

Integrative Network Pharmacology, Molecular Docking, and in Vitro Validation of Senna Auriculata Phytoconstituents As Potential Multi-Target Candidates For Alzheimer's Disease

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Abstract

Background: Alzheimer's disease is a progressive neurodegenerative disorder characterized by cholinergic deficit, amyloid-beta and tau pathology, and oxidative stress, for which currently approved therapies offer only modest symptomatic relief without altering disease progression. Medicinal plants rich in polyphenolic constituents represent a promising source of multi-target anti-Alzheimer's agents. **Objective:** To evaluate the anti-Alzheimer's disease potential of the whole-plant ethanolic extract of *Senna auriculata* through an integrated in-silico and in-vitro approach. **Methods:** Reported phytoconstituents of *S. auriculata* were retrieved from the IMPPAT database and their canonical structures standardized from PubChem. ADME and drug-likeness properties were predicted using SwissADME, and molecular targets were predicted with SwissTargetPrediction. Alzheimer's disease-associated targets from GeneCards and OMIM were intersected with the predicted targets by Venn analysis, and the common targets were subjected to protein–protein interaction network analysis in STRING, visualization in Cytoscape, hub identification with CytoHubba, and Gene Ontology and KEGG pathway enrichment analyses. Molecular docking of prioritized constituents with hub proteins was performed using CB-Dock2 and AutoDock Vina, followed by normal mode analysis of the lead complexes with iMODS. In-vitro, SA-EE was evaluated for DPPH and ABTS radical scavenging activity, acetylcholinesterase and butyrylcholinesterase inhibition by Ellman's method, and cytoprotection against hydrogen peroxide-induced oxidative stress in SH-SY5Y neuroblastoma cells by MTT assay. **Results:** Network pharmacology identified AKT1 and SRC as principal hub targets, and the flavonoid glycoside rutin emerged as the lead constituent, forming energetically favorable complexes with both hubs whose stability was supported by normal mode analysis. In-vitro, SA-EE exhibited concentration-dependent DPPH and ABTS radical scavenging activity, inhibited acetylcholinesterase by up to 91.00% at 100 µg/mL ($R^2 = 0.9259$) and butyrylcholinesterase by up to 81.44% at 600 µg/mL ($R^2 = 0.9616$), and protected SH-SY5Y cells against H_2O_2 -induced oxidative stress. **Conclusion:** SA-EE possesses appreciable dual cholinesterase-inhibitory, antioxidant, and cytoprotective activities that are consistent with its network pharmacology profile, positioning *S. auriculata* as a promising multi-target natural source warranting further isolation of active constituents and in-vivo evaluation in Alzheimer's disease research.

Keywords: *Senna auriculata*; Alzheimer's disease; network pharmacology; molecular docking; acetylcholinesterase; butyrylcholinesterase; oxidative stress; rutin.

1. INTRODUCTION

Alzheimer's disease is a progressive neurodegenerative condition characterized by memory impairment, cognitive decline, and changes in behavior, which severely affect daily functioning (Reitz et al., 2011). It is the most common cause of dementia, accounting for approximately 60–80% of dementia cases, with an estimated 24 million people affected worldwide (Erkkinen et al., 2017). Global burden analyses indicate that about 0.7% of the world's population approximately 51.6 million individuals was living with dementia in 2019, more than double the number affected in 1990 (Javadi et al., 2021). Between 1990 and 2019, the global incidence and prevalence of AD and other dementias increased by 147.95% and 160.84%, respectively (Li et al., 2022), and the number of dementia patients worldwide is projected to reach 152 million by mid-century (Zhang et al., 2021).

The development of AD is complex, involving a mix of genetic, environmental, and lifestyle contributors (Zhang et al., 2021). Critical theories include the cholinergic hypothesis, which focuses on the degeneration of basal forebrain cholinergic neurons and the consequent reduction in synaptic acetylcholine availability (Yang et al., 2012), the accumulation of amyloid-beta plaques and hyperphosphorylated tau neurofibrillary tangles (Tamagno et al., 2021), and oxidative stress in neuronal damage (Kim & Sharma, 2023; Perluigi et al., 2023). Reactive oxygen species are produced by standard metabolic processes such as oxidative phosphorylation, which is the primary source of cellular superoxide anion ($O_2^{\bullet-}$), hydroxyl radicals ($\bullet OH$), and hydrogen peroxide (H_2O_2). Maintaining a proper balance of ROS is crucial for physiological signaling and immune responses; however, when ROS levels become excessive, they initiate oxidation of lipids, proteins, DNA, and other vital molecules, resulting in

oxidative stress that is linked to neurodegenerative diseases, atherosclerosis, accelerated aging, and cancer (Kim & Sharma, 2023). Among the various ROS, H₂O₂ is known to promote apoptosis in different cells, acts as a precursor of highly reactive radicals that readily move in and out of cells and tissues, and is implicated in tau hyperphosphorylation and the creation of harmful neurofibrillary tangles in brain cells (Perluigi et al., 2023; Tamagno et al., 2021). Thus, the overproduction of free radicals compromises the internal antioxidant defense mechanisms and necessitates external antioxidants from dietary sources, such as vitamins C and E and polyphenols, which act as "free radical scavengers" to prevent and repair damage caused by ROS (Santos et al., 2018). Natural resources rich in phenolic compounds and pigments enhance antioxidant activity (Kumar et al., 2024).

Currently, the primary treatment options for AD are cholinesterase inhibitors such as donepezil and NMDA receptor antagonists such as memantine (Ahmed et al., 2021; Liu et al., 2019). Although these drugs can alleviate symptoms, they do not alter the course of the disease (Marucci et al., 2020). There is an urgent need for treatments that tackle the underlying causes of Alzheimer's disease for lasting benefits. Bioactive compounds derived from plants — including phenols, glycosides, flavonoids, and alkaloids — have shown significant promise in preventing plaque formation and enhancing cholinergic transmission (Kumar et al., 2024; Santos et al., 2018).

Senna auriculata Roxb. (syn. *Cassia auriculata* L.), commonly known as 'avaram' and widely distributed in the dry and hot deciduous regions of the Indian subcontinent, is an important medicinal shrub of the family Fabaceae (Bhakshu et al., 2023; Donagaon4, 2026). Traditionally utilized in Ayurvedic and Siddha systems of medicine for the management of diabetes, rheumatic pains, fever, urinary complaints, and abdominal disorders (Bhakshu et al., 2023; Nille et al., 2021), *S. auriculata* is rich in bioactive constituents, including alkaloids, anthraquinones, flavone glycosides, saponins, phenols, terpenoids, flavonoids, tannins, and sterols, together with specific compounds such as epicatechin, ferulic acid, quercetin, quercetin-3-O-rutinoside, and proanthocyanidin B1 (Donagaon4, 2026; Nille et al., 2021). Extracts of the plant exhibit a wide spectrum of pharmacological activities, including antioxidant, anti-inflammatory, antidiabetic, hepatoprotective, nephroprotective, and antimicrobial effects (Baluchamy & Arivoli, 2023; Bhakshu et al., 2023; Nille et al., 2021). The antioxidant and anti-inflammatory properties of its phenolic constituents are of particular relevance to neurodegeneration, since these activities counter two of the principal drivers of AD pathology (Kumar et al., 2024).

Human neuroblastoma SH-SY5Y cells, which exhibit neuronal properties and a characteristic susceptibility to oxidative insults, are widely employed to replicate the human neuronal response to excess ROS in neurodegenerative disease research; in the present study, these cells were used to evaluate the cytoprotective effect of the *S. auriculata* ethanolic extract against H₂O₂-induced oxidative stress using the MTT assay (Mosmann, 1983).

Network pharmacology, as first introduced by Hopkins, merges systems and computational biology to elucidate the connections between metabolites, targets, and diseases, facilitating drug discovery and providing insights into drug–organism interactions (Li et al., 2023; Noor et al., 2022). Integrating bioinformatics and network pharmacology through computational predictions is a robust approach to uncovering the relationships between biological mechanisms, complex diseases, and drug effects (Noor et al., 2022). Molecular docking technology further investigates the interactions between small molecules and larger biological macromolecules (Eberhardt et al., 2021; Liu et al., 2022). This study uniquely combines network pharmacology with molecular docking and in vitro validation to explore how *Senna auriculata* may treat AD, focusing on identifying essential compounds and targets for effective multi-target strategies (Gangwal et al., 2026; Khare et al., 2024). The findings from this research could offer additional understanding of the molecular processes and treatment strategies for AD and its associated effects.

2. Materials and Methods

2.1 In Silico Analysis

2.1.1 Screening of Bioactive Compounds in Whole Plant of *Senna auriculata*

The bioactive compounds reported from the whole plant of *Senna auriculata* were identified through a comprehensive literature search and by consulting the Indian Medicinal Plants, Phytochemistry and Therapeutics database. The scientific name *Senna auriculata* was used as the search keyword to retrieve the phytochemical constituents associated with the plant. IMPPAT is a manually curated database of 1,742 Indian

medicinal plants and 9,596 phytochemicals with standard chemical identifiers and structural information (Mohanraj et al., 2018), with IMPPAT 2.0 expanding this to 4,010 plants and 17,967 phytochemicals with plant-part level associations (Vivek-Ananth et al., 2023). The identified phytochemicals were compiled and standardized for subsequent computational analysis. The corresponding canonical chemical structures and SMILES representations of the compounds were retrieved from the PubChem database (Kim et al., 2022). The resulting non-redundant compound dataset was subsequently used for further *in silico* screening, target prediction, network pharmacology, molecular docking, and pharmacological analysis.

2.1.2 ADME and Drug-Likeness Prediction

The pharmacokinetic and drug-likeness properties of the identified phytoconstituents of the whole plant of *Senna auriculata* were evaluated using the SwissADME web server (Daina et al., 2017). The compounds were assessed for physicochemical properties, including molecular weight, lipophilicity, aqueous solubility, gastrointestinal absorption, blood-brain barrier permeability, P-glycoprotein substrate status, cytochrome P450 enzyme interactions, Lipinski's rule of five and bioavailability characteristics. SwissADME integrates multiple computational models to provide rapid prediction of pharmacokinetic properties and drug-likeness of small molecules (Daina et al., 2017).

2.1.3 Molecular Target Prediction

The potential molecular targets of the identified whole plant of *S. auriculata* phytoconstituents were predicted using SwissTargetPrediction, a web server that predicts the probable protein targets of bioactive small molecules based on the similarity principle through reverse screening (Daina et al., 2019). Each phytoconstituent was submitted individually, and the predicted protein targets were retrieved based on ligand similarity and known bioactive compound–target relationships. The predicted targets were consolidated into a single dataset, and duplicate entries were removed to obtain a non-redundant target list.

2.1.4 Alzheimer's Disease-Associated Target Identification

Alzheimer's disease-associated genes were retrieved using the keyword "Alzheimer's disease" from the GeneCards human gene compendium (Stelzer et al., 2016) and the Online Mendelian Inheritance in Man knowledgebase of human genes and phenotypes (Amberger et al., 2018). The retrieved gene lists were compiled, standardized and duplicate entries were removed to generate a consolidated Alzheimer's disease-associated target dataset. The resulting disease-associated targets were subsequently compared with the predicted targets of whole plant of *S. auriculata* phytoconstituents.

2.1.5 Identification of Common Targets

The predicted targets of whole plant of *S. auriculata* phytoconstituents were compared with Alzheimer's disease-associated targets using the Venny tool (Jia et al., 2021). The two datasets were uploaded to the platform, and the overlapping targets were identified using Venn diagram analysis, which is a widely used approach for identifying shared elements among multiple datasets in bioinformatics (Jia et al., 2021). The common targets were considered potential molecular targets linking *S. auriculata* phytoconstituents with Alzheimer's disease and were subsequently used for PPI network and enrichment analyses.

2.1.6 Protein–Protein Interaction Network Analysis

The identified common targets were subjected to protein–protein interaction network analysis using the STRING database, which collects, scores and integrates all publicly available sources of protein–protein interaction information together with computational predictions (Szklarczyk et al., 2020). The target proteins were submitted to STRING, and the interaction network was generated using the appropriate organism and interaction parameters, providing functional association networks for biological analysis.

2.1.7 Network Visualization and Hub Gene Identification

The STRING-derived PPI network was imported into Cytoscape, an open-source software environment for integrating biomolecular interaction networks with molecular data into a unified conceptual framework (Shannon et al., 2003), for network visualization and topological analysis. The CytoHubba plugin was subsequently used to rank network nodes according to their topological properties, employing eleven scoring methods to identify hub objects and sub-networks (Chin et al., 2014). Degree centrality, betweenness centrality and closeness centrality were considered for identification of highly connected and topologically important hub proteins.

2.1.8 Gene Ontology Enrichment Analysis

The identified common targets were subjected to Gene Ontology enrichment analysis to characterize their functional roles, using the standardized controlled vocabulary for biological process, cellular component and molecular function (Ashburner et al., 2000). Biological process terms were used to identify biological events associated with the targets, cellular component terms to determine their predominant cellular localization, and molecular function terms to characterize their biochemical activities. GO terms with an FDR < 0.05 were considered statistically significant.

2.1.9 KEGG Pathway Enrichment Analysis

The common targets were subjected to Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis to identify significantly associated signaling and metabolic pathways (Kanehisa, 2000). The target genes were mapped against the KEGG pathway database, and enriched pathways were identified based on the representation of the common targets within individual pathways. Pathways satisfying the predefined statistical significance criterion were considered significantly enriched and were used for subsequent mechanistic evaluation.

2.2. Molecular Docking

2.2.1 Protein Structure Retrieval and Preparation

The three-dimensional structures of the selected hub proteins were retrieved from the Protein Data Bank, the single global archive of experimentally determined macromolecular structures (Berman, 2000). Protein structures with suitable structural information were selected for molecular docking. The receptor structures were prepared by removing unnecessary molecules and retaining the protein structure required for ligand-binding analysis. The prepared structures were subsequently used as receptor molecules for docking.

2.2.2 Ligand Structure Preparation

The chemical structures of the selected *S. auriculata* phytoconstituents were obtained from publicly available chemical databases, including PubChem (Kim et al., 2022). The structures were checked and prepared in suitable formats for molecular docking. The ligand structures were subsequently used for interaction analysis with the selected hub proteins.

2.2.3 Molecular Docking of *Senna auriculata* Bioactive Constituents with Hub Proteins

Molecular docking was performed to investigate the potential interactions between the bioactive constituents identified from the whole plant of *Senna auriculata* and the hub proteins identified through network pharmacology analysis. The three-dimensional structures of the selected phytoconstituents were retrieved from the PubChem database and prepared using UCSF Chimera version 1.17.1 (Pettersen et al., 2004). Ligand preparation involved verification of the molecular structures, addition of missing atoms and polar hydrogens, and energy minimization to obtain energetically optimized ligand conformations.

The three-dimensional structures of the selected hub proteins were retrieved from the Protein Data Bank (Berman, 2000). The receptor structures were prepared using BIOVIA Discovery Studio, which involved removal of crystallographic water molecules and co-crystallized ligands. The potential binding pockets of the selected target proteins were identified using the PrankWeb server (Jendele et al., 2019). Further protein preparation was performed using AutoDock Tools, including addition of polar hydrogens, assignment of Kollman charges and definition of appropriate atom types.

Initial protein–ligand interactions and potential binding cavities were investigated using CB-Dock/CB-Dock2, an automated blind-docking platform that integrates binding-cavity detection with molecular docking (Liu et al., 2022). The predicted binding cavities and docking poses were used to identify suitable ligand-binding regions within the selected hub proteins. In addition, molecular docking was performed using AutoDock Vina (Eberhardt et al., 2021), with the grid-box center and dimensions defined according to the predicted active-site or binding-pocket coordinates. The docking poses were ranked according to their predicted binding affinity, expressed in kcal/mol, with more negative values indicating more favourable predicted binding interactions. The resulting protein–ligand complexes were further evaluated based on binding affinity, hydrogen-bond interactions, hydrophobic interactions and interacting amino acid residues. The final docked complexes were visualized and analysed using BIOVIA Discovery Studio Visualizer and UCSF Chimera. The use of molecular docking and subsequent structural analysis is consistent with previous computational investigations of phytoconstituents from *S. auriculata*, including the docking of its methanolic leaf-extract compounds against α -amylase for antidiabetic potential (Dukhyil, 2021), and the network pharmacology and docking study of Karamarathodi et al.

2.2.4 Normal Mode Analysis of Selected *Senna auriculata* Protein–Ligand Complexes

The selected protein–ligand complexes obtained from molecular docking were subjected to normal mode analysis to investigate their structural flexibility and conformational dynamics. Based on the favorable docking interactions, the AKT1–Rutin and SRC–Rutin complexes were selected for further computational analysis. The complexes were analysed using the iMODS server, which employs an elastic network model to evaluate the intrinsic collective motions of macromolecular structures (López-Blanco et al., 2014).

The NMA included evaluation of root mean square fluctuation, deformability, B-factor, eigenvalue, variance distribution and covariance matrix. RMSF analysis was used to assess residue-level flexibility, while the deformability profile was used to identify regions with greater potential for structural deformation. The B-factor profile was analysed to evaluate the relative mobility of individual residues. Eigenvalue analysis was used to assess the energy required for the corresponding collective motions, whereas the variance distribution was used to determine the contribution of individual normal modes to the overall motion of the complex. The covariance matrix was used to evaluate correlated and anti-correlated movements among residues. The iMODS analysis was performed using its default parameters, with an elastic network model applied to maintain the structural integrity of the protein while permitting coordinated conformational movements (López-Blanco et al., 2014).

2.3. In Vitro Analysis

2.3.1 MTT Cytotoxicity Assay

The cytotoxicity of SA-EE against the human neuroblastoma SH-SY5Y cell line was evaluated using the colorimetric MTT assay originally described by Mosmann (Mosmann, 1983). The SH-SY5Y cells were used as the cellular model, and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide was used as the colorimetric reagent. The assay is based on the reduction of the yellow, water-soluble MTT reagent to insoluble purple formazan crystals by metabolically active cells (Mosmann, 1983). The intensity of the resulting purple colour is proportional to cellular metabolic activity and was quantified by measuring the absorbance at 570 nm using a microplate reader.

The SH-SY5Y cells were cultured in DMEM and Ham's F-12 medium supplemented with 10% fetal bovine serum and 1% antibiotic-antimycotic solution and maintained at 37 °C in a humidified atmosphere containing 5% CO₂. The cells were seeded into 96-well plates at an approximate density of 20,000 cells/well and allowed to adhere for approximately 24 h. Following cell attachment, the cells were treated with H₂O₂ (800 µM) alone or with H₂O₂ (800 µM) in combination with different concentrations of *Senna auriculata* extract (6.25, 12.5 and 25 µg/mL). Untreated cells were maintained as the control group. Following 24 h of treatment, the spent medium was removed and MTT reagent was added to achieve a final concentration of approximately 0.5 mg/mL. The plate was protected from light and incubated for approximately 3 h at 37 °C to allow the formation of formazan crystals.

After incubation, the MTT-containing medium was removed and 100 µL of solubilization solution, such as DMSO, was added to each well to dissolve the formazan crystals. The plate was gently shaken to facilitate complete dissolution, and the absorbance was subsequently measured at 570 nm using a microplate reader. The cell viability was calculated relative to the untreated control using the following equation:

$$\text{Percentage Inhibition} = \frac{100 - \{\text{Absorbance of Control} - \text{Absorbance of Sample}\}}{\text{Absorbance of Control}} \times 100$$

The MTT assay was used to determine the effect of H₂O₂-induced oxidative stress and to evaluate the response of SH-SY5Y cells following treatment with different concentrations of *S. auriculata* extract. The resulting cell viability values were used to assess the potential protective or cytotoxic effects of SA-EE under the experimental conditions. Direct microscopic observations of the treated cells after 24 h of incubation were also recorded to assess morphological changes associated with the treatments (Mosmann, 1983).

2.3.2 DPPH Radical Scavenging Assay

The free-radical scavenging activity of SA-EE was evaluated using the 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay following the method of Brand-Williams et al. with appropriate modification (Brand-Williams et al., 1995). A 500 µM DPPH solution was prepared in ethanol, and SA-EE was evaluated at concentrations ranging from 10 to 50 µg/mL. Different concentrations of the extract were mixed with the DPPH solution and incubated for 30 min in the dark at room temperature. The absorbance was measured at 517 nm using a UV–

visible spectrophotometer. Ascorbic acid was used as the reference standard. The percentage of DPPH radical scavenging activity was calculated relative to the control, and the IC₅₀ value was determined from the concentration–response relationship. The DPPH method is based on the reduction of the DPPH radical by antioxidant molecules, resulting in a decrease in absorbance (Brand-Williams et al., 1995).

$$\text{DPPH Radical Scavenging Activity (\%)} = \frac{\{\text{Absorbance of Control} - \text{Absorbance of Sample}\}}{\text{Absorbance of Control}} \times 100$$

2.3.3 ABTS Radical Scavenging Assay

The antioxidant activity of SA-EE was evaluated using the ABTS [2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] radical cation scavenging assay following the method of Re et al. with appropriate modification (Re et al., 1999). ABTS was prepared at a concentration of 7 mM and reacted with 2.45 mM potassium persulfate to generate the ABTS radical cation. The mixture was maintained in the dark at room temperature for 12–16 h. Prior to use, the ABTS radical solution was diluted with ethanol to obtain an absorbance of approximately 0.70 at 734 nm. SA-EE was evaluated at concentrations ranging from 5 to 25 µg/mL. The extract was mixed with the ABTS radical solution and incubated for 30 min in the dark at room temperature, followed by measurement of absorbance at 734 nm. Ascorbic acid was used as the reference standard. The percentage radical scavenging activity and IC₅₀ value were calculated from the concentration–response relationship. The ABTS assay is based on the decolorization of the preformed ABTS radical cation in the presence of antioxidant compounds (Re et al., 1999).

$$\text{ABTS Radical Scavenging Activity (\%)} = \frac{\{\text{Absorbance of Control} - \text{Absorbance of Sample}\}}{\text{Absorbance of Control}} \times 100$$

2.3.4 Acetylcholinesterase Inhibitory Assay

The inhibitory activity of SA-EE against acetylcholinesterase was evaluated using the colorimetric method originally described by Ellman et al. (Ellman et al., 1961). Acetylcholinesterase obtained from electric eel was used as the enzyme source, acetylthiocholine iodide as the substrate, and 5,5'-dithiobis-(2-nitrobenzoic acid) as the chromogenic reagent. The reaction mixture contained 100 mM potassium phosphate buffer (pH 7.0), 2 mM DTNB, 0.8 mM ATCI and 5 µg/mL AChE in a final volume of 1000 µL. Different concentrations of SA-EE ranging from 20 to 100 µg/mL were pre-incubated with the enzyme at room temperature for 30 min. The enzymatic reaction was subsequently performed at 37 °C, and the absorbance was measured at 412 nm using a UV–visible microplate reader. The percentage inhibition of AChE was calculated relative to the untreated enzyme control, and the IC₅₀ value was determined from the concentration–response curve. The Ellman method quantifies cholinesterase activity through the formation of a yellow-coloured product generated from the reaction of thiocholine with DTNB (Ellman et al., 1961).

2.3.5 Butyrylcholinesterase Inhibitory Assay

The inhibitory activity of SA-EE against butyrylcholinesterase was determined using the same Ellman-type colorimetric principle (Ellman et al., 1961). Human serum was used as the enzyme source, butyryl thiocholine iodide was used as the substrate, and DTNB was used as the chromogenic reagent. The reaction mixture contained 100 mM potassium phosphate buffer (pH 7.0), 2 mM DTNB and 0.8 mM BTCl, together with the human serum enzyme source, in a final volume of 1000 µL. SA-EE was evaluated at concentrations ranging from 120 to 600 µg/mL. The extract was pre-incubated with the enzyme source at room temperature for 30 min, followed by enzymatic reaction at 37 °C. Absorbance was measured at 412 nm, and the percentage inhibition was calculated relative to the untreated enzyme control. The IC₅₀ value was determined from the concentration–response relationship (Ellman et al., 1961).

3. Results

3.1 In Silico ADME and Drug-Likeness Analysis of Phytoconstituents Identified from *Senna auriculata* Whole Plant

Senna auriculata whole plant contains diverse phytoconstituents, including flavonoids, flavonoid glycosides, anthraquinone derivatives, steroids, long-chain hydrocarbons, alcohols and fatty acids, with molecular weights ranging from 208.21 to 610.50 g/mol. The major identified phytoconstituents were subjected to in silico ADME profiling using SwissADME, which revealed substantial variation in physicochemical and pharmacokinetic properties (Table 1). Consensus LogP values ranged from –2.74 for rutin to 12.03 for nonacosane, while predicted solubility and gastrointestinal absorption varied considerably among the compounds. Highly polar glycosylated flavonoids such as rutin and quercetin-3-glucoside showed favourable predicted solubility but low

gastrointestinal absorption, whereas highly lipophilic constituents, including nonacosane and β -sitosterol, exhibited poor predicted solubility and absorption. In contrast, kaempferol, cyanidin chloride, cianidanol, chrysophanol, rubiadin, emodin and anthraquinone showed high predicted gastrointestinal absorption and no Lipinski violations. Kaempferol exhibited the most balanced predicted ADME profile, with a molecular weight of 286.24 g/mol, consensus LogP of 1.20, high gastrointestinal absorption, absence of P-gp substrate activity and a bioavailability score of 0.55 (Table 1). These findings identify kaempferol and several other constituents as promising candidates for subsequent experimental validation, although computational predictions should be interpreted cautiously.

Table 1. In silico physicochemical, pharmacokinetic and drug-likeness profiles of major phytoconstituents identified from *Senna auriculata* whole plant.

S. No.	Phytoconstituent	MW (g/mol)	Consensus LogP	Solubility	GI Absorption	P-gp Substrate	Lipinski Violations	Bioavailability Score
1	Rutin	610.50	-2.74	Soluble	Low	No	3	0.17
2	Kaempferol	286.24	1.20	Soluble	High	No	0	0.55
3	Cyanidin chloride	322.69	0.48	Soluble	High	No	0	0.55
4	β -Sitosterol	414.71	7.78	Poorly soluble	Low	No	1	0.55
5	Cianidanol	290.27	0.70	Soluble	High	Yes	0	0.55
6	Chrysophanol	254.24	2.42	Moderately soluble	High	No	0	0.55
7	Rubiadin	256.25	2.30	Moderately soluble	High	No	0	0.55
8	Nonacosane	408.80	12.03	Insoluble	Low	Yes	1	0.55
9	Emodin	270.24	1.88	Soluble	High	No	0	0.55
10	Nonacosan-6-one	422.82	10.73	Insoluble	Low	Yes	1	0.55
11	1-Tetradecanol	214.39	4.86	Moderately soluble	High	No	0	0.55
12	Anthraquinone	208.22	2.82	Moderately soluble	High	No	0	0.55
13	Quercetin-3-glucoside	464.38	-1.34	Soluble	Low	No	2	0.17
14	β -Sitosterol- β -D-glucoside	576.85	5.64	Poorly soluble	Low	No	1	0.55
15	Stearic acid	284.48	6.34	Moderately soluble	High	No	1	0.85
16	Palmitic acid	256.42	5.54	Moderately soluble	High	No	1	0.85
17	Oleic acid	282.47	6.07	Moderately soluble	High	No	1	0.85
18	Linolenic acid	278.43	5.52	Moderately soluble	High	Yes	1	0.85
19	Linoleic acid	280.45	5.78	Moderately soluble	High	No	1	0.85
20	Flavylium	208.21	3.04	Soluble/poorly soluble*	High	Yes	0	0.55

3.2 Target Prediction of *Senna auriculata* Phytoconstituents and Identification of Alzheimer's Disease-Associated Targets

To identify potential targets associated with Alzheimer's disease (AD), disease-related genes were retrieved from the OMIM and GeneCards databases, while potential targets of the identified *Senna auriculata* phytoconstituents were predicted using the SwissTargetPrediction database. A total of 1,900 predicted target entries were obtained for the 19 investigated phytoconstituents. After removal of duplicate entries, 506 unique protein targets associated with *S. auriculata* phytoconstituents were identified. Similarly, consolidation and removal of duplicate entries from the AD-related datasets yielded 20,580 unique AD-associated genes. The potential targets of *S. auriculata* and AD-associated genes were subsequently compared using Venny, and the overlapping targets were visualized using a Venn diagram (Figure 1). The analysis identified 487 common targets shared between *S. auriculata* phytoconstituents and AD. These overlapping targets suggest that the phytoconstituents of *S. auriculata* may potentially exert anti-Alzheimer's effects through interactions with multiple AD-associated molecular targets, supporting a possible multi-component and multi-target mechanism of action. The 487 common targets were therefore considered candidate therapeutic targets and were subsequently subjected to protein-protein interaction network analysis, hub-target identification, Gene Ontology and Kyoto Encyclopedia of Genes and Genomes pathway enrichment analyses to elucidate the potential molecular mechanisms underlying the anti-Alzheimer's activity of *S. auriculata*.

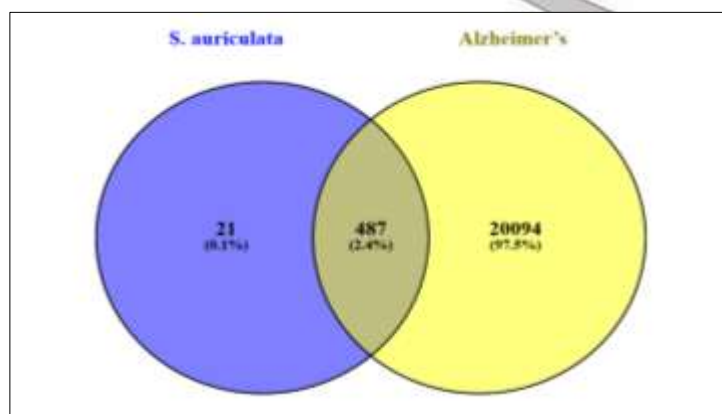


Figure 1. Identification of common targets between *Senna auriculata* phytoconstituents and Alzheimer's disease. (A) Venn diagram illustrating the overlap between *S. auriculata*-associated potential targets and Alzheimer's disease-associated genes.

3.3. Construction and Analysis of the Target Protein–Protein Interaction Network

The 487 common targets of *Senna auriculata* phytoconstituents and Alzheimer's disease (AD) were used to construct a protein–protein interaction (PPI) network using STRING and visualized in Cytoscape (Figure 2A). The resulting network comprised 480 nodes and 8,035 edges, with an average node degree of 33.479, clustering coefficient of 0.226, network density of 0.035, and characteristic path length of 2.491, indicating extensive interconnectivity among the candidate targets. The network contained two connected components, with the majority of proteins forming an interconnected interaction network (Figure 2A).

Hub targets were subsequently identified using the CytoHubba plugin in Cytoscape based on degree centrality. The top 20 hub genes were AKT1, TNF, ALB, EGFR, CTNNB1, SRC, STAT3, BCL2, HSP90AA1, ESR1, HIF1A, CASP3, PPARG, PTGS2, MAPK3, HSP90AB1, EP300, MTOR, SIRT1, and GSK3B (Figure 2B). Among these, AKT1 exhibited the highest degree centrality (degree = 214), followed by TNF (201), ALB (192), EGFR (175), CTNNB1 (171), SRC (167), STAT3 (166), BCL2 (158), HSP90AA1 (157), and ESR1 (153) (Table 2).

The degree, betweenness centrality and closeness centrality values were further evaluated to identify topologically important proteins within the network (Table 2). Among the top 20 hub genes, the mean degree, betweenness centrality and closeness centrality values were 154.45, 0.0289 and 0.5857, respectively. Six hub genes—AKT1, TNF, ALB, EGFR, CTNNB1 and SRC—exhibited values above the corresponding mean values for these centrality measures. AKT1 showed the highest degree centrality (214), together with high betweenness (0.0535) and closeness (0.6367), indicating a prominent topological position within the network. TNF similarly demonstrated high degree, betweenness (0.0514) and closeness (0.6242), whereas ALB exhibited the highest betweenness centrality (0.0680) among the hub genes, together with high degree (192) and closeness (0.6137) (Table 2).

The identification of AKT1, TNF, ALB, EGFR, CTNNB1 and SRC as highly central nodes suggests their potential importance within the *S. auriculata*–AD target network. The additional presence of STAT3, BCL2, HSP90AA1, ESR1, HIF1A, CASP3, PPARG, PTGS2 and MAPK3 among the hub genes further indicates the potential involvement of interconnected processes related to inflammatory signaling, cell survival, apoptosis, oxidative stress and intracellular signal transduction. Collectively, the PPI analysis supports a potential multi-target mechanism underlying the activity of *S. auriculata* against AD rather than an effect mediated by a single molecular target. The identified hub genes were subsequently subjected to Gene Ontology and KEGG pathway enrichment analyses and molecular docking to further investigate their potential mechanistic relevance to AD.

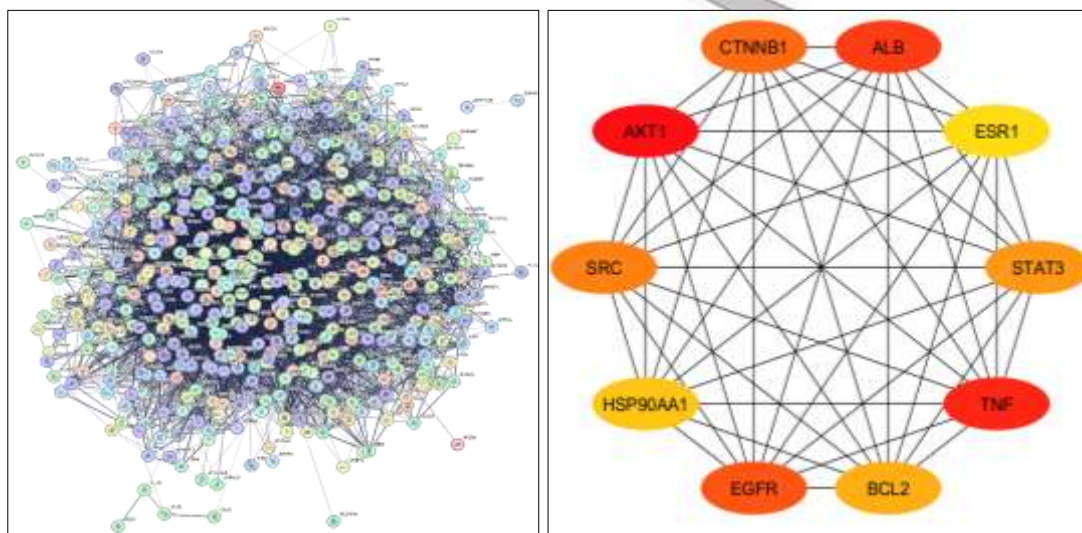


Figure 2. Protein-protein interaction network and identification of hub targets associated with *Senna auriculata* and Alzheimer's disease. (A) STRING-derived PPI network of the common targets. (B) Top 20 hub genes identified using the CytoHubba plugin based on degree centrality.

Table 2. Top 10 hub genes identified from the PPI network based on centrality measures

Rank	Hub gene	Degree (DC)	Betweenness centrality (BC)	Closeness centrality (CC)
1	AKT1	214	0.0535	0.6367
2	TNF	201	0.0514	0.6242
3	ALB	192	0.0680	0.6137
4	EGFR	175	0.0316	0.6020
5	CTNNB1	171	0.0321	0.5982
6	SRC	167	0.0509	0.5945
7	STAT3	166	0.0218	0.5952
8	BCL2	158	0.0224	0.5901
9	HSP90AA1	157	0.0282	0.5850
10	ESR1	153	0.0274	0.5843

3.4. Gene Ontology and Kyoto Encyclopedia of Genes and Genomes Pathway Enrichment Analyses

Functional enrichment analysis was performed on the 487 common targets shared between *Senna auriculata* phytoconstituents and Alzheimer's disease (AD)-associated targets. Gene Ontology (GO) enrichment analysis categorized the significantly enriched terms into biological process (BP), cellular component (CC), and molecular function (MF), with fold enrichment and $-\log_{10}$ P-value represented by bar length and color gradient, respectively (Figure 3A–C). The enriched BP terms were predominantly associated with responses to organic cyclic compounds, oxygen-containing compounds, lipids, hormones and nitrogen-containing compounds, as well as protein phosphorylation, small-molecule metabolism, intracellular signal transduction, regulation of cell communication and cellular stress responses. Among these, responses to organic cyclic compounds, oxygen-containing compounds, lipids and hormones showed relatively higher fold enrichment. The major CC terms included membrane rafts, membrane microdomains, receptor complexes, neuronal cell bodies, dendrites, glutamatergic synapses, postsynaptic regions, neuron projections, plasma membrane regions, cell junctions, extracellular exosomes and the endoplasmic reticulum. The enriched MF terms were mainly related to nuclear receptor activity, ligand-activated transcription factor activity, protein kinase activity, protein serine/threonine kinase activity, phosphotransferase activity, transition metal ion binding and nucleotide binding, with nuclear receptor and ligand-activated transcription factor activities showing relatively high fold enrichment (Figure 3A–C).

KEGG pathway enrichment analysis further revealed the involvement of multiple signaling and metabolic pathways, including the PI3K–Akt, MAPK, Ras, HIF-1, calcium and cAMP signaling pathways, as well as neuroactive ligand–receptor interaction, insulin resistance, lipid and atherosclerosis, metabolic pathways and pathways in cancer (Figure 3D). Among these, the PI3K–Akt, MAPK, Ras, calcium, cAMP and HIF-1 signaling pathways are particularly relevant to AD because of their established roles in neuronal survival, synaptic signaling, cellular stress responses, inflammation, metabolism and apoptosis. The enrichment of the neuroactive

ligand–receptor interaction pathway further suggests potential involvement of the identified targets in neuronal signaling and neurotransmitter-associated mechanisms. Collectively, the GO and KEGG enrichment profiles indicate that the common targets of *S. auriculata* phytoconstituents and AD are distributed across interconnected biological processes, cellular components and molecular functions, supporting a potential multi-component and multi-target mechanism of action. These findings provide a functional basis for prioritizing hub targets and phytoconstituents for subsequent molecular docking analysis.

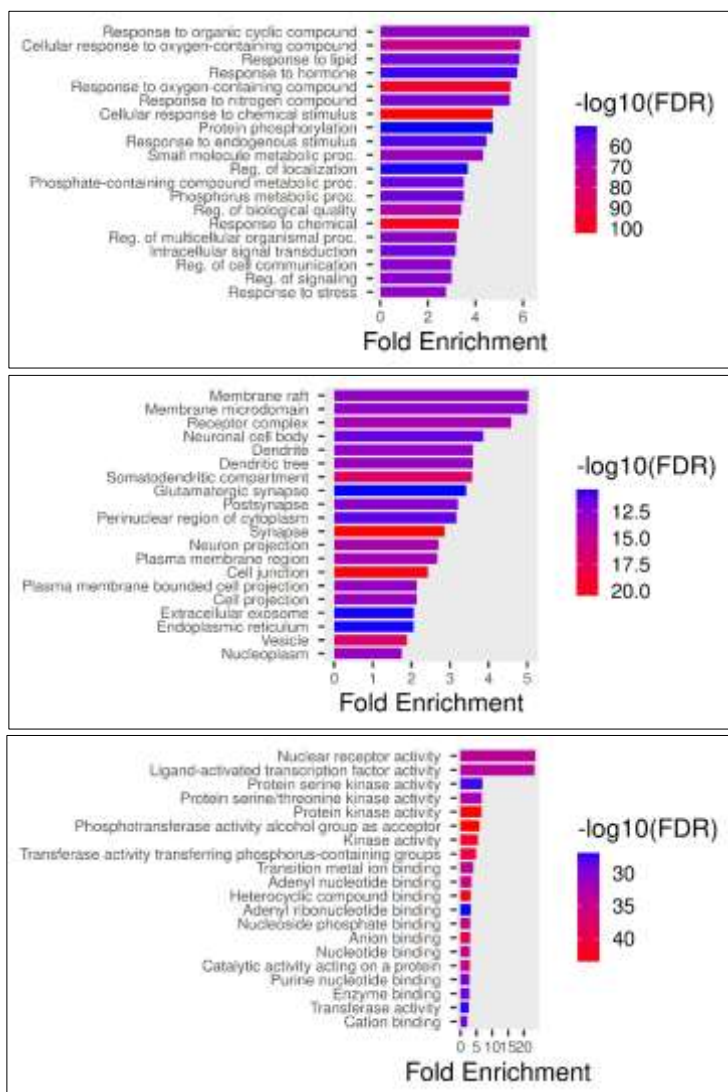


Figure 3. Gene Ontology and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of the 487 common targets associated with *Senna auriculata* phytoconstituents and Alzheimer's disease. (A) Biological process (BP) enrichment analysis. (B) Cellular component (CC) enrichment analysis. (C) Molecular function (MF) enrichment analysis. (D) KEGG pathway enrichment analysis. Bar length represents fold enrichment, while the color gradient indicates $-\log_{10}$ P-value.

3.5. Molecular Docking Analysis

Based on the PPI network and KEGG enrichment analyses, ten hub proteins—AKT1, TNF, EGFR, CTNNB1, SRC, BCL2, STAT3, HSP90AA1, ESR1 and ALB—were selected for molecular docking with the identified *Senna auriculata* phytoconstituents. Docking analysis was performed to evaluate the predicted binding affinities and interaction patterns of the phytoconstituents within the binding sites of the prioritized hub proteins. The binding energies and representative protein–ligand interaction poses are presented in Figures 4–13.

AKT1 exhibited the strongest overall binding interactions among the evaluated targets, with rutin showing the highest predicted affinity (-11.168 kcal/mol), followed by quercetin-3-glucoside (-9.741 kcal/mol), cyanidanol (-9.162 kcal/mol) and kaempferol (-9.070 kcal/mol). In contrast, nonacosane showed the weakest interaction

with AKT1 (–6.397 kcal/mol). Rutin formed hydrogen bonds with ASN-54, ARG-273, ASP-274 and ILE-290, while ASN-54, GLN-79 and SER-205 were recurrent interaction residues among the flavonoid complexes (Figure 4).

For TNF, β -sitosterol showed the highest predicted binding affinity (–8.893 kcal/mol), followed by quercetin-3-glucoside (–8.009 kcal/mol), linolenic acid (–7.980 kcal/mol) and linoleic acid (–7.550 kcal/mol), whereas rutin showed the lowest affinity (–5.586 kcal/mol). The strongest interactions were predominantly characterized by hydrophobic contacts, with β -sitosterol and linolenic acid showing no hydrogen-bond interactions (Figure 5). EGFR displayed favorable interactions with kaempferol (–8.834 kcal/mol), cianidanol (–8.680 kcal/mol), quercetin-3-glucoside (–8.649 kcal/mol) and chrysophanol (–8.606 kcal/mol). ASP-855 and THR-854 were recurrent interaction residues, while kaempferol formed hydrogen bonds with LYS-745, ILE-759, THR-854 and ASP-855 (Figure 6).

CTNNB1 showed comparatively weaker and narrowly distributed docking scores, ranging from –5.451 to –5.887 kcal/mol. Rutin exhibited the highest affinity (–5.887 kcal/mol), followed by kaempferol (–5.814 kcal/mol), with GLN-203 identified as a recurrent interaction residue (Figure 7). In contrast, SRC demonstrated strong predicted interactions with rutin (–10.470 kcal/mol), followed by quercetin-3-glucoside (–9.040 kcal/mol), kaempferol (–9.036 kcal/mol) and cianidanol (–8.770 kcal/mol). Nonacosane showed the lowest affinity (–6.113 kcal/mol). THR-338, MET-341 and ASP-404 were recurrent residues, while cianidanol formed a hydrogen bond with ASP-404 at 2.87 Å (Figure 8).

Among the remaining targets, BCL2 showed its strongest interaction with anthraquinone (–6.947 kcal/mol), followed by rubiadin (–6.761 kcal/mol), chrysophanol (–6.605 kcal/mol) and cianidanol (–6.472 kcal/mol), whereas nonacosane showed the weakest affinity (–4.970 kcal/mol) (Figure 9). STAT3 exhibited its highest affinity with chrysophanol (–5.816 kcal/mol), followed by β -sitosterol- β -D-glucoside (–5.589 kcal/mol), while nonacosane showed the lowest affinity (–3.155 kcal/mol). Chrysophanol interacted within the SH2-domain region involving SER-611, GLU-612 and SER-613 (Figure 10). HSP90AA1 showed the strongest interaction with β -sitosterol- β -D-glucoside (–8.759 kcal/mol), followed by rubiadin (–7.722 kcal/mol) and emodin (–7.582 kcal/mol), whereas nonacosan-6-one exhibited the weakest affinity (–5.058 kcal/mol). Recurrent residues included GLY-137, PHE-138 and SER-52 (Figure 11).

ESR1 exhibited the highest predicted affinity for chrysophanol (–6.152 kcal/mol), followed by β -sitosterol (–5.940 kcal/mol), rubiadin (–5.923 kcal/mol) and emodin (–5.897 kcal/mol), whereas nonacosane showed the lowest affinity (–3.770 kcal/mol). The anthraquinone derivatives showed recurrent interactions involving LYS-122, whereas fatty acids and aliphatic alcohols predominantly occupied hydrophobic regions of the binding site (Figure 12). ALB demonstrated a strong interaction with rubiadin (–9.414 kcal/mol), while emodin showed the lowest affinity among the tested ALB complexes (–8.683 kcal/mol). The interactions of these anthraquinone derivatives with ALB were predominantly hydrophobic and lacked hydrogen-bond interactions (Figure 13).

Overall, the docking analysis revealed considerable variation in the predicted binding affinities across the hub proteins and phytoconstituents. Rutin showed particularly strong interactions with AKT1 and SRC, kaempferol demonstrated favorable binding to EGFR, β -sitosterol showed the strongest interaction with TNF, and rubiadin exhibited a strong interaction with ALB. Anthraquinone derivatives, including chrysophanol, rubiadin and emodin, also demonstrated favorable interactions with several hub proteins. These findings suggest that selected *S. auriculata* phytoconstituents may interact with multiple AD-associated hub proteins through diverse hydrogen-bonding and hydrophobic interactions. However, the docking scores represent computational predictions of binding affinity and should not be interpreted as direct evidence of biological efficacy. The prioritized protein–ligand complexes were therefore considered for further validation through molecular dynamics simulation and other experimental approaches.

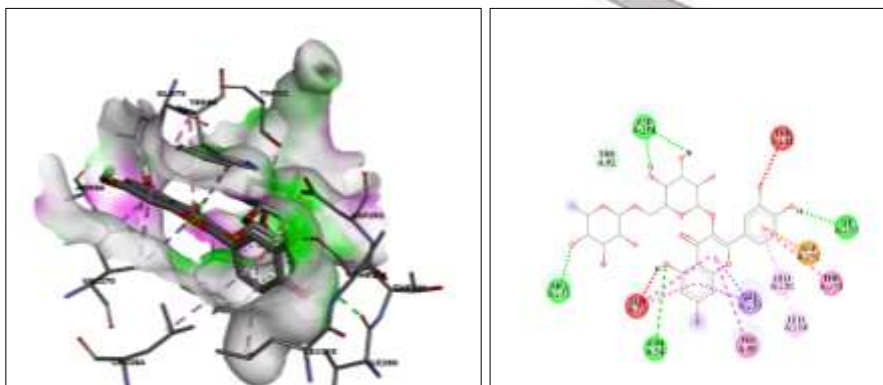


Figure 4. Molecular docking interaction of rutin with AKT1 (PDB: 4EJN) showing the hydrogen-bonding residues ASN-54, ARG-273, ASP-274, and ILE-290 and the lowest binding affinity of -11.168 kcal/mol.

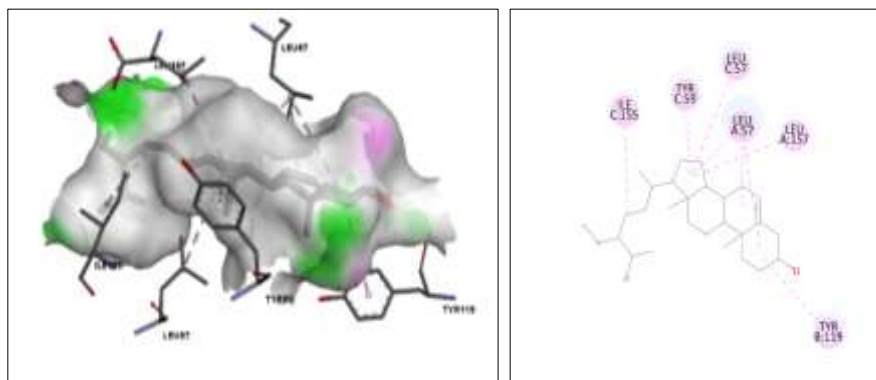


Figure 5. Molecular docking interaction of beta-sitosterol with TNF (PDB: 6OP0) showing the binding affinity of -8.893 kcal/mol in the absence of hydrogen bonds.

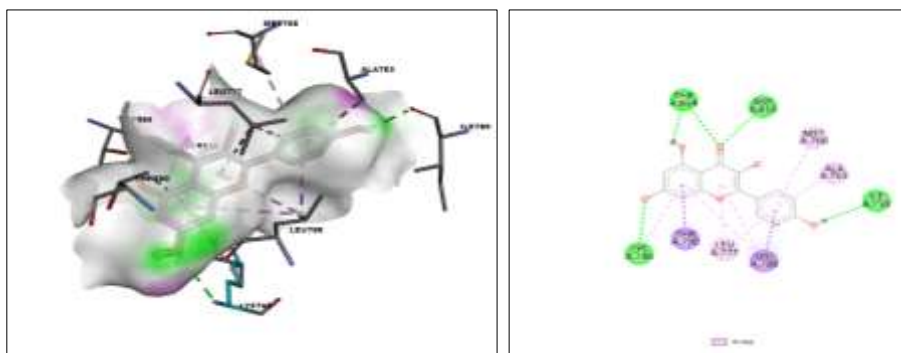


Figure 6. Molecular docking interaction of kaempferol with EGFR (PDB: 8A27) showing the hydrogen-bonding residues LYS-745, ILE-759, THR-854, and ASP-855 and the binding affinity of -8.834 kcal/mol.

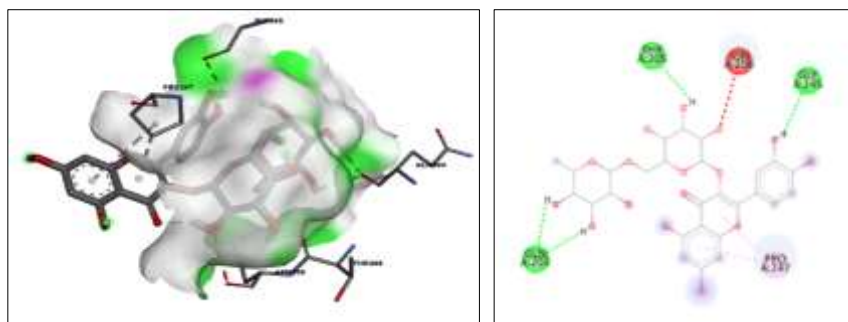


Figure 7. Molecular docking interaction of rutin with CTNNB1 (PDB: 7AFW) showing the hydrogen-bonding residues GLN-203, THR-205, and GLY-245 and the binding affinity of -5.887 kcal/mol.

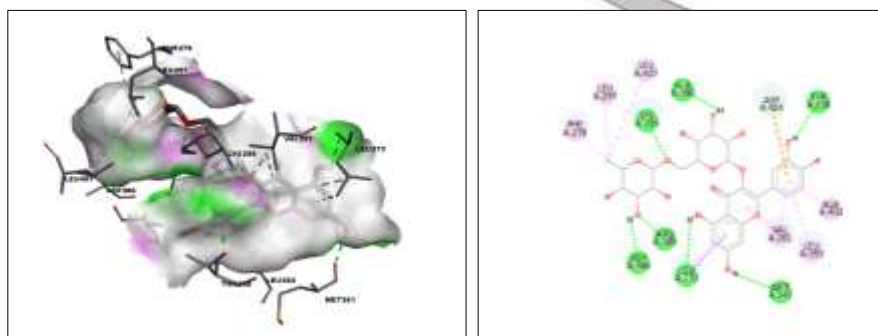


Figure 8. Molecular docking interaction of rutin with SRC (PDB: 2SRC) showing the hydrogen-bonding residues LEU-273, LYS-295, THR-338, MET-341, ASP-386, ARG-388, and ALA-390 and the binding affinity of -10.47 kcal/mol

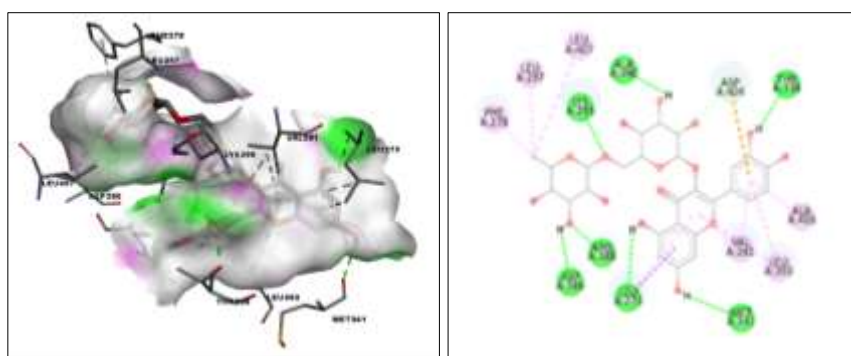


Figure 9. Molecular docking interaction of anthraquinone with BCL2 (PDB: 6GL8) showing the hydrogen-bonding residues LEU-273, LYS-295, THR-338, MET-341, ASP-386, ARG-388, and ALA-390 and the binding affinity of -10.47 kcal/mol

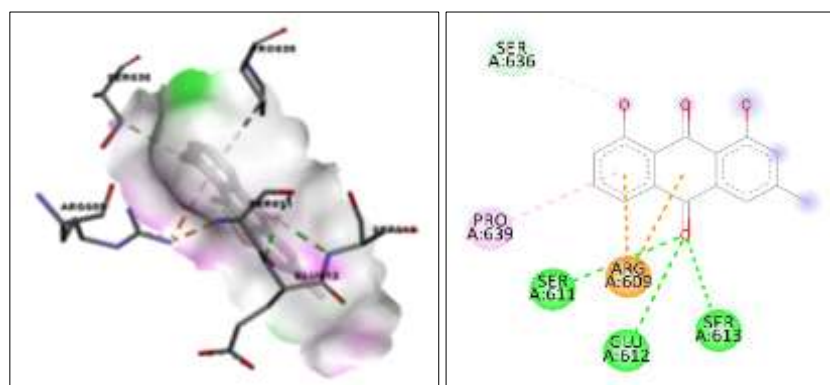


Figure 10. Molecular docking interaction of chrysophanol with STAT3 (PDB: 6NJS) showing the hydrogen-bonding residues SER-611, GLU-612, and SER-613 and the binding affinity of -5.816 kcal/mol.

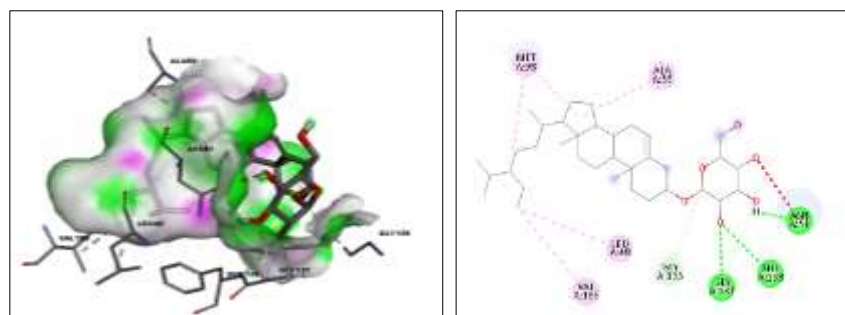


Figure 11. Molecular docking interaction of beta-sitosterol-beta-D-glucoside with HSP90AA1 (PDB: 1BYQ) showing the hydrogen-bonding residues ASN-51, GLY-137, and PHE-138 and the binding affinity of -8.759 kcal/mol.

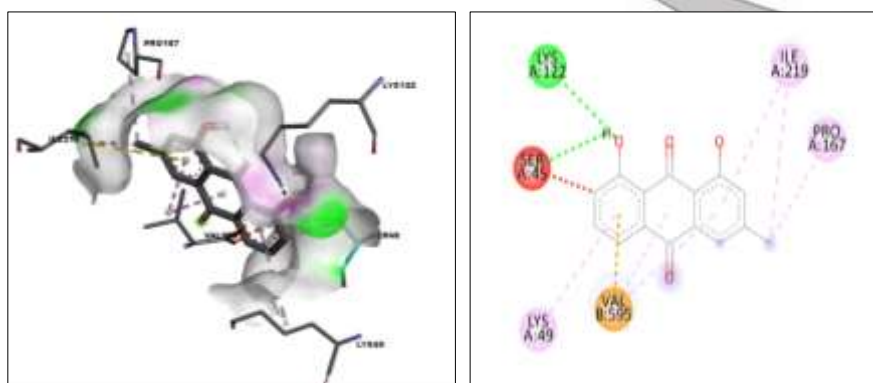


Figure 12. Molecular docking interaction of chrysophanol with ESR1 (PDB: 7BAA) showing the hydrogen-bonding residue LYS-122 and the binding affinity of -6.152 kcal/mol.

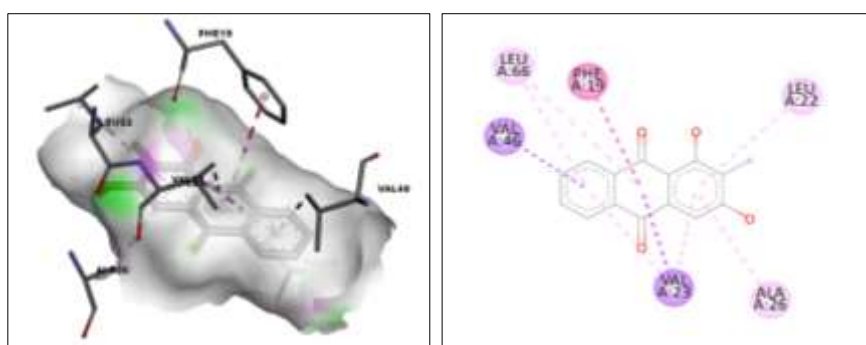


Figure 13. Molecular docking interaction of rubiadin with ALB (PDB: 6YG9) showing the binding affinity of -9.414 kcal/mol in the absence of hydrogen bonds.

3.6. Molecular Dynamics Simulations

3.6.1. Normal Mode Analysis of the AKT1–Rutin Complex

Normal mode analysis (NMA) was performed to characterize the intrinsic flexibility, collective motions and conformational dynamics of the docked AKT1–Rutin complex. The analysis included root mean square fluctuation (RMSF), deformability, eigenvalue, variance distribution, covariance matrix and B-factor profiles, providing complementary information on residue-level mobility and collective motions within the complex (Figure 14).

The RMSF profile showed predominantly low-to-moderate residue fluctuations, with localized increases around residues 40–50, 105–145, 200–215, 300–315, 345–360 and 385–440. Higher fluctuations were observed near residues approximately 45, 140, 305, 390 and 430, particularly toward the C-terminal region, suggesting greater flexibility in specific loop or solvent-exposed regions. Nevertheless, most residues displayed relatively limited fluctuations, indicating preservation of the overall structural framework of AKT1 (Figure 14A).

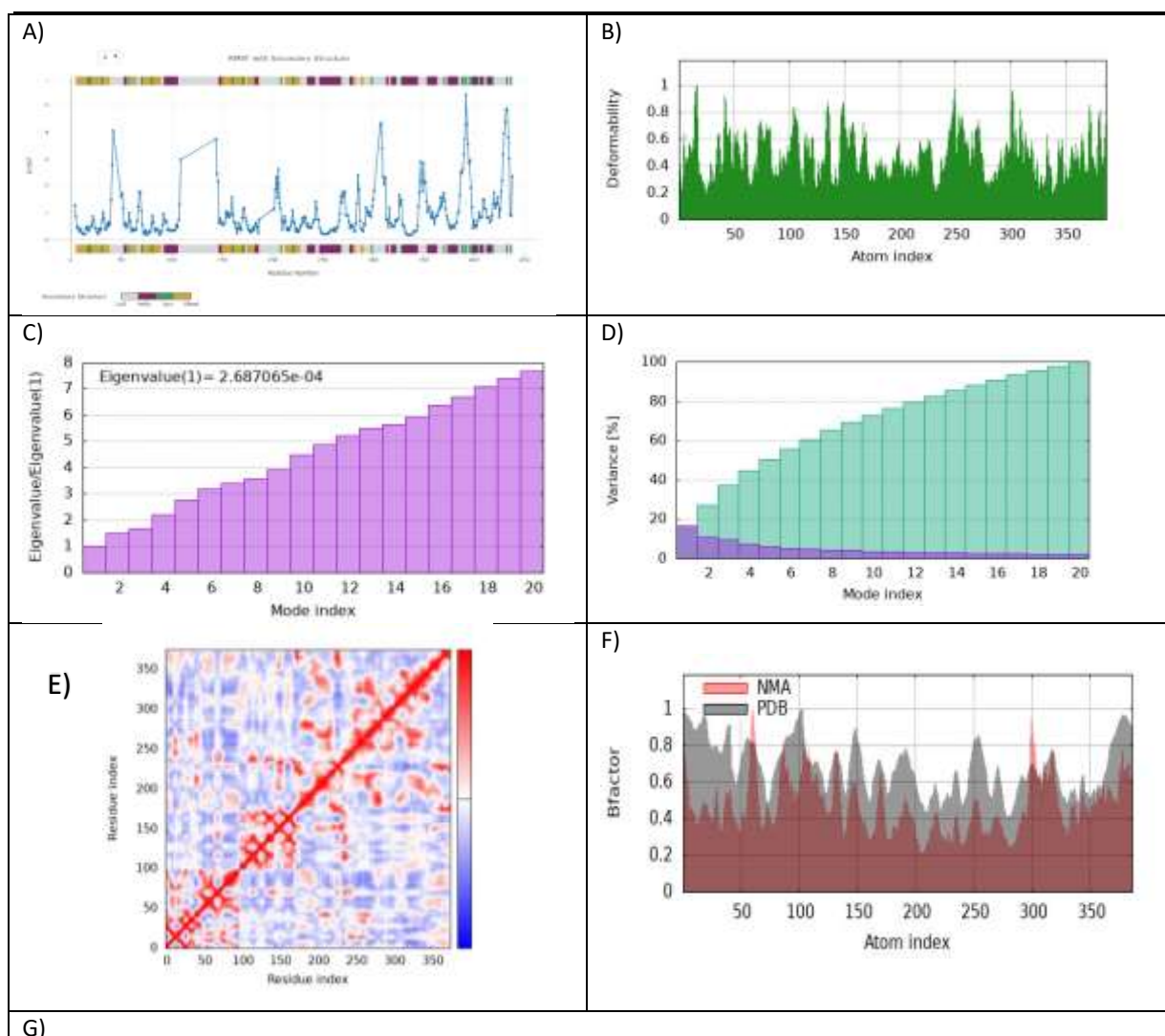
The deformability profile revealed several localized peaks around residues 40–50, 100–150, 245–260, 300–315 and 385–405, indicating regions with a greater intrinsic propensity for conformational adjustment. Rather than showing extensive deformation throughout the protein, the profile indicated that conformational flexibility was concentrated within specific structural regions (Figure 14B).

The first-mode eigenvalue was calculated as 2.687065×10^{-4} , indicating that the corresponding collective motion is energetically accessible. The eigenvalue spectrum showed increasing eigenvalues across successive modes, consistent with the first modes representing relatively low-energy collective motions of the complex (Figure 14C). The first normal mode contributed approximately 16% of the total variance, while the contributions of subsequent modes progressively decreased. The cumulative variance approached approximately 100% by the twentieth mode, indicating that the principal collective motions were captured within the analyzed modes (Figure 14D).

The covariance matrix revealed regions of positively correlated and anti-correlated residue motions. Positively correlated regions indicate residues moving in similar directions, whereas anti-correlated regions represent oppositely directed motions. The distinct off-diagonal patterns suggest coordinated motions between different structural regions of AKT1, while the strong diagonal signal reflects residue self-correlation (Figure 14E).

Comparison of the NMA-derived mobility profile with the experimentally determined PDB B-factor profile showed broadly comparable patterns of residue flexibility, although differences were observed in specific regions. Regions displaying higher predicted mobility corresponded generally to more flexible structural segments, whereas lower mobility was associated with relatively constrained regions (Figure 14F). The three-dimensional representation further illustrated the distribution of residue mobility across the AKT1 structure and indicated that the predicted flexibility was localized rather than associated with generalized structural disruption (Figure 14G).

Collectively, the NMA results indicate that the AKT1–Rutin complex exhibits localized residue flexibility together with coordinated collective motions while retaining the overall structural organization of AKT1. The relatively low first-mode eigenvalue, substantial contribution of the first normal mode to total variance, localized deformability peaks and correlated residue motions suggest that the complex can undergo energetically accessible conformational adjustments following ligand binding. Combined with the favorable docking interaction observed for rutin with AKT1, these findings support the prioritization of the AKT1–Rutin complex for further investigation. However, NMA alone cannot establish the stability of a protein–ligand complex under physiological conditions; therefore, conventional trajectory-based molecular dynamics simulations incorporating RMSD, RMSF, radius of gyration, hydrogen-bond persistence and binding free-energy analyses would provide stronger evidence of complex stability and binding behavior.



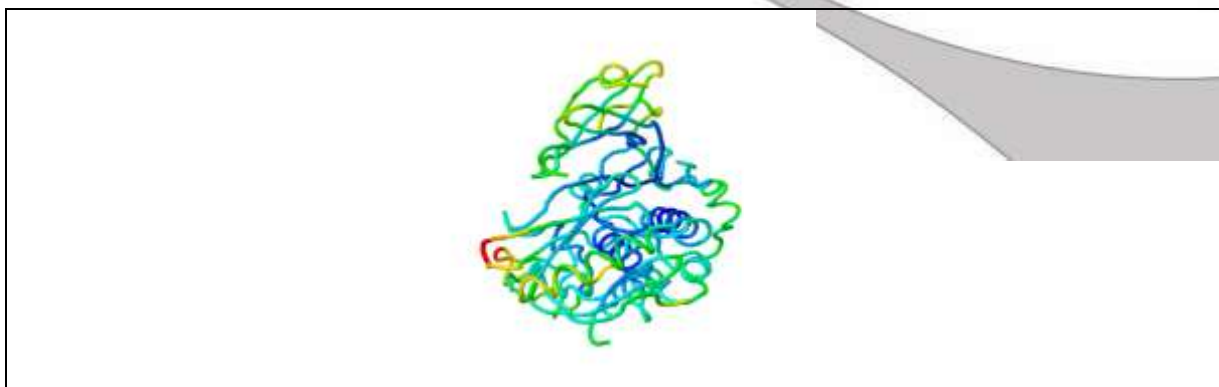


Figure 14. Normal mode analysis of the AKT1–Rutin complex. (A) Residue-wise RMSF profile showing the predicted flexibility of AKT1 residues; (B) deformability profile indicating regions with greater propensity for conformational deformation; (C) eigenvalue distribution of the first 20 normal modes; (D) variance contribution of individual normal modes; (E) covariance matrix showing correlated and anti-correlated residue motions; (F) comparison of NMA-predicted and experimentally determined PDB B-factor profiles; and (G) three-dimensional representation of the AKT1–Rutin complex showing the distribution of residue mobility.

3.6.2. Normal Mode Analysis of the SRC–Rutin Complex

Normal mode analysis (NMA) was performed to characterize the conformational flexibility, collective motions, deformability and structural dynamics of the docked SRC–Rutin complex. The analysis included root mean square fluctuation (RMSF), deformability, eigenvalue, variance distribution, covariance matrix, B-factor comparison and three-dimensional structural representation (Figure 15).

The RMSF profile showed predominantly low-to-moderate residue fluctuations across the SRC–Rutin complex, with localized increases around residues 140–150, 190–205, 330–345, 410–425 and the C-terminal region. The most prominent fluctuation occurred toward the C-terminal region, reaching approximately 5.6 RMSF units, with additional peaks around residues 140 and 195. These regions may correspond to flexible loops or solvent-exposed structural segments capable of localized conformational adjustment. In contrast, relatively low fluctuations across substantial portions of SRC indicate preservation of its overall structural framework following rutin binding (Figure 15A).

The deformability profile revealed several localized peaks in the N-terminal region and around approximately 70–120, 160–200, 300–350 and 400–450 atom indices, indicating regions with greater intrinsic flexibility. The absence of extensive peaks throughout the protein suggests that conformational flexibility is predominantly localized to specific structural regions (Figure 15B).

The first-mode eigenvalue was 8.610557×10^{-5} , indicating that the dominant collective motion represented by this mode is energetically accessible. The progressive increase in eigenvalues across subsequent modes suggests that the initial modes correspond to relatively low-energy collective motions of the SRC–Rutin complex (Figure 15C). The first normal mode contributed approximately 20% of the total variance, while the contributions of successive modes gradually decreased. The cumulative variance approached approximately 100% by the twentieth mode, indicating that the major collective motions were captured within the first 20 modes (Figure 15D).

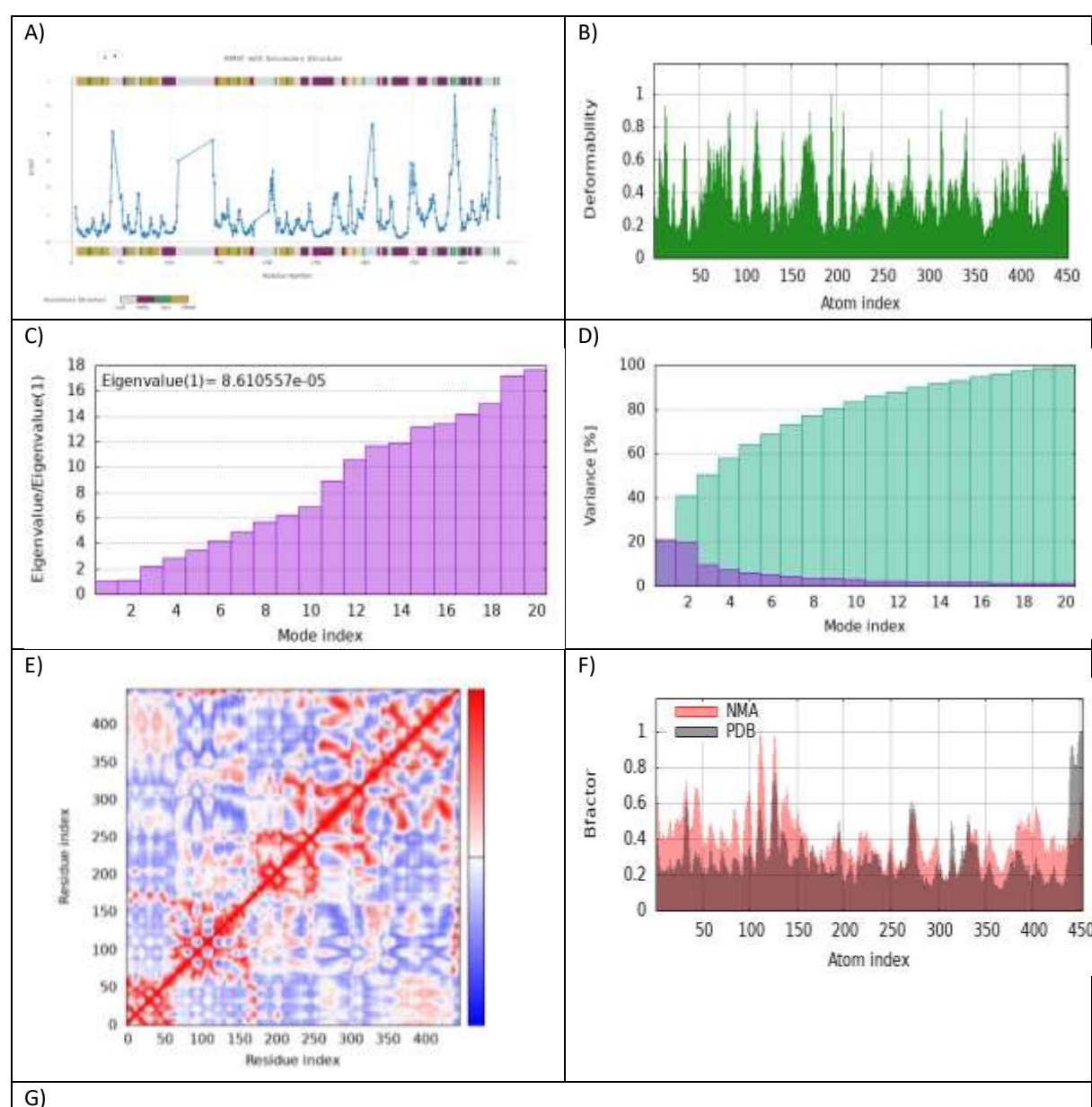
The covariance matrix demonstrated distinct positively and negatively correlated regions, representing coordinated and anti-correlated residue movements, respectively. Several well-defined blocks of correlated motion were observed within the central and lower regions of the SRC structure, together with coordinated motions involving more distal regions. These patterns indicate that residue movements within SRC are coordinated rather than independent and may contribute to conformational adaptation following ligand interaction (Figure 15E).

Comparison of the NMA-predicted mobility with the experimentally determined PDB B-factor profile showed broadly similar mobility patterns across several regions, although localized differences were observed. Increased predicted mobility was particularly evident around the 100–150 region and toward the C-terminal region, whereas several central regions exhibited comparatively lower mobility. The overall correspondence between

the predicted and experimental mobility profiles provides supportive evidence for the consistency of the predicted conformational dynamics (Figure 15F).

The three-dimensional representation of the SRC–Rutin complex showed an organized protein architecture comprising relatively constrained structural regions together with flexible loop and terminal segments. The distribution of residue mobility was predominantly localized to exposed regions, without evidence of generalized structural disruption (Figure 15G).

Collectively, the NMA results indicate that the SRC–Rutin complex exhibits localized residue flexibility and coordinated collective motions while retaining the overall structural organization of SRC. The low first-mode eigenvalue (8.610557×10^{-5}), substantial contribution of the first normal mode to total variance, localized deformability peaks and correlated residue motions indicate that the complex can undergo energetically accessible conformational adjustments. Together with the favorable docking affinity of -10.470 kcal/mol for the SRC–Rutin complex, these findings support its prioritization for further investigation in the context of the potential anti-Alzheimer's activity of *Senna auriculata*. However, NMA alone does not establish dynamic stability under physiological conditions; therefore, conventional trajectory-based molecular dynamics simulations and complementary analyses, including RMSD, RMSF, radius of gyration, hydrogen-bond persistence and binding free-energy calculations, would provide further evidence regarding complex stability and binding behavior.



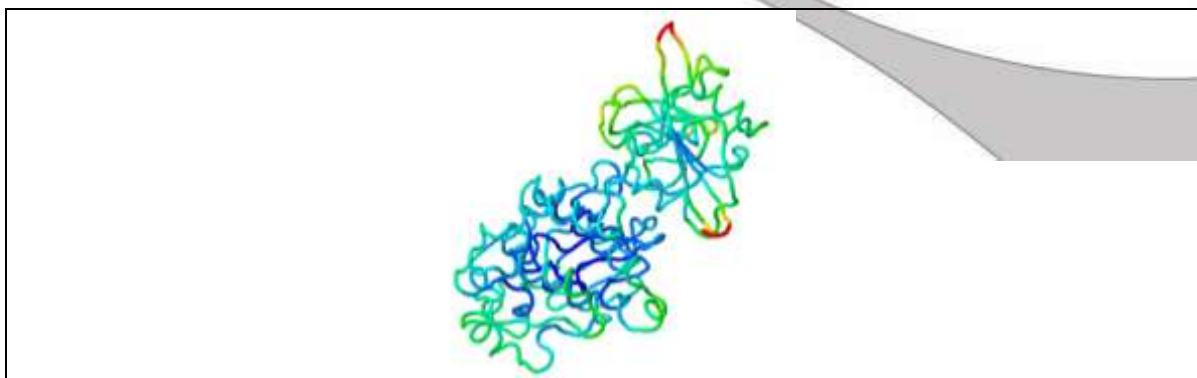


Figure 15. Normal mode analysis of the SRC–Rutin complex. (A) Residue-wise RMSF profile showing the predicted flexibility of SRC residues; (B) deformability profile indicating regions with greater propensity for conformational deformation; (C) eigenvalue distribution of the first 20 normal modes; (D) variance contribution of individual normal modes; (E) covariance matrix showing correlated and anti-correlated residue motions; (F) comparison of NMA-predicted and experimentally determined PDB B-factor profiles; and (G) three-dimensional representation of the SRC–Rutin complex showing the distribution of residue mobility.

3.7 In Vitro Studies

3.7.1 Cytotoxicity of *Senna auriculata* in SH-SY5Y Cells

The cytotoxicity of the whole-plant extract of *Senna auriculata* was evaluated in SH-SY5Y cells using the MTT assay following 24 h exposure to concentrations ranging from 6.25 to 100 µg/mL. Untreated cells served as the control, and cell viability was determined relative to the control based on the reduction of MTT to formazan by metabolically active cells. The mean absorbance, percentage cell viability and reduction in viability are presented in Table 3.

A gradual concentration-dependent decrease in cell viability was observed, with viability decreasing from 98.81% at 6.25 µg/mL to 92.27% at 100 µg/mL. The corresponding viabilities at 12.5, 25 and 50 µg/mL were 98.40%, 96.60% and 94.52%, respectively. Even at the highest concentration tested, the reduction in viability was only 7.73%, with approximately 92% of cells remaining viable. These findings indicate that the extract produced only a mild reduction in SH-SY5Y cell viability over the tested concentration range. As cell viability did not approach 50% inhibition, an IC₅₀ value could not be reliably determined and was therefore reported as not applicable (NA).

Microscopic examination further supported the MTT findings. Control and extract-treated cells retained predominantly adherent, elongated and neuronal-like morphology, without an apparent concentration-dependent reduction in the overall cell population. Representative microscopic images of SH-SY5Y cells treated with different concentrations of *S. auriculata* extract are presented in Figure 16.

Collectively, the MTT assay and morphological observations indicate that the *S. auriculata* whole-plant extract exhibited low cytotoxicity toward SH-SY5Y cells at concentrations of 6.25–100 µg/mL following 24 h exposure. The absence of substantial loss of cell viability supports the selection of these concentrations for subsequent cellular investigations. However, the MTT assay primarily assesses cell viability and does not by itself establish neuroprotective or anti-Alzheimer's activity. Further evaluation using an appropriate AD-related cellular injury model, together with biochemical and molecular markers of neuronal injury, oxidative stress or amyloid-associated pathology, would be required to establish neuroprotective efficacy.

Table 3. Effect of different concentrations of *Senna auriculata* whole-plant extract on the viability of SH-SY5Y cells following 24 h treatment, as determined by the MTT assay.

S. auriculata concentration (µg/mL)	Mean absorbance	Cell viability (%)	Reduction in viability (%)
Untreated control	1.2220	100.00	0.00
6.25	1.2075	98.81	1.19
12.5	1.2025	98.40	1.60
25	1.1805	96.60	3.40
50	1.1550	94.52	5.48

100	1.1275	92.27	7.73
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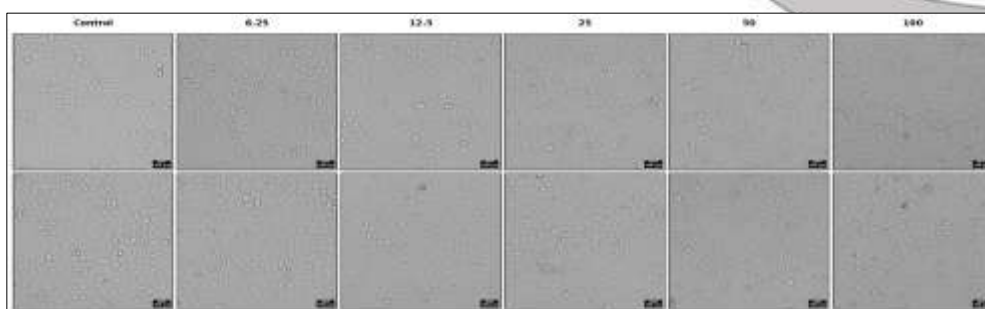


Figure 16. Effect of *S. auriculata* on cell viability at different concentrations.

Cell viability (%) of cells treated with increasing concentrations of *S. auriculata* (6.25, 12.5, 25, 50, and 100 µg/mL) compared with the untreated control. Data are presented as mean values with illustrative error bars

3.8 Antioxidant Studies

3.8.1. DPPH Radical Scavenging Assay of *Senna auriculata* Ethanolic Extract

1,1-Diphenyl-2-picrylhydrazyl (DPPH) is a stable free radical widely used to evaluate the radical-scavenging capacity of antioxidant compounds. Upon accepting an electron or hydrogen atom from an antioxidant, DPPH is reduced to its non-radical form, resulting in a measurable decrease in absorbance. The ethanolic extract of *Senna auriculata* (SA-EE) demonstrated a concentration-dependent increase in DPPH radical-scavenging activity, with inhibition increasing from 15.03% at 10 µg/mL to 66.05% at 50 µg/mL (Figure 17). The extract exhibited an IC₅₀ value of 34.72 µg/mL (Table 4; Figure 17), compared with 6.24 µg/mL for the reference standard ascorbic acid. Thus, SA-EE demonstrated appreciable DPPH radical-scavenging activity, although its potency was lower than that of ascorbic acid under the experimental conditions.

Table 4. DPPH radical scavenging activity of *Senna auriculata* ethanolic extract (SA-EE) at different concentrations.

Concentration of SA-EE (µg/mL)	OD at 540 nm	DPPH radical scavenging (%)
10	0.503	15
20	0.387	35
30	0.299	49
40	0.261	56
50	0.201	66
IC ₅₀	34.72 µg/mL	

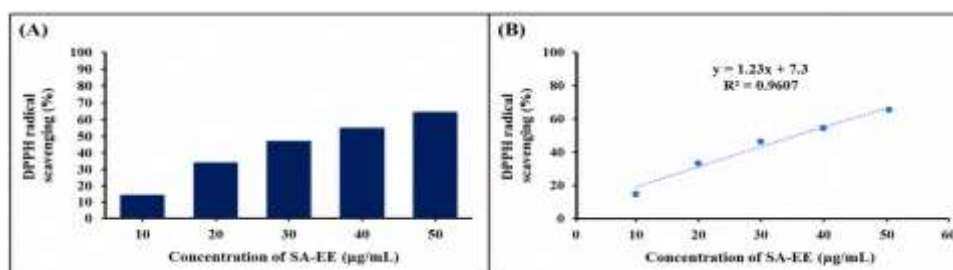


Figure 17. DPPH radical-scavenging activity of *Senna auriculata* ethanolic extract (SA-EE). (A) Concentration-dependent DPPH radical-scavenging activity of SA-EE at 10–50 µg/mL. (B) Linear regression analysis showing the relationship between SA-EE concentration and percentage DPPH radical scavenging ($y = 1.23x + 7.3$; $R^2 = 0.9607$).

3.8.2. ABTS Radical Scavenging Assay

The ethanolic extract of *Senna auriculata* (SA-EE) exhibited concentration-dependent scavenging of the ABTS radical cation (ABTS•+) over the concentration range of 5–25 µg/mL (Figure 18). The extract showed an IC₅₀ value of 19.59 µg/mL, compared with 3.48 µg/mL for the reference standard ascorbic acid (Table 5). The

observed scavenging activity is attributable to the ability of antioxidant constituents to donate electrons or hydrogen atoms, thereby reducing ABTS•+ and decreasing its characteristic blue-green coloration.

Comparison of the two antioxidant assays revealed a lower IC₅₀ for SA-EE in the ABTS assay (19.59 µg/mL) than in the DPPH assay (34.72 µg/mL), indicating greater apparent radical-scavenging activity toward the ABTS•+ system under the present experimental conditions. This difference may reflect the broader reactivity of the ABTS radical system toward diverse classes of antioxidant constituents present in the extract. The concentration–response relationship and regression analysis used to determine the IC₅₀ value are presented in Figure 18. Overall, the ABTS assay confirms the appreciable antioxidant potential of SA-EE, although its activity remained lower than that of ascorbic acid.

Table 5. ABTS radical scavenging activity and IC₅₀ value of *Senna auriculata* ethanolic extract (SA-EE) at different concentrations.

Concentration of SA-EE (µg/mL)	OD at 734 nm	ABTS Radical Scavenging (%)
Control	0.609	0.00
5	0.569	6.57
10	0.487	20.03
15	0.379	37.77
20	0.312	48.77
25	0.197	67.65

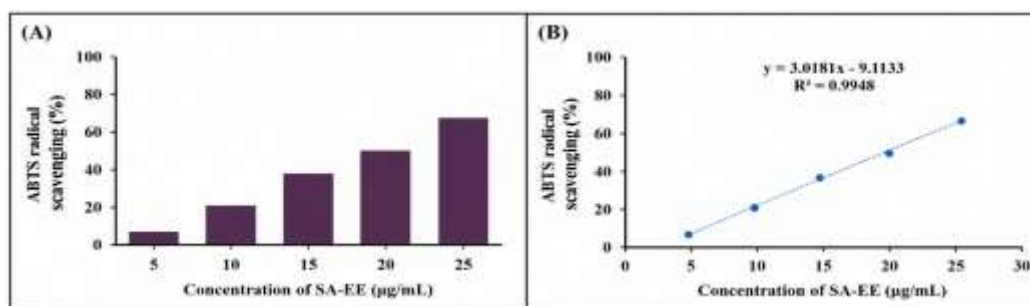


Figure 18. ABTS radical-scavenging activity of *Senna auriculata* ethanolic extract (SA-EE). (A) Concentration-dependent ABTS radical-scavenging activity of SA-EE at 5–25 µg/mL. (B) Linear regression analysis showing the relationship between SA-EE concentration and ABTS radical-scavenging activity ($y = 3.0181x - 9.1133$; $R^2 = 0.9948$).

3.9. Acetylcholinesterase Inhibitory Activity

Acetylcholinesterase (AChE) catalyzes the hydrolysis of acetylcholine, and its inhibition represents an established therapeutic strategy for increasing acetylcholine availability in the synaptic cleft and managing Alzheimer's disease. In the present study, the AChE inhibitory activity of the ethanolic extract of *Senna auriculata* (SA-EE) was evaluated using Ellman's colorimetric method. SA-EE exhibited a concentration-dependent inhibition of AChE, with inhibition increasing from 33.17% at 20 µg/mL to 91.00% at 100 µg/mL, with intermediate inhibition values of 60.64%, 71.89% and 77.27% at 40, 60 and 80 µg/mL, respectively (Figure 28). Correspondingly, absorbance decreased from 0.832 at 20 µg/mL to 0.112 at 100 µg/mL, compared with a control absorbance of 1.245 (Table 6).

Regression analysis demonstrated a strong positive concentration–response relationship, with the equation $y = 0.6614x + 27.108$ and an R^2 value of 0.9259 (Figure 19). The progressive increase in AChE inhibition with increasing extract concentration indicates appreciable inhibitory activity of SA-EE under the experimental conditions. These findings provide preliminary experimental evidence supporting further investigation of *S. auriculata* extract and its constituent phytochemicals as potential sources of AChE-inhibitory agents relevant to Alzheimer's disease.

Table 6. Acetylcholinesterase inhibitory activity of *Senna auriculata* ethanolic extract (SA-EE) at different concentrations.

Concentration of SA-EE (µg/mL)	OD at 412 nm	AChE inhibition (%)
Control	1.245	0.00
20	0.832	33.17

40	0.490	60.64
60	0.350	71.89
80	0.283	77.27
100	0.112	91.00

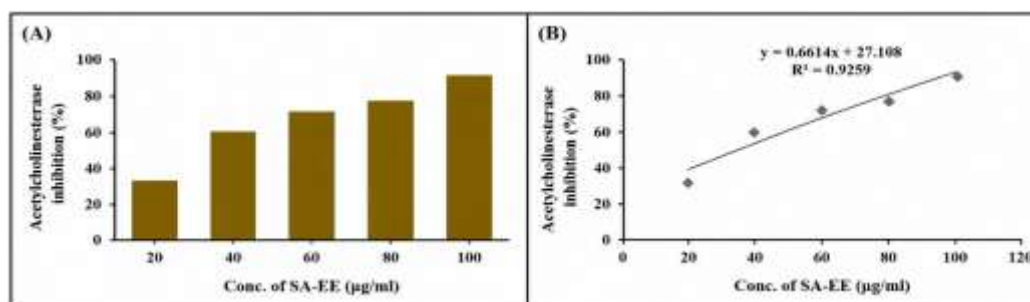


Figure 19. Acetylcholinesterase inhibitory activity of *Senna auriculata* ethanolic extract (SA-EE). (A) Concentration-dependent inhibition of acetylcholinesterase activity by SA-EE at 20–100 µg/mL. (B) Linear regression analysis showing the relationship between SA-EE concentration and percentage acetylcholinesterase inhibition ($y = 0.6614x + 27.108$; $R^2 = 0.9259$).

3.10. Butyrylcholinesterase Inhibitory Activity

Butyrylcholinesterase (BChE) is a cholinesterase enzyme involved in the hydrolysis of choline-containing esters and is considered a relevant therapeutic target in Alzheimer's disease because its inhibition may contribute to the preservation of cholinergic neurotransmission. In the present study, the BChE inhibitory activity of *Senna auriculata* ethanolic extract (SA-EE) was evaluated at concentrations of 120–600 µg/mL using an in vitro enzyme inhibition assay. SA-EE exhibited a clear concentration-dependent increase in BChE inhibition, with inhibition values of 8.19%, 28.09%, 34.78%, 53.51%, and 81.44% at 120, 240, 360, 480, and 600 µg/mL, respectively (Figure 20A). The corresponding absorbance values decreased progressively from 0.549 to 0.111, compared with a control absorbance of 0.598 (Table 7). More than 50% inhibition was achieved at 480 µg/mL, while the maximum inhibition of 81.44% was observed at 600 µg/mL.

Regression analysis demonstrated a strong positive relationship between SA-EE concentration and BChE inhibition, with the regression equation $y = 0.1433x - 10.368$ and $R^2 = 0.9616$ (Figure 20B). The high coefficient of determination indicates a strong concentration-dependent response within the tested concentration range. Overall, SA-EE demonstrated appreciable in vitro BChE inhibitory activity. Together with the AChE inhibitory activity observed in the present study, these findings support the cholinesterase inhibitory potential of SA-EE and warrant further investigation of its bioactive phytoconstituents and the molecular mechanisms underlying enzyme inhibition.

Table 7. Butyrylcholinesterase inhibitory activity of *Senna auriculata* ethanolic extract (SA-EE)

Concentration of SA-EE (µg/mL)	OD at 412 nm	Butyrylcholinesterase inhibition (%)
Control	0.598	0.00
120	0.549	8.19
240	0.430	28.09
360	0.390	34.78
480	0.278	53.51
600	0.111	81.44

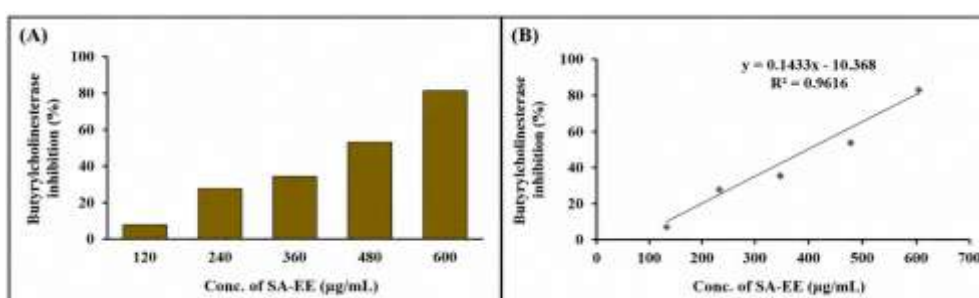


Figure 20. Butyrylcholinesterase inhibitory activity of *Senna auriculata* ethanolic extract (SA-EE). (A) Concentration-dependent inhibition of butyrylcholinesterase activity by SA-EE at 120–600 µg/mL. (B) Linear regression analysis showing the relationship between SA-EE concentration and percentage butyrylcholinesterase inhibition ($y = 0.1433x - 10.368$; $R^2 = 0.9616$).

4. Discussion

This study integrated network pharmacology, molecular docking, normal mode analysis, and in-vitro experimentation to evaluate the potential anti-Alzheimer's activity of the whole-plant ethanolic extract of *Senna auriculata*. The computational workflow identified the phytoconstituent repertoire of the plant through the IMPPAT database (Mohanraj et al., 2018; Vivek-Ananth et al., 2023), screened the compounds for drug-likeness and pharmacokinetic suitability with SwissADME (Daina et al., 2017), and predicted their macromolecular targets with SwissTargetPrediction (Daina et al., 2019). Overlap analysis of the predicted targets with Alzheimer's disease-associated genes retrieved from GeneCards and OMIM (Amberger et al., 2018; Stelzer et al., 2016) yielded a set of common targets whose protein–protein interaction network was analyzed through STRING (Szklarczyk et al., 2020) and Cytoscape (Shannon et al., 2003), with hub proteins ranked by topological algorithms in CytoHubba (Chin et al., 2014) and functional annotation performed by Gene Ontology and KEGG pathway enrichment analyses (Ashburner et al., 2000; Kanehisa, 2000). Molecular docking with CB-Dock2 and AutoDock Vina (Eberhardt et al., 2021; Liu et al., 2022) prioritized the flavonoid glycoside rutin, whose complexes with the hubs AKT1 and SRC were further examined by normal mode analysis using the iMODS server (López-Blanco et al., 2014).

The experimental arm of the study substantiated these predictions. SA-EE exhibited concentration-dependent radical-scavenging activity in both the ABTS and DPPH assays, with IC_{50} values of 19.59 µg/mL and 34.72 µg/mL, respectively, compared with 3.48 µg/mL for the ascorbic acid reference standard. The extract also produced strong, dose-dependent inhibition of both cholinesterases, reaching 91.00% AChE inhibition at 100 µg/mL and 81.44% BChE inhibition at 600 µg/mL, and exerted cytoprotective effects against hydrogen peroxide-induced oxidative stress in SH-SY5Y neuroblastoma cells.

AD is a multifactorial disease in which cholinergic deficit, amyloid and tau pathology, and oxidative stress act synergistically, and single-target drugs have repeatedly failed to meaningfully modify its course (Liu et al., 2019; Yang et al., 2012). Network pharmacology was introduced precisely to address this complexity, shifting analysis from single compounds acting on single targets to the systems-level behavior of multi-component interventions (Li et al., 2023; Noor et al., 2022). The present approach — phytochemical retrieval, target prediction, disease-target overlap, PPI construction, hub identification, and enrichment analysis — is now a well-established strategy for prioritizing anti-AD candidates from medicinal plants, having been applied successfully to *Madhuca longifolia* (Khare et al., 2024), *Gmelina arborea* (Gangwal et al., 2026), the multi-herb formulation *Mecasin* (Jo et al., 2025), and *Fibraurea recisa* (Wang et al., 2022). The convergence of the present network topology with these independent studies is notable: AKT1 repeatedly emerges as a top-ranked hub given its central role in neuronal survival, insulin/growth-factor signaling, and activity-dependent synaptic plasticity, where ROS-mediated oxidative modification of AKT1 has been linked to synaptic dysfunction in AD (Khare et al., 2024; Wang et al., 2022). The identification of SRC, a Src-family non-receptor tyrosine kinase, as a further hub is equally mechanistically compelling, since Src-family kinases — particularly the closely related Fyn — physically associate with tau and phosphorylate it at N-terminal tyrosine residues, and mediate amyloid-beta-induced synaptotoxicity through NMDA receptor signaling (Liu et al., 2020; Nygaard et al., 2014; Sheng et al., 2012).

In analogous network pharmacology studies of medicinal plants against AD, KEGG enrichment consistently implicates the PI3K-Akt, MAPK, FoxO, and TNF signaling pathways, which coordinate neuronal survival, inflammatory regulation, and cellular stress responses (Gangwal et al., 2026; Jo et al., 2025; Wang et al., 2022). The pathway landscape identified for *S. auriculata* — centered on hubs that bridge neuroinflammation and neuronal survival — is therefore consistent with an extract that acts through coordinated modulation of cell-survival kinases and inflammatory mediators rather than through a single molecular event.

Among the phytoconstituents ranked by the docking analysis, the flavonoid glycoside rutin (quercetin-3-O-rutinoside), a documented constituent of *S. auriculata* (Nille et al., 2021), emerged as the lead compound, with the AKT1–rutin and SRC–rutin complexes selected for detailed normal mode analysis on the basis of their favorable binding interactions. This finding is strongly supported by the independent literature. Rutin and/or its metabolites are able to cross the blood–brain barrier and have been shown to modify cognitive and behavioral

symptoms in models of neurodegeneration through effects on amyloid-beta processing, aggregation and action, metal chelation, and the oxidant–antioxidant balance (Habtemariam, 2016). In Tau-P301S mice, rutin prevented tau hyperphosphorylation through restoration of PP2A activity, normalized the NF- κ B-driven neuroinflammatory response, and rescued synapse loss by preventing microglial engulfment of synapses (Sun et al., 2021). At the enzyme level, a computational study of 20 plant-derived bioactive molecules against AChE identified rutin as the superior candidate, with a docking score of –12.335 and a binding energy of –73.313 kcal/mol, further supporting its selection for stability analysis (Vijayakumar et al., 2017). Broadly, rutin's neuroprotective profile — reduction of proinflammatory cytokines, improvement of antioxidant enzyme activities, restoration of mitochondrial complex enzyme function, and anti-apoptotic effects (Enogieru et al., 2018) — aligns precisely with the antioxidant, anti-inflammatory, and cholinesterase-inhibitory activities measured in SA-EE, suggesting that rutin may be a principal contributor to the extract's multi-target pharmacology. Its relatively low bioavailability nevertheless remains a recognized translational hurdle that would require formulation strategies in any future development (Zhang & Wang, 2025).

The antioxidant findings corroborate the computational prioritization. SA-EE scavenged the preformed ABTS radical cation and the DPPH radical in a concentration-dependent manner, with the ABTS IC₅₀ (19.59 μ g/mL) approximately 1.8-fold lower than the DPPH IC₅₀ (34.72 μ g/mL). This differential is expected: the ABTS assay measures the activity of both lipophilic and hydrophilic antioxidants and reacts with a wider range of electron- and hydrogen-donating species, whereas DPPH is more selective toward hydrogen-donating, lipophilic antioxidants (Brand-Williams et al., 1995; Re et al., 1999). The lower ABTS IC₅₀ therefore indicates that the activity of SA-EE reflects the combined contribution of multiple classes of phytoconstituents — flavonoids, phenolic acids, and glycosides — rather than a single compound class, a pattern consistent with the extract's known chemical complexity (Nille et al., 2021). Although both IC₅₀ values exceed that of the reference standard ascorbic acid (3.48 μ g/mL), the observed activity is within the range reported for many plant extracts evaluated under identical conditions and is meaningful in the context of AD, where oxidative stress is an early and self-propagating driver of amyloid aggregation, tau hyperphosphorylation, and neuronal loss (Cheignon et al., 2017; Kim & Sharma, 2023; Perluigi et al., 2023).

The most promising experimental finding is the potent, concentration-dependent inhibition of both cholinesterases. SA-EE inhibited AChE with a clear dose–response (33.17% at 20 μ g/mL rising to 91.00% at 100 μ g/mL; $y = 0.6614x + 27.108$, $R^2 = 0.9259$) and inhibited BChE from 8.19% at 120 μ g/mL to 81.44% at 600 μ g/mL ($y = 0.1433x - 10.368$, $R^2 = 0.9616$), as determined by the Ellman colorimetric method (Ellman et al., 1961). The dual-inhibition profile is pharmacologically significant. Whereas AChE activity declines or remains stable in the AD brain, BChE activity progressively increases, and both enzymes are legitimate therapeutic targets (Greig et al., 2001). Dual cholinesterase inhibition has been proposed to provide more sustained cognitive benefit than AChE-selective inhibition and to reduce the formation of amyloidogenic complexes (Ballard, 2002), and accumulating evidence indicates that dual-target cholinesterase inhibitors achieve better therapeutic effects with fewer side effects than AChE-selective agents (Zhou & Huang, 2021). Moreover, AChE colocalizes with amyloid plaques and may directly promote amyloid aggregation, providing a rationale for cholinesterase inhibition that extends beyond cholinergic restoration (Kumar et al., 2024).

The potency of SA-EE is best appreciated in the context of other plant-derived cholinesterase inhibitors. Screening of 80 traditional Chinese medicinal plants identified extracts of *Coptis chinensis* with IC₅₀ values as low as 0.031 μ g/mL — up to 100-fold more potent than galantamine (IC₅₀ = 4.33 μ g/mL) — and attributed the activity to synergistic combinations of isoquinoline alkaloids (Kaufmann et al., 2016). In another study, aqueous extracts of *Acacia nilotica* and *Rhamnus prinoides* inhibited AChE with IC₅₀ values of 0.079 and 0.201 mg/mL, respectively, compared with 0.0004 mg/mL for galanthamine (Crowch & Okello, 2009). Crude plant extracts generally achieve inhibition through the additive or synergistic action of multiple secondary metabolites (Kaufmann et al., 2016; Santos et al., 2018), which is consistent with the present finding that both the antioxidant and cholinesterase-inhibitory activities of SA-EE derive from a complex mixture rather than a single constituent. Against this landscape, the near-complete inhibition of AChE (91%) and substantial inhibition of BChE (81%) at the upper concentrations tested place *S. auriculata* among the more promising plant-derived cholinesterase inhibitor sources reported to date.

The SH-SY5Y neuroblastoma model was used to evaluate whether the antioxidant activity of SA-EE translates into cellular protection against hydrogen peroxide-induced oxidative injury. H₂O₂ is a particularly relevant insult in AD research because it is the principal cellular source of hydroxyl radicals, induces neuronal apoptosis, and has been implicated in tau hyperphosphorylation and neurofibrillary tangle formation (Kim & Sharma, 2023;

Perluigi et al., 2023). The observed maintenance of cell viability and of normal morphology in extract-treated cells exposed to 800 μM H_2O_2 indicates that the phenolic and flavonoid constituents of SA-EE — including rutin — can intercept ROS at the cellular level before they damage lipids, proteins, and DNA, an effect consistent with the reduction of proinflammatory cytokines, improvement of antioxidant enzyme activities, and restoration of mitochondrial complex function documented for rutin (Enogieru et al., 2018; Habtemariam, 2016). These results are preliminary but mechanistically coherent: they demonstrate that the extract's radical-scavenging chemistry, quantified in the DPPH and ABTS assays, operates within a living neuronal cell model under oxidative challenge.

The present findings extend earlier pharmacological work on *S. auriculata*. Previous investigations have documented the antioxidant and antibacterial activities of the methanolic leaf extract (Baluchamy & Arivoli, 2023), the antidiabetic potential of leaf-extract compounds by molecular docking against α -amylase (Dukhyil, 2021), and the broad traditional and phytochemical profile of the plant, which includes alkaloids, flavonoids, saponins, tannins, and phenolic glycosides (Bhakshu et al., 2023; Donagaon4, 2026; Nille et al., 2021). The present study adds a mechanistically distinct dimension by demonstrating, for the first time to our knowledge, that the whole-plant ethanolic extract possesses dual cholinesterase-inhibitory and neuronal cytoprotective activities — properties directly relevant to AD — and by anchoring these activities to specific hub targets (AKT1, SRC) and a defined lead constituent (rutin) through a reproducible network pharmacology workflow.

Future work should isolate and characterize the individual constituents of SA-EE, confirm the rutin–AKT1 and rutin–SRC interactions through biophysical and cellular assays, and subject the prioritized complexes to molecular dynamics simulations to complement the normal mode analysis (López-Blanco et al., 2014). The differential BChE selectivity observed at higher concentrations warrants mechanistic studies of the extract's binding mode at the BChE active and peripheral sites (Greig et al., 2001; Zhou & Huang, 2021). Preclinical evaluation in transgenic AD models, together with bioavailability-enhancing formulations given rutin's known pharmacokinetic limitations (Zhang & Wang, 2025), would represent the logical next step toward clinical relevance.

In conclusion, this study demonstrates that the whole-plant ethanolic extract of *S. auriculata* combines appreciable ABTS and DPPH radical-scavenging activity, potent concentration-dependent dual inhibition of acetylcholinesterase and butyrylcholinesterase, and cytoprotective activity against oxidative stress in SH-SY5Y cells, with network pharmacology identifying AKT1 and SRC as principal hubs and rutin as the lead phytoconstituent. These convergent computational and experimental findings position *S. auriculata* as a promising, multi-target natural source for further development in Alzheimer's disease research.

Study limitations. The present study integrates computational target prediction, molecular docking, normal mode analysis and in vitro assays; however, computational target prediction and docking do not establish target engagement or therapeutic efficacy. In particular, the identification of rutin as a computationally prioritized constituent does not demonstrate that rutin is the principal active constituent of the crude extract in the absence of direct chemical quantification. Similarly, normal mode analysis provides information on intrinsic collective motions but does not replace conventional trajectory-based molecular dynamics simulations. The in vitro findings should therefore be interpreted as preliminary pharmacological evidence requiring confirmation using purified constituents, target-engagement studies, mechanistic cellular assays and appropriate in vivo Alzheimer's disease models.

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