GMR
The Original

INTEGRATED NETWORK PHARMACOLOGY, MOLECULAR DOCKING, AND IN-VITRO VALIDATION REVEAL POTENTIAL NOOTROPIC PHYTOCONSTITUENTS FROM SELECTED INDIAN MEDICINAL PLANTS AGAINST COGNITIVE IMPAIRMENT

Mahesh Bandappa Manke*[1], Dr. Omprakash Gadgeappa Bhusnure[2]

[1*]Research Scholar, Channabasweshwar Pharmacy College (Degree), Latur - 413512, Maharashtra, India, Email: mahesh.bmanke@gmail.com, ORCID ID – 0000-0003-0384-3364.
[2]Research Guide, Channabasweshwar Pharmacy College (Degree), Latur - 413512, Maharashtra, India, Email: omprakashbhusnure@gmail.com, ORCID ID – 0000-0001-7794-5123.
*Corresponding Author: Mahesh Bandappa Manke, Email: mahesh.bmanke@gmail.com

ABSTRACT
Cognitive impairment, a progressive neurodegenerative condition including dementia and Alzheimer's disease that poses a significant global health challenge. Current therapeutic strategies primarily offer symptomatic relief and fail to arrest effective disease progression, underscoring the need for novel multi-target therapeutic approaches. The present study was planned to evaluate potential nootropic phytoconstituents from *Allium cepa*, *Curcuma longa*, and *Coleus forskohli*i, through integrated network pharmacology, molecular docking, and in-vitro validation studies. Network pharmacology study identified the PI3K-Akt signaling pathway as a central neuroprotective mechanism, along with EGFR, ErbB, estrogen, prolactin, and HIF-1 signaling pathways involved in neuronal survival, synaptic plasticity, and cognitive function. Molecular docking study revealed strong binding affinities of curcumin, quercetin, and rosmarinic acid towards key protein targets including AKT1, BCL2, EGFR, STAT3, and SRC. In-vitro validation revealed significant antioxidant and anti-inflammatory activities of curcumin, quercetin, and rosmarinic acid, supporting their therapeutic potential for the treatment of cognitive impairment. The integrated computational and experimental findings indicate that these phytoconstituents may modulate multiple pathological processes associated with cognitive impairment, including oxidative stress, neuroinflammation, apoptosis, and amyloid-related pathways. The findings suggest that curcumin, quercetin, and rosmarinic acid are promising multi-target nootropic phytoconstituents with therapeutic potential for the treatment of cognitive impairment.

KEYWORDS: Cognitive impairment, Network pharmacology, Nootropics, Molecular docking.

1. INTRODUCTION

Cognitive impairment is a progressive neurodegenerative disorder characterized by deterioration in memory, learning, reasoning, behavior and overall daily functioning. Alzheimer's disease (AD) is the most common cause of cognitive impairment, accounting for approximately 60–70% of cases worldwide, with incidence increasing significantly with age [1,2]. Current pharmacological treatments provide only symptomatic relief and do not effectively prevent or reverse disease progression, emphasizing the need for novel, disease-modifying therapeutic approaches [3,4]. The pathophysiology of cognitive impairment is multi-factorial and involves amyloid-β plaque deposition, tau hyperphosphorylation, oxidative stress, mitochondrial dysfunction, neuroinflammation, synaptic loss, cholinergic deficits, and genetic and signaling pathway dysregulation [5,6]. Due to this complexity, single-target drugs have limited efficacy, leading to growing interest in multi-target strategies. Network pharmacology integrates pharmacology, bioinformatics, and systems biology to analyze drug–target–pathway interactions at a systems level, offering a holistic framework for understanding and treating complex diseases [7]. This approach is particularly relevant to herbal medicines, which contain multiple bioactive phytoconstituents acting on diverse targets. Plant-derived phytochemicals such as flavonoids, polyphenols, alkaloids, terpenoids, and organosulfur compounds demonstrate neuroprotective and nootropic effects by reducing oxidative stress, suppressing neuroinflammation, inhibiting amyloid aggregation, modulating neurotransmitters, and enhancing synaptic plasticity [8].

Based on reported neuroprotective evidences, this study focuses on *Allium cepa* (*A. cepa*), *Curcuma longa* (*C. longa*), and *Coleus forskohlii* (*C. forskohlii*) for their potential nootropic activity. Nootropic agents can positively influence cognitive processes, including memory retention, learning ability, concentration, motivation, and higher-order executive functions. *A. cepa* contains quercetin, kaempferol, and organosulfur compounds with antioxidant and anti-inflammatory actions; quercetin crosses the blood brain barrier and shows anti-amyloid effects, while kaempferol supports neuroprotection and anti-inflammatory signaling [9]. *C. longa* contains curcumin and related curcuminoids that exhibit antioxidant, anti-

inflammatory, anti-amyloid, and anti-tau aggregation properties, modulating multiple pathways involved in neurodegeneration [10]. *C. forskohlii* contains rosmarinic acid and luteolin, which reduce oxidative stress, inhibit amyloid toxicity, and regulate microglial activation, thereby supporting cognitive function [11]. Although these medicinal plants show individual neuroprotective effects, their multi-target mechanisms in cognitive impairment remain insufficiently understood. Therefore, this study was planned to investigate the nootropic and neuroprotective potential of selected Indian medicinal plants through an integrated approach involving network pharmacology, molecular docking, and in-vitro validation to identify key bioactive phytoconstituents, molecular targets, and signaling pathways associated with cognitive function and neurodegeneration. The process of network pharmacology based research study is shown in Figure 1.

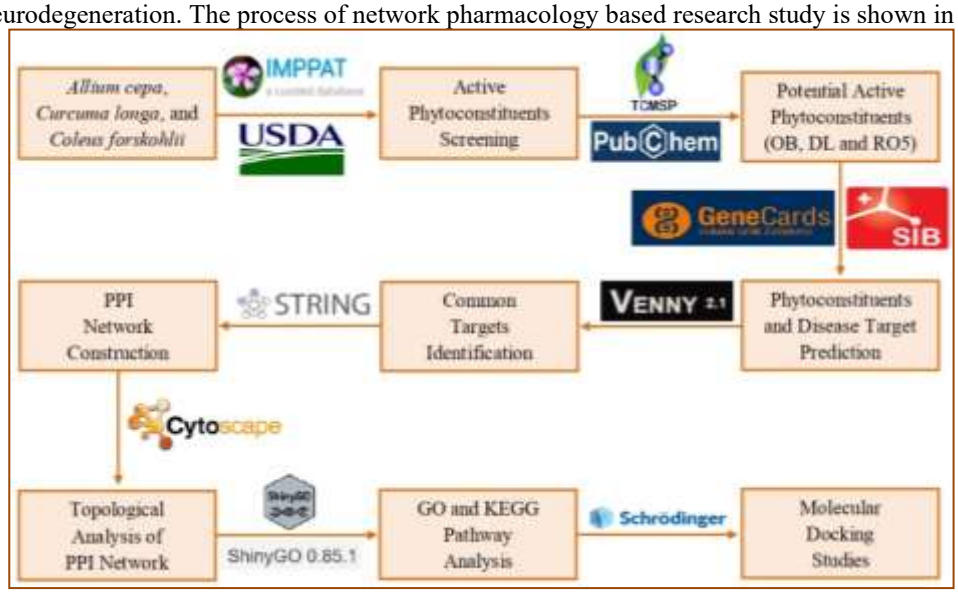


Figure 1. The flowchart of the network pharmacology based research study.

2. MATERIALS AND METHODS

2.1 Bioactive Compounds Screening

The medicinal plants were selected on the basis of previous studies [12]. Information on their biologically active phytoconstituents was collected through literature review and several databases, including Indian Medicinal Plants, Phytochemistry and Therapeutics 2.0 (IMPPAT 2.0, https://cb.imsc.res.in/imppat/), Dr. Duke's Phytochemical and Ethnobotanical Database (https://phytochem.nal.usda.gov/), and the Traditional Chinese Medicine Systems Pharmacology database (TCMSP, available online: https://www.tcmsp-e.com). These platforms were used to identify chemical constituents of *Allium cepa*, *Curcuma longa*, and *Coleus forskohlii*. Active phytoconstituents were selected based on oral bioavailability (OB) ≥ 30%, drug-likeness (DL) ≥ 0.18, and Lipinski's rule of five. Two phytoconstituents with OB < 30% were additionally included due to reported neuroprotective and pharmacological activities. After removing duplicates, chemical structures and canonical SMILES were retrieved from the PubChem database (https://www.drugbank.ca) [13].

2.2 Compound and Disease Target Prediction

The SMILES of each bioactive compound was uploaded to Swiss Target Prediction (http://www.swisstargetprediction.ch/), a free online tool used to predict possible protein targets of small molecules. The species was set to "Homo sapiens". The target data for each bioactive compound was downloaded in CSV format, and only targets with a prediction probability ≥ 0.1 were selected for further analysis. Genes related to memory impairment were collected from the GeneCards database (https://www.genecards.org/) by searching the keywords 'Cognitive impairment', 'Neurodegeneration', and 'Alzheimer's disease' and targets with a GIFTS score ≥50 were selected for further analysis [14].

2.3 Common Targets Identification

Common targets were identified using the Venny 2.1.0 online tool (https://bioinfogp.cnb.csic.es/tools/venny/), which compares different datasets to find overlapping targets. The predicted targets of the selected phytoconstituents and disease-related targets associated with memory impairment were uploaded to the tool, and the overlapping targets were obtained as the output [15].

2.4 Core Target Network Construction of PPI and Identification of Hub Targets

The common targets identified using the Venny 2.1.0 tool were uploaded to the STRING 12.0 database (https://string-db.org) to construct a protein-protein interaction (PPI) network, with "Homo sapiens" selected as the species and a medium confidence interaction score (>0.400). The STRING 12.0 database was used to analyze interactions among memory impairment–related proteins. Cytoscape (Version 3.7.2) refined the PPI network based on node connectivity, with highly connected nodes shown larger. Hub targets were identified using the CytoHubba plugin based on degree, betweenness, and closeness centrality, highlighting potential therapeutic targets [16].

2.5 Compound-Target-Pathway Network

The compound-target and pathway-target networks were merged to build the compound-target-pathway network by using Cytoscape. The nodes in the network represent the active chemicals, potential targets, and signaling pathways. The edges depict the relationship between these three types of nodes and how they interact with each other [16].

2.6 Gene Ontology and Pathway Enrichment Analysis

Functional enrichment analysis of the identified core target proteins was conducted using ShinyGO (v0.85). This platform facilitated both Gene Ontology (GO) functional annotation categorized into molecular functions (MF), biological processes (BP), cellular components (CC) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis. To elucidate the nootropic mechanisms of phytoconstituents from *A. cepa*, *C. longa*, and *C. forskohli*i, significance was determined using a False Discovery Rate (FDR) threshold of < 0.05. The top 20 enriched terms for each GO category and KEGG pathway were prioritized based on fold enrichment. These findings were visualized via bubble charts, where bubble size and color represent gene counts, FDR adjusted p-values, and fold enrichment levels [17].

2.7 Molecular Docking Studies

Molecular docking was performed using the XP Glide in Schrodinger software to evaluate interactions between isorhamnetin, quercetin, kaempferol, luteolin, curcumin, bisdemethoxycurcumin, and rosmarinic acid with five hub proteins. Ligand structures were retrieved from PubChem, while protein structures were obtained from PDB [3O96 (AKT1), 5O0K (BCL2), 6NJS (STAT3), 4WKQ (EGFR), 7NG7 (SRC)]. Proteins were prepared using the protein preparation wizard prior to docking analysis [18].

2.8 In-vitro Antioxidant and Anti-inflammatory Activity

2.8.1 Drugs and chemicals

All organic solvents and chemicals of analytical grade were purchased from Labware Chemicals, Latur, Maharashtra, India

2.8.2 Antioxidant Assay

2.8.2.1 DPPH Radical Scavenging Assay

A 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) solution (0.004% w/v) was prepared using 95% methanol. Stock solutions of curcumin, quercetin, and rosmarinic acid were prepared at a concentration of 1 mg/mL in 95% methanol. Freshly prepared DPPH solution was dispensed into test tubes, followed by the addition of curcumin, quercetin, and rosmarinic acid. Serial dilutions were then carried out to obtain concentrations ranging from 20 to 100 µg/mL, and the final volume in each test tube was adjusted to 3 mL. The reaction mixtures were incubated for 10 minutes, after which the absorbance was measured at 515 nm using a UV-Visible spectrophotometer. Ascorbic acid was used as the reference standard and was dissolved in double-distilled water to prepare a stock solution of 1 mg/mL, followed by similar serial dilutions (20-100 µg/mL). A control was prepared containing the same volume of solvent without any test compound or reference standard [19].

DPPH scavenging effect (%) = (A0 – A1) / A1 × 100

Where A0 was the absorbance of the control (blank) and A1 was the absorbance of the test compounds or standard.

2.8.2.2 H2O2 Radical Scavenging Assay

A hydrogen peroxide (H2O2) solution (2 mM) was prepared in phosphate buffer (pH 7.4), and its concentration was determined spectrophotometrically at 230 nm. Solutions of curcumin, quercetin, and rosmarinic acid (20-100 µg/mL) prepared in distilled water were added to hydrogen peroxide solution (0.6 mL, 2 mM). The reaction mixtures were incubated for 20 minutes, after which the absorbance was measured at 230 nm against a blank containing phosphate buffer without hydrogen peroxide [20].

The percentage scavenging of hydrogen peroxide was calculated using the following formula:

H2O2 scavenging effect (%) = (A0 – A1) / A1 × 100

Where A0 represents the absorbance of the control (without H2O2), and A1 represents the absorbance in the presence of the test compounds or standard.

2.8.3 Anti-inflammatory Assay

2.8.3.1 BSA Denaturation Assay

The 0.05 mL of each test compound at concentrations of 20, 40, 60, 80, and 100 µg/mL was mixed with 0.45 mL of 1% aqueous BSA solution. The pH of the reaction mixture was adjusted to 6.3 using a small volume of 1N hydrochloric acid. The samples were incubated at room temperature for 20 minutes, followed by heating at 55°C for 30 minutes in a water bath. After cooling, the absorbance was measured at 660 nm using UV spectrophotometer. Diclofenac sodium was used as the standard reference drug and dimethyl sulfoxide (DMSO) served as the vehicle control [21].

The percentage inhibition of protein denaturation was calculated using the following equation:

% Inhibition = $(A_0 - A_1) / A_0 \times 100$

Where A0 represents the absorbance of the control, and A1 represents the absorbance in the presence of the test compounds or standard.

2.8.3.2 EA Denaturation Assay

The reaction mixture consisted of 0.2 mL of fresh egg albumin, 2.8 mL of phosphate-buffered saline (PBS, pH 6.4), and 0.6 mL of test compound solutions prepared in 0.2% DMSO, to obtain final concentrations of 20, 40, 60, 80, and 100 µg/mL. The samples were incubated at 37°C for 10 minutes, followed by heating at 70°C for 20 minutes in a water bath. After cooling, the absorbance was recorded at 660 nm using UV spectrophotometer. A negative control consisted of egg albumin, PBS, and 0.2% DMSO without test compounds, while diclofenac sodium was used as the positive control [21].

The percentage inhibition of protein denaturation was calculated using the following equation:

% Inhibition = $(A_0 - A_1) / A_0 \times 100$

Where A0 represents the absorbance of the control, and A1 represents the absorbance in the presence of the test compounds or standard.

2.8.4 Statistical analysis

The experimental results derived in the study were expressed as the mean ± SD from three parallel measurements. Linear regression analysis was used to calculate the IC50 values.

3. RESULTS

3.1 Bioactive Compounds Screening

Phytochemical data of *A. cepa*, *C. longa*, and *C. forskohlii* were collected from literature and databases, identifying 158 compounds (77, 60, and 21 respectively). Based on OB and DL screening, 11 bioactive compounds were selected, bis-demethoxycurcumin, campesterol, cycloartenol, cycloeucalenol, isorhamnetin, kaempferol, luteolin, phytosterol, quercetin, curcumin, and rosmarinic acid. Their physicochemical properties are summarized in Table 1.

Table 1. The pharmacokinetic properties of 11 phytoconstituents.

Molecule ID	Phytoconstituent	MW	OB (%)	DL	Number of Lipinski's rule of 5 violations	Lipinski's rule of 5
MOL000940	Bis-Demethoxycurcumin	308.35	77.38	0.26	0	Passed
MOL012254	Campesterol	400.76	37.58	0.71	1	Passed
MOL003578	Cycloartenol	426.8	38.69	0.78	1	Passed
MOL009653	Cycloeucalenol	426.8	39.73	0.79	1	Passed
MOL000354	Isorhamnetin	316.28	49.6	0.31	0	Passed
MOL000422	Kaempferol	286.25	41.88	0.24	0	Passed
MOL000006	Luteolin	286.25	36.16	0.25	0	Passed
MOL001532	Phytosterol	414.79	36.91	0.75	1	Passed
MOL000098	Quercetin	302.25	46.43	0.28	0	Passed
MOL000090	Curcumin	368.41	5.15	0.41	0	Passed
MOL011865	Rosmarinic Acid	360.34	1.38	0.35	0	Passed

3.2 Compound and Disease Target Prediction

Target prediction of selected drug-like compounds using SwissADME yielded 44, 14, 16, 11, 67, 65, 65, 28, 66, 47, and 31 targets for bis-demethoxycurcumin, campesterol, cycloartenol, cycloeucalenol, isorhamnetin, kaempferol, luteolin, phytosterol, quercetin, curcumin, and rosmarinic acid, respectively. After de-duplication, 226 unique targets were obtained, along with 3,964 cognitive impairment related genes from GeneCards database.

3.3 Common Targets Identification

A total of 188 common targets were identified between the complete set of cognitive impairment related targets and the total targets associated with all phytoconstituents (Figure 2). These common targets were determined using the Venny 2.1.0 online tool, which facilitates the analysis of Venn diagrams to explore relationships among different datasets.

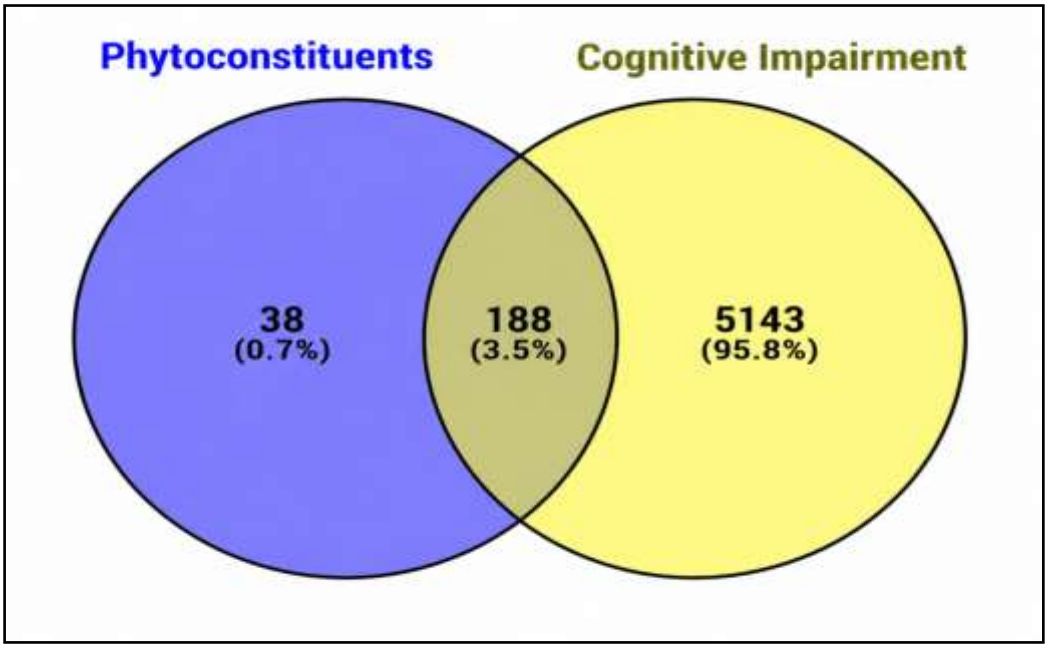


Figure 2. Venn diagram showing the intersection between the predicted phytoconstituents targets (226) and the cognitive impairment related targets (5331).

3.4 Core Target PPI Network Construction

The core target protein–protein interaction (PPI) network was established using the STRING database based on 188 key targets (Figure 3). The constructed network comprised 188 nodes and 2172 edges. Network analysis revealed that the average node degree was 23.1, while the average local clustering coefficient was 0.538. Furthermore, the PPI enrichment

p-value was < 1.0e−16, indicating that the observed number of interactions was significantly higher than expected by random chance, suggesting strong functional associations among the target proteins.

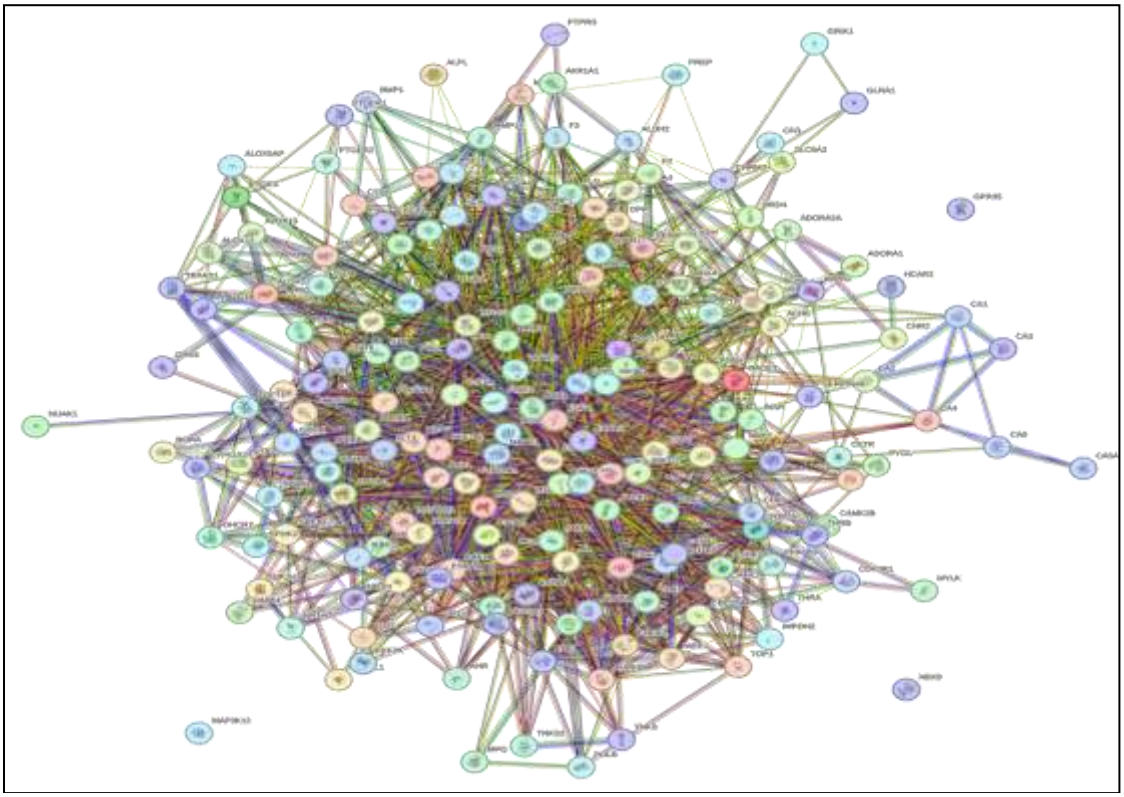


Figure 3. Core target PPI network by STRING database.

3.5 Compounds-Target Network Construction and Identification of Hub Targets

The compound–target network was constructed using Cytoscape, identifying 11 compounds interacting with 188 key targets, illustrating complex multi-target relationships (Figure 4). CytoHubba analysis in Cytoscape identified the top 20 hub nodes based on degree of connectivity, including AKT1, BCL2, EGFR, STAT3, SRC, HSP90AA1, ESR1, PTGS2, MMP9, PPARG, GSK3B, ERBB2, PARP1, MTOR, EP300, MAPK1, MMP2, MCL1, KDR, and PIK3R1. Node color (red to yellow) and size represented connectivity, with AKT1 showing the highest degree (92) and PIK3R1 the lowest (44). Candidate potential targets evaluated through CytoHubba based on degree shown in Figure 5A. Overlap analysis further identified AKT1, BCL2, EGFR, STAT3, and SRC as key hub targets shown in Figure 5B.

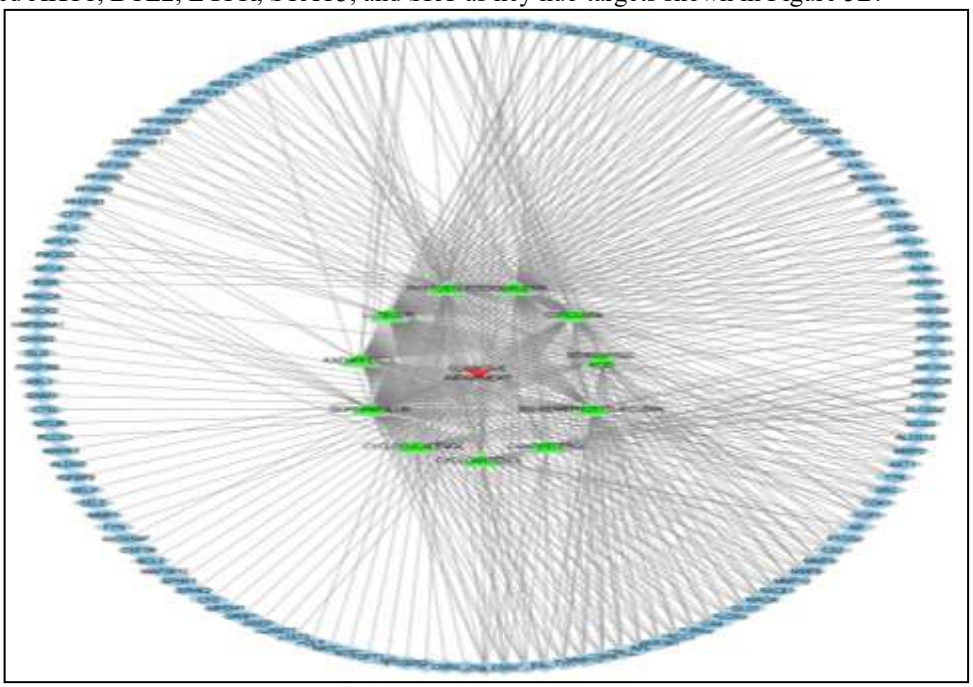


Figure 4. Compound–target network construction by Cytoscape.

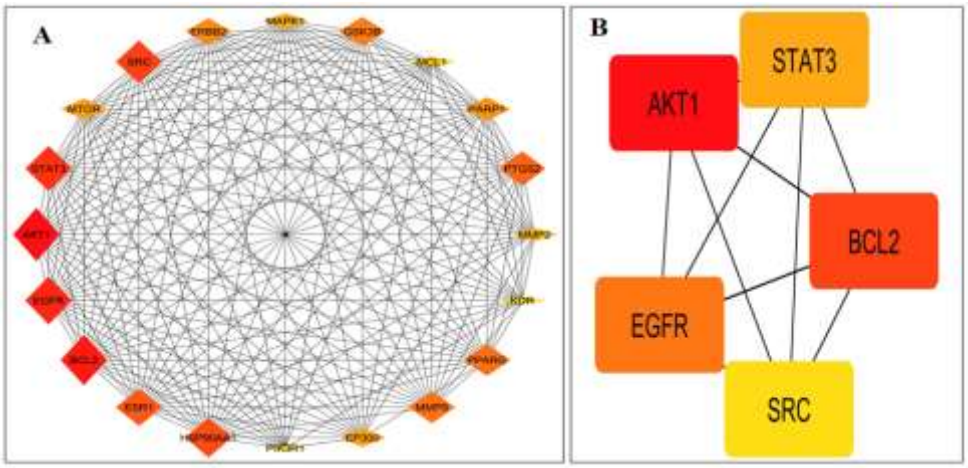


Figure 5. (A) Potential candidate targets were identified using CytoHubba based on degree centrality. (B) The PPI network was optimized using Cytoscape.

3.6 Compound-Target-Pathway Network Construction

Compound–target and pathway–target networks were integrated to visualize interactions among bioactive compounds, targets, and disease pathways, indicating potential synergistic effects. CytoHubba ranked compounds by connectivity, identifying isorhamnetin (67), quercetin (66), kaempferol (65), luteolin (65), curcumin (47), bis-demethoxycurcumin (44), and rosmarinic acid (30) as top seven for docking with hub targets AKT1, BCL2, EGFR, STAT3, and SRC. The compound–target–pathway network was constructed using Cytoscape and is shown in Figure 6.

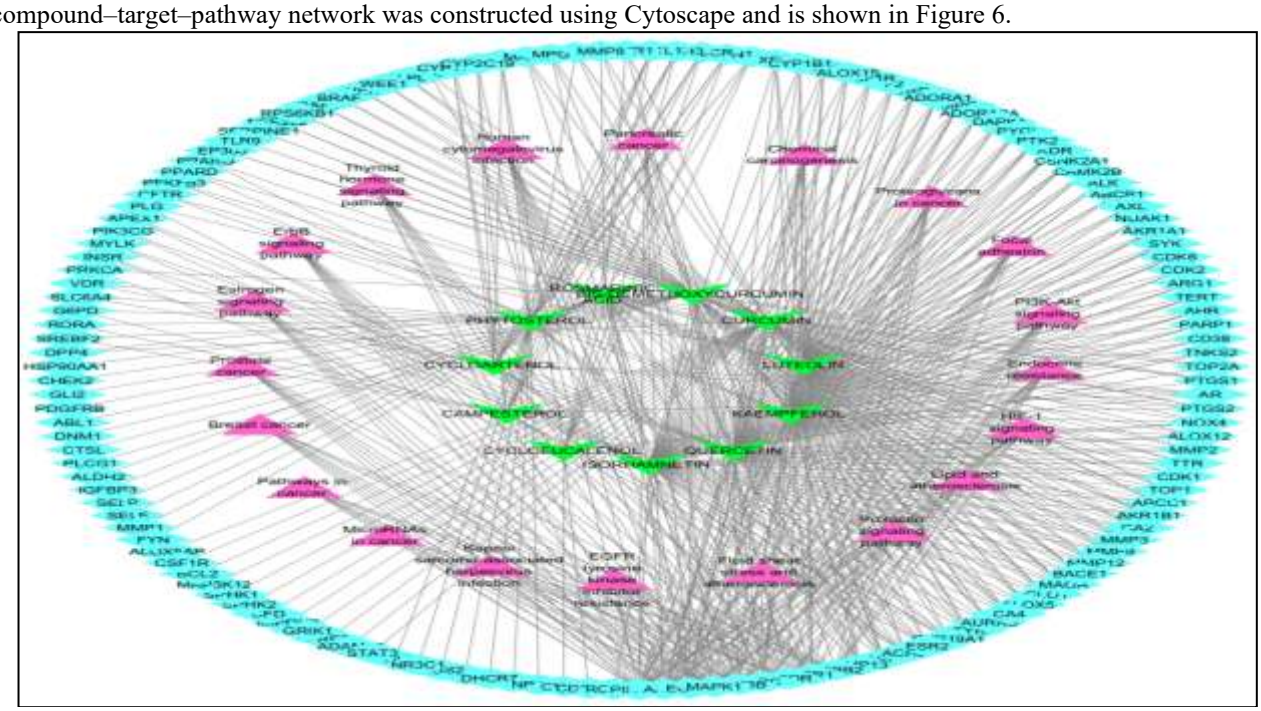


Figure 6. Compound–target-pathway network construction by Cytoscape.

3.7 Gene Ontology (GO) and KEGG Pathway Enrichment Analysis

Gene ontology (GO) and KEGG enrichment analyses were performed to characterize target bioactivity. The targets were enriched in 1000 biological processes (BP), 157 cellular components (CC), 284 molecular functions (MF), and 150 KEGG pathways (FDR < 0.05, gene count > 2), visualized using bubble plots. Top 20 MF terms included protein kinase activity, transcription factor binding, and enzyme binding (Figure 7A). CC terms were mainly associated with membrane rafts, nucleus, mitochondrion, and transcriptional complexes (Figure 7B). BP terms were enriched in responses to hormones, stress, and regulation of apoptosis and signaling (Figure 7C). KEGG analysis highlighted signaling and cancer-related pathways, including PI3K-Akt, EGFR, HIF-1, and endocrine resistance pathways (Figure 7D).

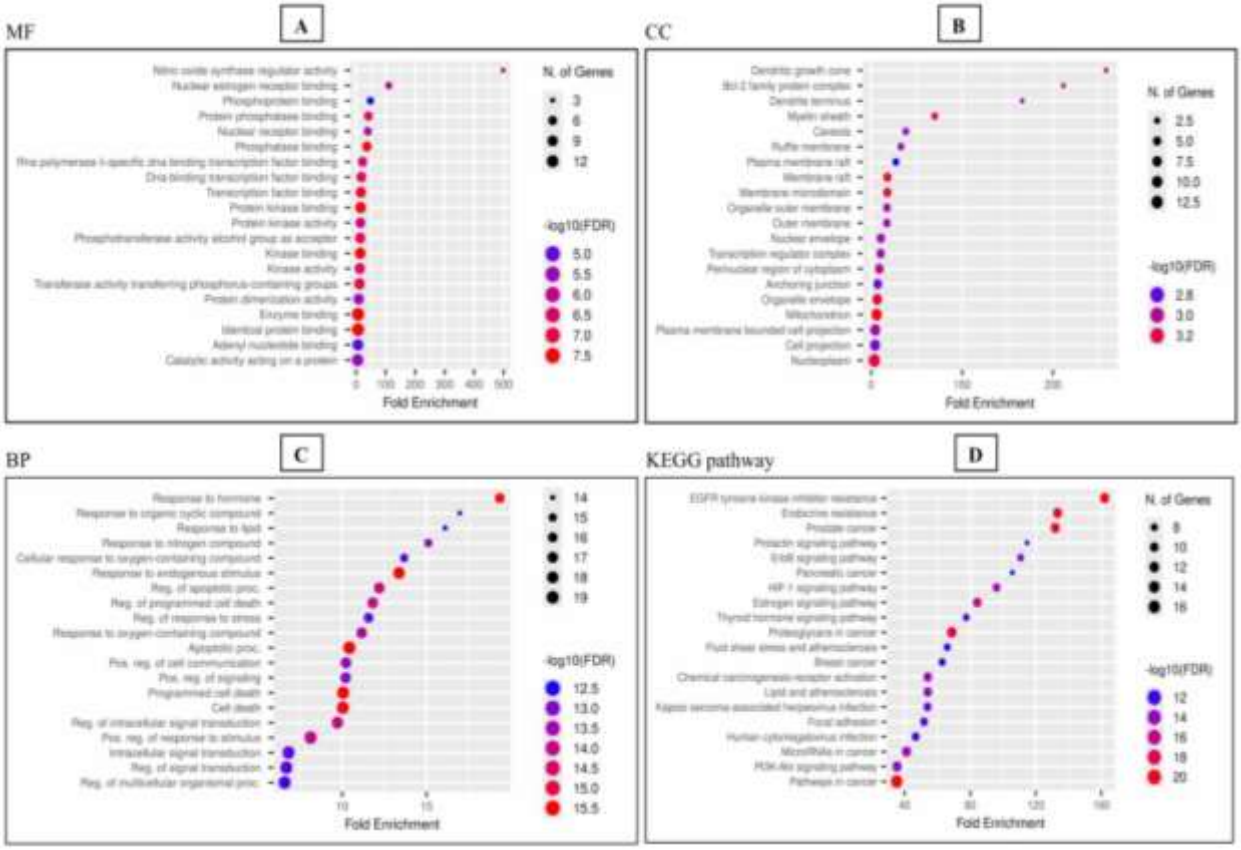


Figure 7. Bubble map of enriched gene functional annotations and signaling pathways: (A) Molecular functions, (B) Cellular components, (C) Biological processes, and (D) KEGG pathways. The dot size indicates gene count, and color represents false discovery rate (FDR).

3.8 Molecular Docking Studies

Molecular docking analysis was performed to evaluate the binding affinity of seven phytoconstituents consisting of isorhamnetin, quercetin, kaempferol, luteolin, curcumin, bisdemethoxycurcumin, and rosmarinic acid against the five hub proteins (AKT1, BCL2, STAT3, EGFR, and SRC). Overall, the docking scores ranged from −3.02176 to −8.25257 kcal/mol, indicating that all selected phytoconstituents interacted with the target proteins to varying degrees. Among the tested phytoconstituents, curcumin exhibited the strongest binding affinity toward AKT1 (−7.95556 kcal/mol) and SRC (−7.84217 kcal/mol). Rosmarinic acid showed the highest binding affinity toward BCL2 (−8.25257 kcal/mol), STAT3 (−4.92336 kcal/mol), and EGFR (−5.96716 kcal/mol) among all the investigated phytoconstituents. Isorhamnetin, kaempferol, luteolin, and quercetin exhibited moderate binding affinities across all target proteins. Among them, curcumin and rosmarinic acid exhibited the strongest overall binding affinities, while quercetin showed consistent interactions with all five target proteins, supporting their potential role in the treatment of cognitive impairment through multi-target mechanisms. Therefore, curcumin, quercetin, and rosmarinic acid were selected for further in-vitro validation studies. The detailed docking scores are presented in Table 2.

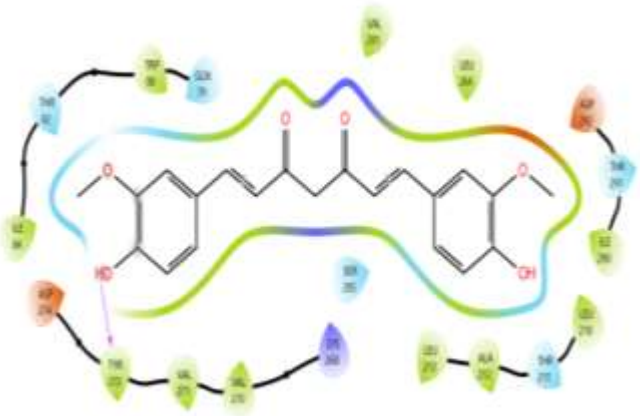
Table 2. Molecular docking results.

Compound	Docking Score (kcal/mol)				
	AKT1	BCL2	STAT3	EGFR	SRC
Isorhamnetin	−6.30037	−6.82906	−3.15704	−3.25135	−6.9256
Quercetin	−5.93011	−6.2345	−3.335	−3.41962	−6.67614
Kaempferol	−6.46056	−6.66528	−3.02176	−3.10775	−6.70361
Luteolin	−6.07446	−5.76028	−3.77021	−3.94248	−6.87348
Curcumin	−7.95556	−6.36649	−3.06732	−4.74634	−7.84217

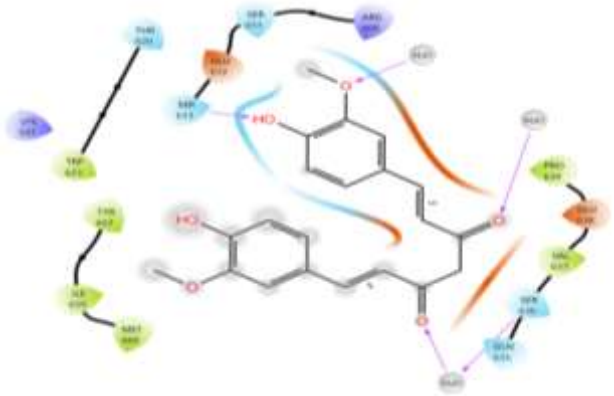
Bisdemethoxycurcumin	−7.85552	−5.93214	−3.81345	−4.67364	−7.40269
Rosmarinic acid	−7.11988	−8.25257	−4.92336	−5.96716	−6.05031

The corresponding two-dimensional (2D) interactions between curcumin, quercetin, and rosmarinic acid and the target proteins are shown in Figures 8, 9 and 10 respectively.

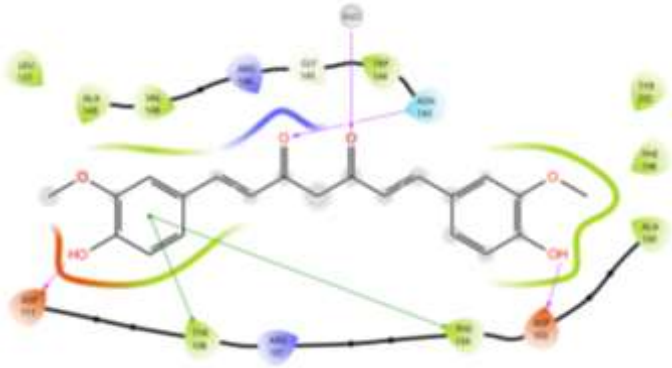
AKT Serine/Threonine Kinase1 (ATK1)



Signal transducer and activator of transcription3 (STAT3)



B-Cell Lymphoma 2 (BCL2)



Epidermal Growth Factor Receptor (EGFR)

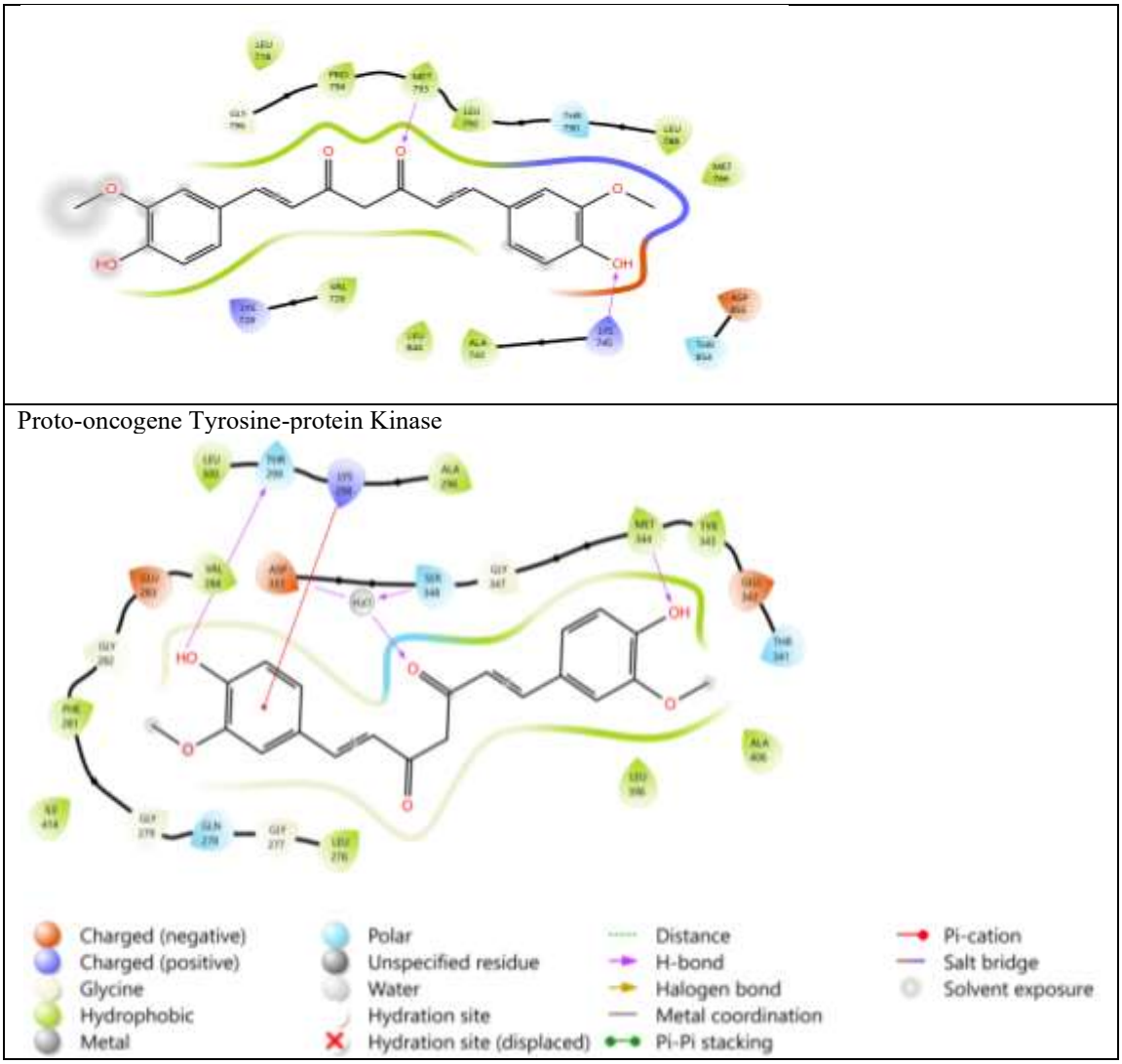
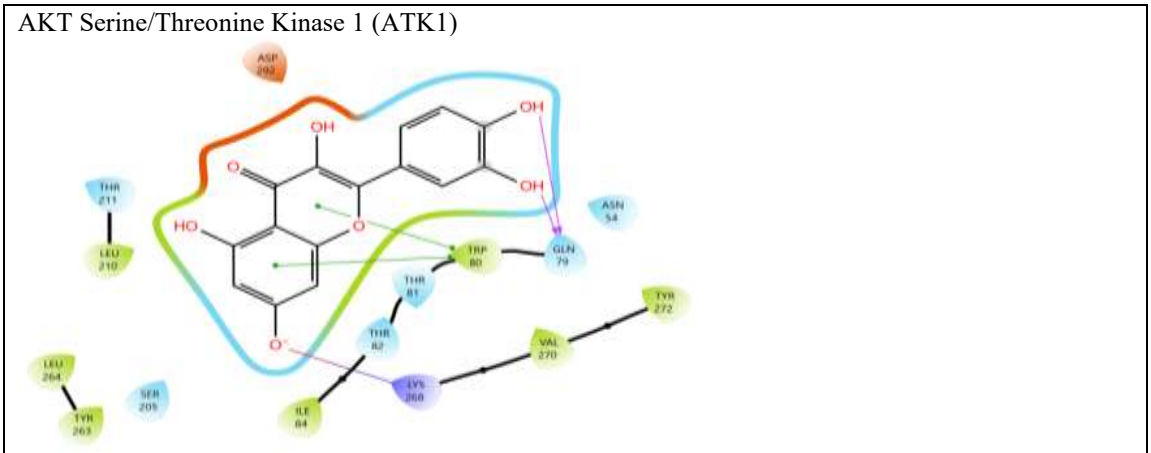


Figure 8. 2D interaction diagram of curcumin with the identified targets.



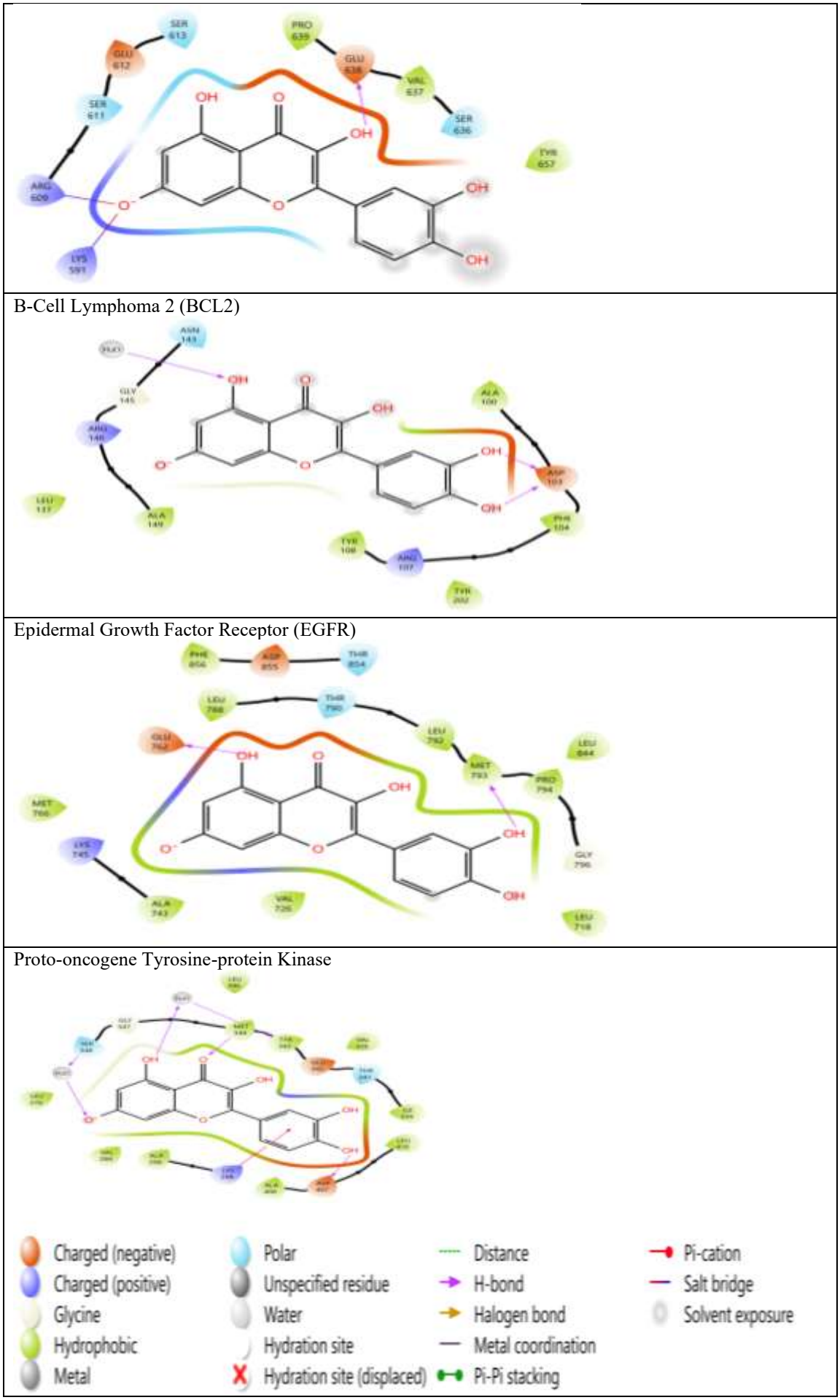
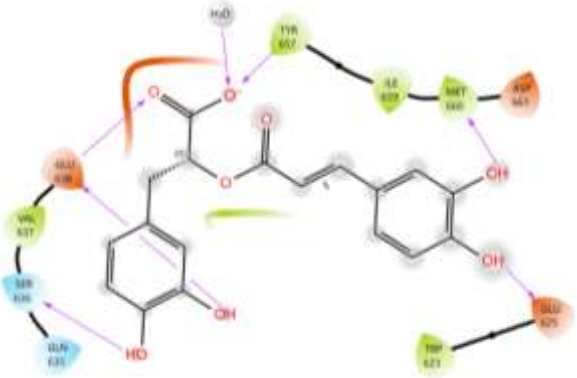
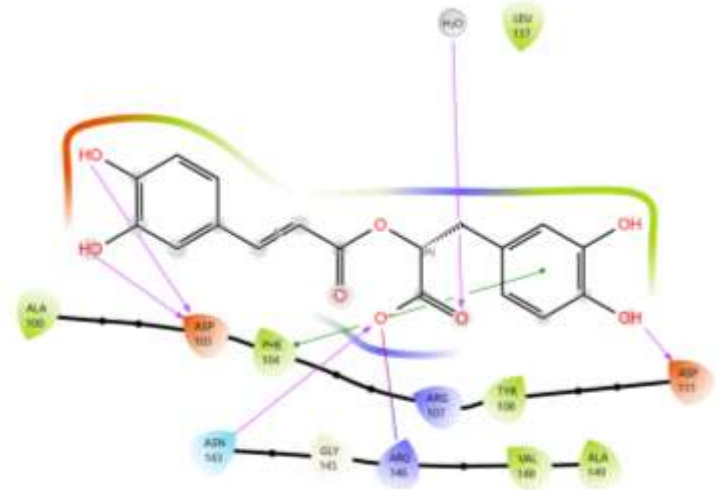
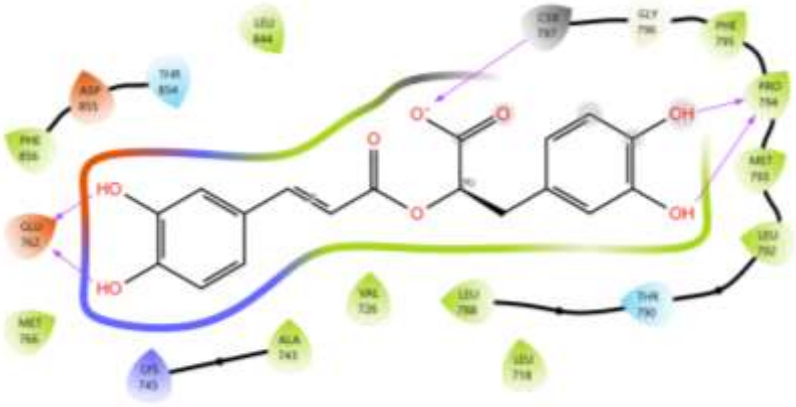


Figure 9. 2D interaction diagram of quercetin with the identified targets.

AKT Serine/Threonine Kinase 1 (ATK1) 
Signal transducer and activator of transcription 3 (STAT3) 
B-Cell Lymphoma 2 (BCL2) 
Epidermal Growth Factor Receptor (EGFR) 
Proto-oncogene Tyrosine-protein Kinase

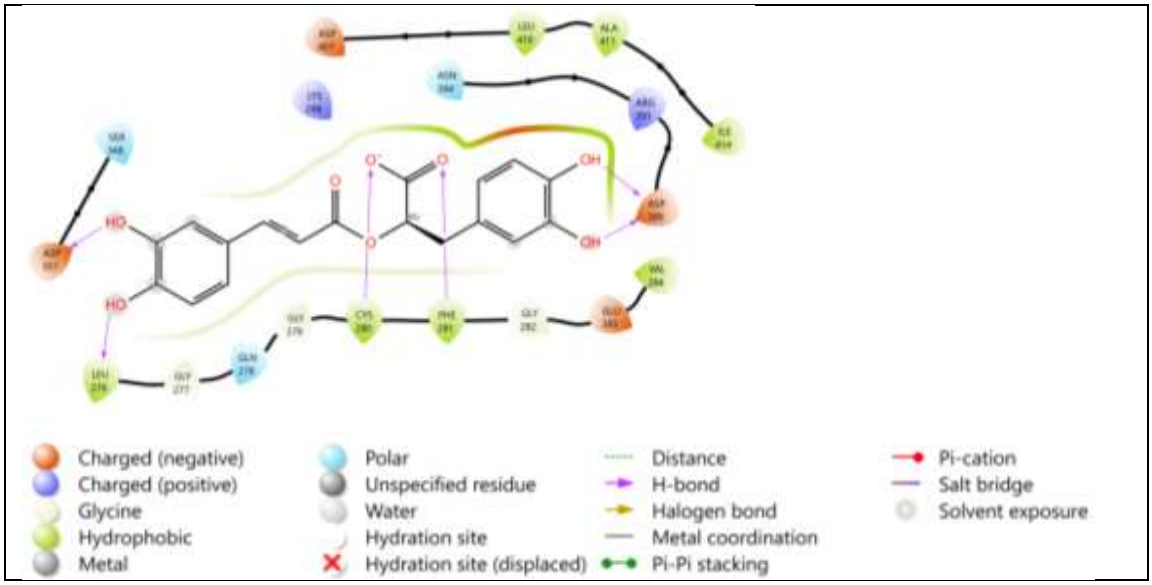


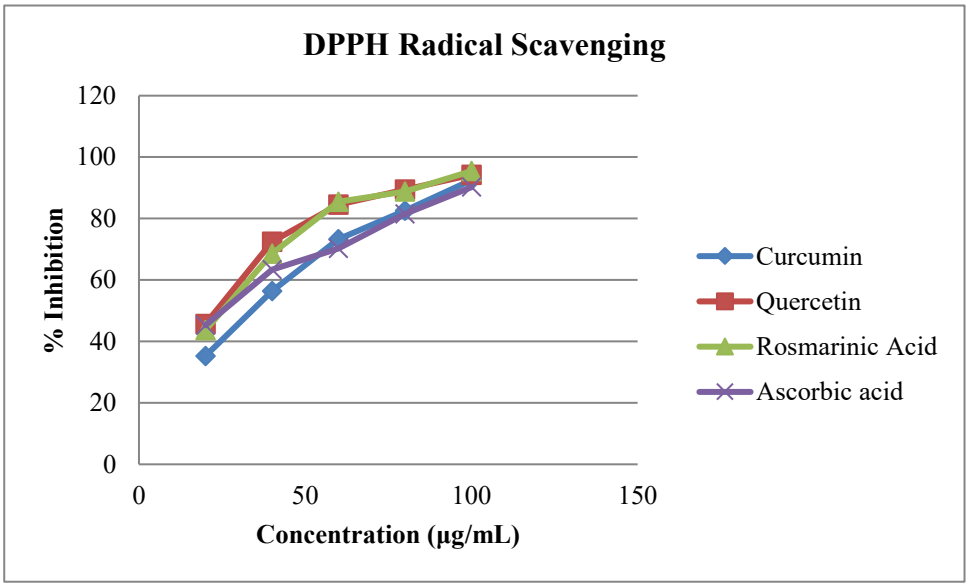
Figure 10. 2D interaction diagram of rosmarinic acid with the identified targets.

3.9 In-vitro Antioxidant and Anti-inflammatory Activity

3.9.1 Antioxidant Assay

The DPPH free radical scavenging activity was obtained for curcumin, quercetin and rosmarinic acid with IC50 values of 34.44, 12.20 and 17.39 µg/mL, respectively, compared with the standard ascorbic acid, which showed an IC50 value of 22.73 µg/mL (Figure 11A). Also, curcumin, quercetin and rosmarinic acid revealed concentration-dependent hydrogen peroxide scavenging activity, with IC50 values of 22.84, 9.36 and 15.07 µg/mL, respectively, whereas ascorbic acid exhibited an IC50 value of 17.42 µg/mL (Figure 11B).

(A)



(B)

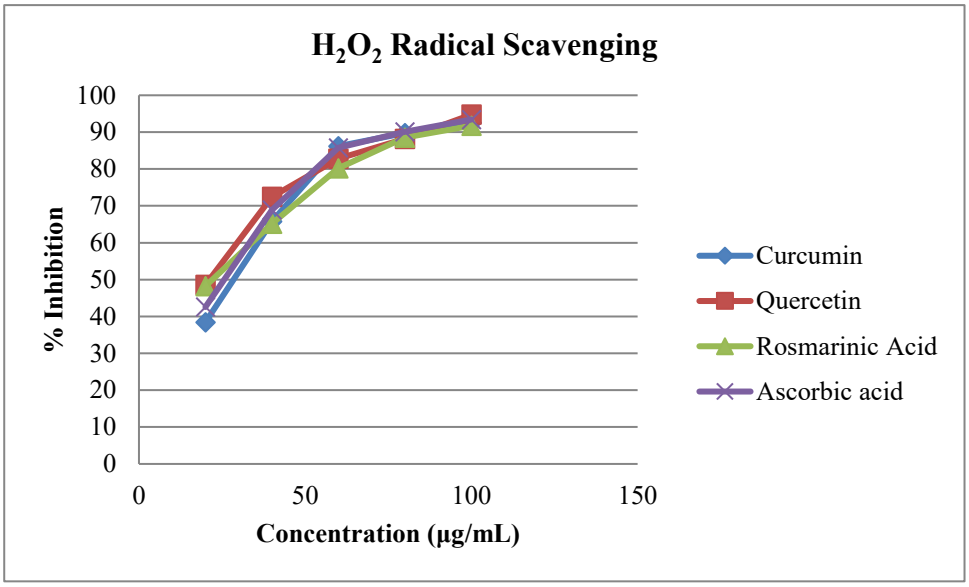
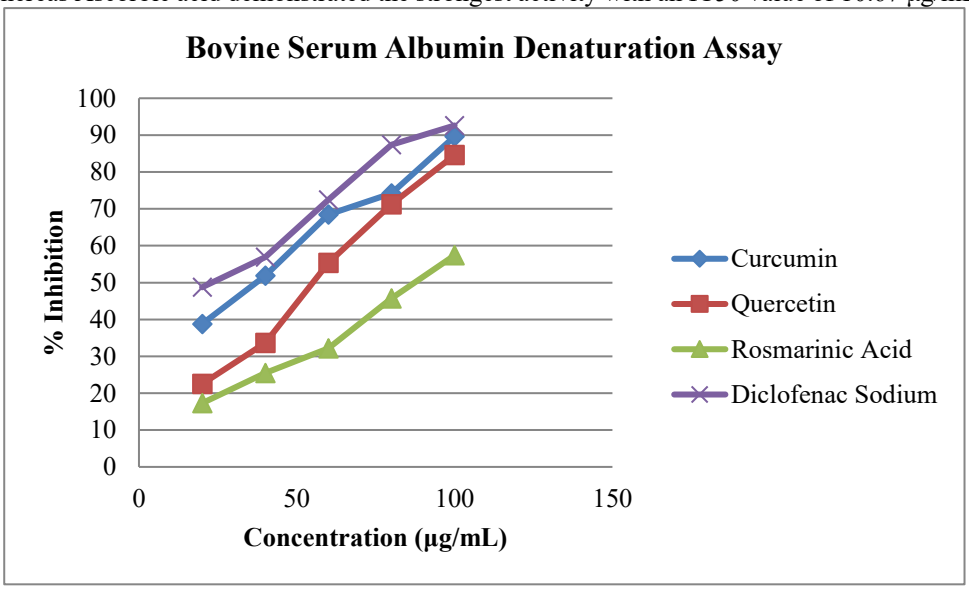


Figure 11. Effect of various concentrations of curcumin, rosmarinic acid, quercetin and ascorbic acid on (A) DPPH radicals scavenging (B) H2O2 radicals scavenging.

3.9.2 Anti-inflammatory Assay

The bovine serum albumin (BSA) denaturation assay demonstrated that curcumin, quercetin and rosmarinic acid possessed inhibitory activity with IC50 values of 36.51, 55.76 and 88.71 µg/mL, respectively, while the reference standard, Diclofenac sodium, exhibited a lower IC50 value of 23.49 µg/mL (Figure 12A). Similarly, in the egg albumin (EA) denaturation assay, curcumin, quercetin and rosmarinic acid produced IC50 values of 27.88, 44.94, and 64.84 µg/mL, respectively, whereas Ascorbic acid demonstrated the strongest activity with an IC50 value of 10.67 µg/mL (Figure 12B).

(A)



(B)

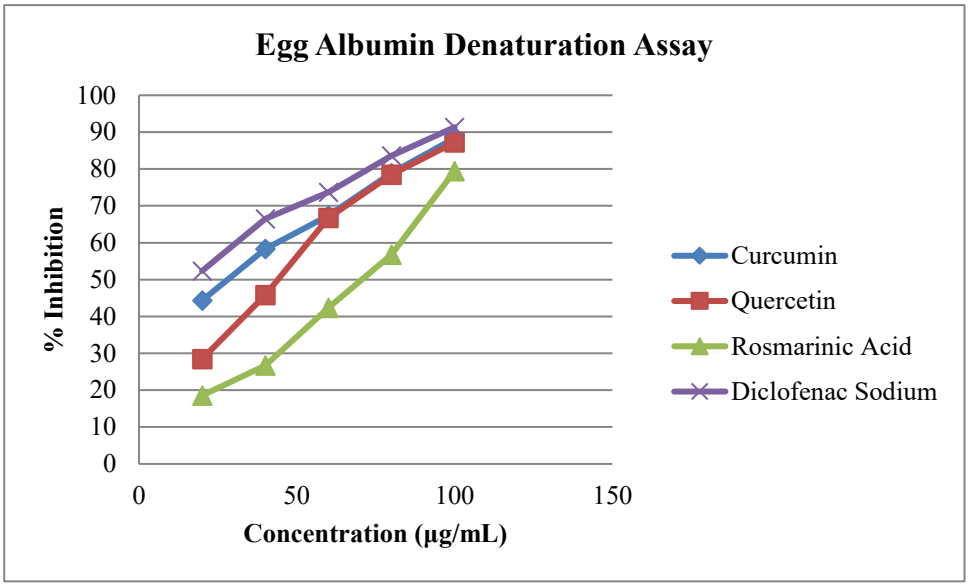


Figure 12. Effect of various concentrations of curcumin, rosmarinic acid, quercetin and ascorbic acid on (A) BSA denaturation assay (B) Egg albumin denaturation assay.

4. DISCUSSION

The present study employed integrated network pharmacology, molecular docking, and in-vitro experimental validation to investigate the molecular basis of the nootropic potential of phytoconstituents from *A. cepa, C. longa,* and *C. forskohlii*. KEGG pathway enrichment analysis identified the PI3K-Akt signaling pathway as the most significantly enriched pathway, highlighting its critical role in neuroprotection and cognitive function. The PI3K-Akt pathway regulates neuronal survival, synaptic plasticity, glucose metabolism, autophagy, and apoptosis, while its dysregulation contributes to amyloid-β accumulation, tau hyperphosphorylation, oxidative stress, and neuroinflammation during AD progression [22]. Akt activation suppresses GSK-3β activity, thereby reducing tau pathology, and promotes CREB and BDNF mediated neurotrophic signaling, which are essential for learning and memory [23]. These findings suggest that modulation of PI3K-Akt signaling is a key mechanism underlying the neuroprotective effects of the identified phytoconstituents.

The enrichment of the ErbB signaling and EGFR tyrosine kinase inhibitor resistance pathways further emphasizes the importance of receptor tyrosine kinase-mediated signaling in neuronal maintenance and survival. EGFR-mediated activation of PI3K-Akt and MAPK pathways regulates neurogenesis, synaptic plasticity, and inflammatory responses, and its dysregulation has been linked to cognitive decline [24]. Similarly, enrichment of Estrogen and Prolactin signaling pathways indicates the involvement of neuroendocrine mechanisms in cognitive regulation. Estrogen exerts antioxidant, anti-inflammatory, and anti-apoptotic effects while influencing cholinergic neurotransmission and amyloid precursor protein processing [25,26], whereas prolactin promotes neurogenesis and neuronal survival through JAK-STAT and PI3K-Akt signaling [27]. The HIF-1 signaling pathway was also significantly enriched, plays an important role in protecting neurons against hypoxia, oxidative stress, and metabolic dysfunction through enhanced antioxidant defense and cellular adaptation mechanisms [28].

Among the selected plants identified phytoconstituents, curcumin exhibited potent antioxidant, anti-inflammatory, and anti-amyloidogenic activities, whereas quercetin and rosmarinic acid showed significant neuroprotective effects through the attenuation of oxidative stress, suppression of neuroinflammation, and inhibition of apoptosis [29-31]. Molecular docking analysis further revealed that these phytoconstituents displayed strong binding affinities toward key therapeutic targets, including AKT1, BCL2, EGFR, STAT3, and SRC which are significantly involved in neuronal survival, synaptic plasticity, apoptosis regulation, and inflammatory signaling [32]. The favorable docking interactions, together with the in-vitro experimental validation results, highlight the multi-target neuroprotective potential of these phytoconstituents. The present study findings suggest that curcumin, quercetin, and rosmarinic acid may protect against cognitive impairment through the coordinated modulation of multiple signaling pathways associated with neuronal survival, synaptic function, and neuroinflammation, thereby supporting their potential as promising therapeutic agents for the treatment of AD and cognitive impairment.

5. CONCLUSION

An integrated network pharmacology, molecular docking, and in-vitro experimental validation approach investigated the nootropic potential of phytoconstituents derived from selected Indian medicinal plants, including *Allium cepa*, *Curcuma longa*, and *Coleus forskohlii*. The findings identified curcumin, quercetin, and rosmarinic acid as key bioactive phytoconstituents capable of modulating multiple molecular targets and signaling pathways associated with cognitive impairment, particularly the PI3K-Akt, EGFR, ErbB, Estrogen, Prolactin, and HIF-1 signaling pathways. Molecular docking study revealed strong interactions of curcumin, quercetin, and rosmarinic acid towards key protein targets involved in neuronal survival, neuroinflammation, and cognitive function, including AKT1, BCL2, EGFR, STAT3, and SRC, while in-vitro antioxidant and anti-inflammatory evaluations further supported their neuroprotective potential.

However, additional in-vivo studies using animal models are necessary to establish the therapeutic efficacy and translational potential of these phytoconstituents in preventing and managing neurodegenerative disorders.

FUNDING:
Not applicable.

ACKNOWLEDGMENTS:
We sincerely express our gratitude to all those who contributed to this work directly or indirectly.

CONFLICTS OF INTEREST:
The authors declare that they have no conflicts of interest.

REFERENCES
1. McCollum, L.; Karlawish, J. Cognitive impairment evaluation and management. Med. Clin. North Am. 2020, 104, 807–825, https://doi.org/10.1016/j.mcna.2020.06.007.
2. Joshi, S.; Morley, J.E. Cognitive impairment. Med. Clin. North Am. 2006, 90, 769–787, https://doi.org/10.1016/j.mcna.2006.05.014.
3. Cummings, J.; Lee, G.; Nahed, P.; Kambar, M.E.Z.N.; Zhong, K.; Fonseca, J.; Taghva, K. Alzheimer's disease drug development pipeline: 2022. Alzheimers Dement. (N Y) 2022, 8, e12295, https://doi.org/10.1002/trc2.12295.
4. Karch, C.M.; Goate, A.M. Alzheimer's disease risk genes and mechanisms of disease pathogenesis. Biol. Psychiatry 2015, 77, 43–51, https://doi.org/10.1016/j.biopsych.2014.05.006.
5. Zhang, J.; Kong, G.; Yang, J.; Pang, L.; Li, X. Pathological mechanisms and treatment progression of Alzheimer's disease. Eur. J. Med. Res. 2025, 30, 625, https://doi.org/10.1186/s40001-025-02886-9.
6. Manke, M.B.; Kalshetti, M.P.; Giram, P.S.; Bhusnure, O.G. Alzheimer's disease and its management: A review. Curr. Trends Pharm. Pharm. Chem. 2025, 7, 79–86, https://doi.org/10.18231/j.ctppc.v.7.i.3.1.
7. Hopkins, A.L. Network pharmacology: The next paradigm in drug discovery. Nat. Chem. Biol. 2008, 4, 682–690, https://doi.org/10.1038/nchembio.118.
8. Li, S.; Zhang, B. Traditional Chinese medicine network pharmacology: Theory, methodology and application. Chin. J. Nat. Med. 2013, 11, 110–120, https://doi.org/10.1016/S1875-5364(13)60037-0.
9. Chakraborty, A.J.; Uddin, T.M.; Matin Zidan, B.M.R.; Mitra, S.; Das, R.; Nainu, F.; Dhama, K.; Roy, A.; Hossain, M.J.; Khusro, A.; Emran, T.B. Allium cepa: A treasure of bioactive phytochemicals with prospective health benefits. Evid. Based Complement. Alternat. Med. 2022, 2022, 4586318, https://doi.org/10.1155/2022/4586318.
10. Islam, M.R.; Rauf, A.; Akter, S.; Akter, H.; Al-Imran, M.I.K.; Fakir, M.N.H.; Thufa, G.K.; Islam, M.T.; Hemeg, H.A.; Abdulmonem, W.A.; Aljohani, A.S.M.; Iriti, M. Neuroprotective potential of curcumin in neurodegenerative diseases: Clinical insights into cellular and molecular signaling pathways. J. Biochem. Mol. Toxicol. 2025, 39, e70369, https://doi.org/10.1002/jbt.70369.
11. Kapoor, T.; Mehan, S.; Suri, M.; Sharma, N.; Kumar, N.; Narula, A.S.; Alshammari, A.; Alasmari, A.F.; Alharbi, M.; Assiri, M.A.; Kalfin, R. Forskolin, an adenylyl cyclase/cAMP/CREB signaling activator restoring myelin-associated oligodendrocyte destruction in experimental ethidium bromide model of multiple sclerosis. Cells 2022, 11, 2771, https://doi.org/10.3390/cells11182771.
12. Roy, A. Role of medicinal plants against Alzheimer's disease. Int. J. Complement. Alt. Med. 2018, 11, 205–208, https://doi.org/10.15406/ijcam.2018.11.00398.
13. Khanna, S.; Selvaraj, D.; Tyagi, M.; Devadharshini; Jayaram, S. An integrated network pharmacology and molecular modelling study of phytoconstituents targeting Alzheimer's disease. Medicine in Omics 2024, 12, 100031, https://doi.org/10.1016/j.meomic.2023.100031.
14. Luo, T.; Lu, Y.; Yan, S.; Xiao, X.; Rong, X.; Guo, J. Network pharmacology in research of Chinese medicine formula: Methodology, application and prospective. Chin. J. Integr. Med. 2020, 26, 72–80, https://doi.org/10.1007/s11655-019-3064-0.
15. Jiao, X.; Jin, X.; Ma, Y.; Yang, Y.; Li, J.; Liang, L.; Liu, R.; Li, Z. A comprehensive application: Molecular docking and network pharmacology for the prediction of bioactive constituents and elucidation of mechanisms of action in component-based Chinese medicine. Comput. Biol. Chem. 2021, 90, 107402, https://doi.org/10.1016/j.compbiolchem.2020.107402.
16. Obaidullah, A.J.; Alanazi, M.M.; Alsaif, N.A.; Alanazi, A.S.; Albassam, H.; Az, A.; Alwassil, O.I.; Alqahtani, A.M.; Tareq, A.M. Network pharmacology- and molecular docking-based identification of potential phytocompounds from Argyreia capitiformis in the treatment of inflammation. Evid. Based Complement. Alternat. Med. 2022, 2022, 8037488, https://doi.org/10.1155/2022/8037488.
17. Shannon, P.; Markiel, A.; Ozier, O.; Baliga, N.S.; Wang, J.T.; Ramage, D.; Amin, N.; Schwikowski, B.; Ideker, T. Cytoscape: A software environment for integrated models of biomolecular interaction networks. Genome Res. 2003, 13, 2498–2504, https://doi.org/10.1101/gr.1239303.
18. Shri, S.R.; Nayak, Y.; Ranganath Pai, S. Molecular docking studies and molecular dynamic simulation analysis: To identify novel ATP-competitive inhibition of glycogen synthase kinase-3β for Alzheimer's disease. F1000Research 2025, 13, 773, https://doi.org/10.12688/f1000research.

19. McCune, L.; Johns, T. Antioxidant activity in medicinal plants associated with the symptoms of diabetes mellitus used by the Indigenous Peoples of the North American boreal forest. J. Ethnopharmacol. 2002, 82, 197–205, https://doi.org/10.1016/S0378-8741(02)00180-0.
20. Ruch, R.J.; Cheng, S.J.; Klaunig, J.E. Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis 1989, 10, 1003–1008, https://doi.org/10.1093/carcin/10.6.1003.
21. Ameena, M.; Meignana, A.I.; Karthikeyan, R.; Rajeshkumar, S. Evaluation of the anti-inflammatory, antimicrobial, antioxidant, and cytotoxic effects of chitosan thiocolchicoside-lauric acid nanogel. Cureus 2023, 15, e46003, https://doi.org/10.7759/cureus.46003.
22. Long, H.Z.; Cheng, Y.; Zhou, Z.W.; Luo, H.Y.; Wen, D.D.; Gao, L.C. PI3K/AKT signal pathway: A target of natural products in the prevention and treatment of Alzheimer's disease and Parkinson's disease. Front. Pharmacol. 2021, 12, 648636, https://doi.org/10.3389/fphar.2021.648636.
23. Pan, J.; Yao, Q.; Wang, Y.; Chang, S.; Li, C.; Wu, Y.; Shen, J.; Yang, R. The role of PI3K signaling pathway in Alzheimer's disease. Front. Aging Neurosci. 2024, 16, 1459025, https://doi.org/10.3389/fnagi.2024.1459025.
24. Sheng, L.; Bayliss, G.; Zhuang, S. Epidermal growth factor receptor: A potential therapeutic target for diabetic kidney disease. Front. Pharmacol. 2021, 11, 598910, https://doi.org/10.3389/fphar.2020.598910.
25. Brinton, R.D. Estrogen-induced plasticity from cells to circuits: Predictions for cognitive function. Trends Pharmacol. Sci. 2009, 30, 212–222, https://doi.org/10.1016/j.tips.2008.12.006.
26. Nesci, S.; Lenaz, G. Impaired mitochondrial bioenergetics under pathological conditions. Life (Basel) 2022, 12, 205, https://doi.org/10.3390/life12020205.
27. Torner, L. Actions of prolactin in the brain: From physiological adaptations to stress and neurogenesis to psychopathology. Front. Endocrinol. (Lausanne) 2016, 7, 25, https://doi.org/10.3389/fendo.2016.00025.
28. Wang, Y.Y.; Huang, Z.T.; Yuan, M.H.; Jing, F.; Cai, R.L.; Zou, Q.; Pu, Y.S.; Wang, S.Y.; Chen, F.; Yi, W.M.; Zhang, H.J.; Cai, Z.Y. Role of hypoxia inducible factor-1α in Alzheimer's disease. J. Alzheimers Dis. 2021, 80, 949–961, https://doi.org/10.3233/JAD-201448.
29. Azzini, E.; Pena-Corona, S.I.; Hernández-Parra, H.; Chandran, D.; Saleena, L.A.K.; Sawikr, Y.; Peluso, I.; Dhumal, S.; Kumar, M.; Leyva-Gómez, G.; Martorell, M.; Sharifi-Rad, J.; Calina, D. Neuroprotective and anti-inflammatory effects of curcumin in Alzheimer's disease: Targeting neuroinflammation strategies. Phytother. Res. 2024, 38, 3169–3189, https://doi.org/10.1002/ptr.8200.
30. Costa, L.G.; Garrick, J.M.; Roquè, P.J.; Pellacani, C. Mechanisms of neuroprotection by quercetin: Counteracting oxidative stress and more. Oxid. Med. Cell. Longev. 2016, 2016, 2986796, https://doi.org/10.1155/2016/2986796.
31. Rong, H.; Liang, Y.; Niu, Y. Rosmarinic acid attenuates β-amyloid-induced oxidative stress via Akt/GSK-3β/Fyn-mediated Nrf2 activation in PC12 cells. Free Radic. Biol. Med. 2018, 120, 114–123, https://doi.org/10.1016/j.freeradbiomed.2018.03.028.
32. Wu, X.; Yang, Z.; Zou, J.; Gao, H.; Shao, Z.; Li, C.; Lei, P. Protein kinases in neurodegenerative diseases: Current understandings and implications for drug discovery. Signal Transduct. Target. Ther. 2025, 10, 146, https://doi.org/10.1038/s41392-025-02179-x