emblica*): The sustainer. *Journal of Applied Pharmaceutical Science*, *2*, 176–183.

Smoot, M. E., Ono, K., Ruscheinski, J., Wang, P.-L., & Ideker, T. (2011). Cytoscape 2.8: New features for data integration and network visualization. *Bioinformatics*, *27*(3), 431–432. https://doi.org/10.1093/bioinformatics/btq675

Takeda, S. (2019). Progression of Alzheimer's disease, tau propagation, and its modifiable risk factors. *Neuroscience Research*, *141*, 36–42. https://doi.org/10.1016/j.neures.2018.08.005

Tang, Y., Li, M., Wang, J., Pan, Y., & Wu, F.-X. (2015). CytoNCA: A Cytoscape plugin for centrality analysis and evaluation of protein interaction networks. *BioSystems*, *127*, 67–72. https://doi.org/10.1016/j.biosystems.2014.11.005

Thenmozhi, A. J., Dhivyabharathi, M., Raja, T. R. W., Manivasagam, T., & Essa, M. M. (2016). Tannoid principles of *Emblica officinalis* renovate cognitive deficits and attenuate amyloid pathologies against aluminum chloride induced rat model of Alzheimer's disease. *Nutritional Neuroscience*, *19*(6), 269–278. https://doi.org/10.1179/1476830515Y.0000000016

Trott, O., & Olson, A. J. (2010). AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of Computational Chemistry*, *31*(2), 455–461. https://doi.org/10.1002/jcc.21334

Van der Schyf, C. J. (2011). The use of multi-target drugs in the treatment of neurodegenerative diseases. *Expert Review of Clinical Pharmacology*, *4*(3), 293–298. (https://doi.org/10.1586/ecp.11.13

Wang, W., Liu, T., Yang, L., Ma, Y., Dou, F., Shi, L., Wen, A., & Ding, Y. (2019). Study on the multi-targets mechanism of Triphala on cardio-cerebral vascular diseases based on network pharmacology. *Biomedicine & Pharmacotherapy = Biomedecine & Pharmacotherapie*, *116*, 108994. https://doi.org/10.1016/j.biopha.2019.108994

Yokozawa, T., Kim, S., Kim, H., Okubo, T., Chu, D.-C., & Juneja, L. (2007). Amla (*Emblica officinalis* Gaertn.) prevents dyslipidaemia and oxidative stress in the ageing process. *The British Journal of Nutrition*, *97*(6), 1187–1195. https://doi.org/10.1017/S0007114507691971