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In Vitro and In Silico Evaluation of the Therapeutic Potential of Ginseng and Its Principal Bioactive Constituents in Ischaemic Stroke

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Abstract

Ischemic stroke (IS) represents a major global health burden and remains a leading cause of disability and mortality, underscoring the urgent need for improved therapeutic strategies. Tianjiangxueshuantong (TJXST) pills are widely prescribed for cerebrovascular disorders and include ginseng as a key herbal constituent; however, the specific contribution and mechanistic role of ginseng in IS therapy have not been comprehensively clarified. The present study aims to systematically evaluate the protective effects of ginseng against IS and to elucidate its underlying molecular mechanisms.To investigate the neuroprotective potential of ginseng, oxygen–glucose deprivation/reperfusion (OGD/R) injury was induced in bEnd.3 endothelial cells, while glutamate-mediated neurotoxicity was established in PC12 cells. Cell viability following ginseng extract treatment was assessed using CCK8 assays. Subsequently, an integrated network pharmacology strategy combined with Gene Expression Omnibus (GEO) database analysis was applied to screen the principal bioactive constituents of ginseng, identify key therapeutic targets in IS, and uncover relevant signaling pathways. Molecular docking was performed to evaluate the binding affinity between core components and hub targets, and surface plasmon resonance (SPR) was employed to experimentally verify these interactions. Furthermore, quantitative real-time PCR (qRT-PCR) and Western blot (WB) analyses were conducted to assess alterations in gene and protein expression levels. SRC knockdown experiments were additionally carried out to determine its functional involvement in the neuroprotective actions of ginseng.The analysis revealed arachidonate, kaempferol, celabenzine, suchilactone, ginsenoside Re, and ginsenoside Rg1 as the principal active ingredients of ginseng. Core intervention targets associated with IS included PTPN11, JAK2, PIK3R1, EGFR, PIK3CA, PIK3CB, SRC, and PTK2. Molecular docking demonstrated stable binding interactions between the identified active components and hub targets. SPR assays further confirmed that the major ginseng constituents exhibited quantifiable binding affinity toward SRC. Both qRT-PCR and WB analyses indicated significant modulation of core target expression in the two cellular injury models. Importantly, ablation of SRC eliminated the protective effects of ginseng extract, highlighting SRC as a pivotal mediator of its neuroprotective activity. Additionally, CCK8 and ELISA assays confirmed enhanced cell survival and suppressed inflammatory responses, evidenced by reduced secretion of TNF-α (Tumor Necrosis Factor-alpha), IL-1β (Interleukin-1 beta), VEGF (Vascular Endothelial Growth Factor), and Ang-1 (Angiopoietin-1), following treatment with the identified active compounds.This study provides a systematic mechanistic insight into how ginseng exerts therapeutic effects in IS. The identification of critical bioactive constituents and their molecular targets offers a valuable foundation for the development of novel, multi-target therapeutic strategies for ischemic stroke.

Keywords: Ginseng, Ischemic stroke, Tianjianxueshuangtong pills, Molecular docking, OGD/R models

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Introduction

Ischemic stroke (IS) accounts for more than 80% of all stroke cases worldwide [1] and is characterized by extensive neuronal loss, inflammatory responses, and damage to neurovascular units [2-4]. These pathological changes often manifest clinically as motor dysfunction,

speech and sensory impairments, visual abnormalities, and cognitive decline, collectively leading to profound reductions in quality of life and functional independence [5-8]. Moreover, the elevated rates of incidence, recurrence, disability, and mortality associated with IS impose substantial socioeconomic burdens on patients, families, and healthcare systems [9-11]. Consequently, the development of more effective therapeutic interventions remains a critical priority.

Current IS treatment strategies primarily focus on re-establishing cerebral perfusion through pharmacological thrombolysis or mechanical thrombectomy [12, 13]. However, these interventions are constrained by several limitations. The narrow therapeutic time window following stroke onset (< 4.5 h) restricts patient eligibility and increases the risk of hemorrhagic complications and contraindications [14]. Additionally, therapies targeting single molecular pathways demonstrate limited clinical efficacy and have failed to substantially reduce IS-related morbidity and mortality [15]. Reperfusion itself may further aggravate neuronal injury by inducing oxidative stress and secondary damage [16]. These challenges emphasize the need for alternative therapeutic approaches that are multi-targeted and exhibit lower toxicity.

Panax ginseng is rich in diverse bioactive compounds and has long been utilized for the management of various disorders, including cardiovascular dysfunction, insomnia, hemorrhagic conditions, and metabolic diseases [17]. Accumulating evidence suggests that ginseng exerts protective effects in cerebrovascular pathologies, including ischemic stroke [18]. Tianjiangxueshuantong pills (TJXSTP), which incorporate ginseng as a principal component, are clinically used for IS, atherosclerosis, and hyperlipidemia [19]. Nevertheless, the precise role and mechanistic contribution of ginseng within IS treatment remain insufficiently investigated. In addition, formulations such as Ginseng Wutou Decoction have been reported to confer neuroprotection by reinforcing blood–brain barrier integrity during ischemia–reperfusion injury [20, 21]. Ginseng has also been shown to alleviate cognitive dysfunction and may play a preventive role in neurodegenerative diseases such as Parkinson's disease. Despite these encouraging findings in cardiovascular and cerebrovascular disorders [20], systematic evaluation of ginseng's therapeutic mechanisms in IS is still lacking.

In the present study, two established in vitro ischemic stroke models were employed to preliminarily assess the protective effects of ginseng against IS-related injury. Subsequently, a combined network pharmacology and GEO database approach was used to comprehensively identify the active components, molecular targets, and signaling pathways involved in ginseng-mediated intervention in IS. Finally, molecular docking and experimental validation were performed to confirm these findings **(Figure 1).** By integrating in vitro experiments with in silico analyses, this study provides a robust framework for investigating the pharmacological basis of ginseng. While cellular assays offer direct evidence of biological effects, computational approaches predict molecular interactions and target networks, together enabling a more comprehensive understanding of ginseng's therapeutic potential in ischemic stroke.

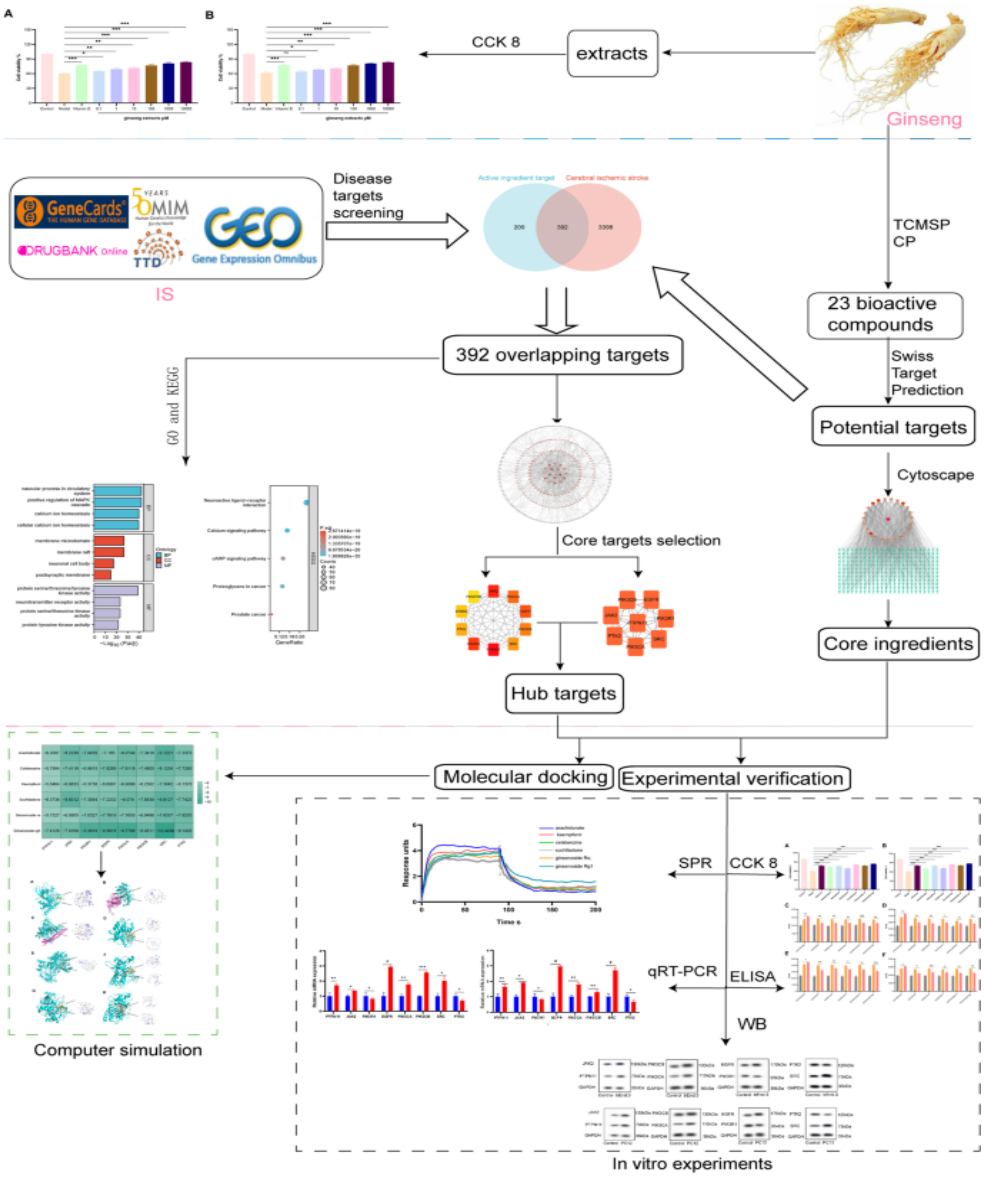


Figure 1. Schematic representation of the experimental and analytical framework of this study. Two cellular ischemic stroke models were employed, including oxygen–glucose deprivation/reperfusion (OGD/R) in bEnd.3 endothelial cells and glutamate-mediated excitotoxic injury in PC12 cells. The cytoprotective effects of ginseng extracts were evaluated using CCK-8 assays. Bioactive constituents of ginseng were screened through the TCMSP and Pharmacopoeia databases, and their putative targets were predicted using SwissTargetPrediction. Genes associated with ischemic stroke were collected from GEO (GSE16561), GeneCards, DisGeNET, DrugBank, and TTD databases. Overlapping targets between compounds and disease were used to construct a protein–protein interaction (PPI) network. Functional enrichment analyses, including Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG), were performed using Metascape to identify key biological functions and signaling pathways. Core compounds and hub targets were subsequently verified by molecular docking (AutoDock Vina) and surface plasmon resonance (SPR). Experimental validation involved quantitative real-time PCR (qRT-PCR) and Western blot analysis of target expression, SRC gene silencing, CCK-8-based cell viability assays, and ELISA quantification of inflammatory cytokines.

Materials and Methods

Establishment of the Oxygen–Glucose Deprivation/Reperfusion (OGD/R) model

Mouse brain microvascular endothelial cells (bEnd.3) obtained from the Shanghai Cell Bank were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10 percent fetal bovine serum and 1 percent penicillin. Cells were maintained under standard conditions at 37 °C in a humidified incubator containing 5% CO_2.

For induction of ischemia-reperfusion injury, bEnd.3 cells in the logarithmic growth phase were subjected to an OGD/R protocol. Briefly, cells were rinsed with PBS and incubated in glucose-free DMEM under hypoxic conditions (95% N_2, 5% CO_2, 1% O_2) for 2 h using a tri-gas incubator. After oxygen–glucose deprivation, normal culture medium was restored, and cells were returned to normoxic conditions (5% CO_2, 37 °C) for an additional 24 h to allow reperfusion. This in vitro model simulates vascular injury during ischemic stroke and is particularly relevant for investigating endothelial dysfunction and blood–brain barrier disruption, which contribute to secondary neuronal damage [3].

Construction of the glutamate-induced PC12 cell injury model

PC12 cells sourced from the Shanghai Cell Bank were cultured in RPMI-1640 medium supplemented with 10 percent fetal bovine serum and 1% penicillin at 37 °C in a 5% CO_2 atmosphere. To induce excitotoxic injury, cells were exposed to 10 mM glutamate, a concentration known to overstimulate glutamate receptors, resulting in excessive calcium influx and neuronal damage, a hallmark pathological feature of ischemic stroke [21].

Although PC12 cells originate from rat adrenal pheochromocytoma, they acquire neuron-like characteristics following nerve growth factor (NGF) stimulation, including neurite extension, neuronal marker expression, and electrophysiological properties. These features closely resemble central nervous system neurons exposed to excitotoxic stress, making PC12 cells a widely accepted in vitro model for studying glutamate-induced neuronal injury and evaluating neuroprotective interventions in ischemic stroke research.

Assessment of ginseng extracts on bend.3 cell viability after OGD/R

bEnd.3 cells were plated into 96-well plates at a density of 1×10^5 cells per well (100 µL/well), with three replicate wells assigned to each experimental condition. Following completion of the OGD/R procedure, cells were treated with either control medium or medium containing ginseng extracts for 24 h.

Cell viability was measured using the CCK-8 assay in accordance with the manufacturer's instructions. Briefly, 10 µL of CCK-8 reagent was added to each well, followed by incubation for two hours at 37 °C in a 5 percent CO_2 environment. Absorbance was recorded at 450 nm using a microplate reader. Each experiment was independently repeated three times to ensure reproducibility. Cell survival was calculated as:

Cell survival rate (%) = [(treatment group − normal group) / (model group − normal group)] × 100%.

All test compounds were dissolved in DMSO and diluted with culture medium to achieve a final DMSO concentration ≤ 0.1%; a corresponding 0.1 percent DMSO vehicle control was included in all assays.

Evaluation of ginseng extracts on glutamate-injured PC12 cell viability

PC12 cells were seeded into 96-well plates at a density of 1×10^5 cells per well (100 µL/well), with three replicates per group. After 24 h of attachment, cells were treated for an additional 24 h with RPMI-1640 medium containing 10 mM glutamate in the presence or absence of different concentrations of ginseng extracts. Vitamin E (10 µM) was used as a positive control.

Cell viability was subsequently determined using the CCK-8 assay following the same protocol described above.

Identification of core bioactive components of ginseng

The chemical constituents of ginseng were collected from the Traditional Chinese Medicine Systems Pharmacology (TCMSP) database (http://lsp.nwu.edu.cn/tcmsp.php) [22]. Candidate active compounds were screened based on oral bioavailability (OB ≥ 30 percent) and drug-likeness (DL ≥ 0.18). To ensure completeness, the screened compounds were supplemented using information from the Pharmacopoeia of the People's Republic of China.

Structural information for each active compound, including SMILES formats, was obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) [23]. Target prediction for the identified compounds was

performed using SwissTargetPrediction [20]. A comprehensive "Ginseng–Active Ingredient–Target" interaction network was constructed using Cytoscape 3.7.2 [24], and network topology analysis was carried out via the NetworkAnalyst plugin to identify the core bioactive constituents of ginseng.

Collection of Ischemic Stroke (IS)–related targets

Gene expression datasets associated with ischemic stroke were retrieved from the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/) by searching the keyword "IS." Among the available datasets, GSE16561 was selected for downstream analysis. Raw expression data were log-transformed, filtered, and normalized to identify microarray-based predictor genes relevant to IS pathogenesis.

In parallel, IS-associated genes were systematically collected from multiple disease- and drug-related databases, including GeneCards (https://www.genecards.org/), DisGeNET (https://www.disgenet.org/), DrugBank (https://www.drugbank.ca/), and the Therapeutic Target Database (TTD; http://db.idrblab.net/ttd/). All genes obtained from these sources were integrated, and duplicate entries were removed to generate a comprehensive list of IS-related targets.

The ginseng extract powder used in this investigation was a United States Pharmacopeia (USP)–certified standard (product number: USP-1291708; specification: 1.5 g; manufacturer: USP). The lyophilized powder complied with USP quality standards. For preparation, 1.5 g of the extract was dissolved in sterile distilled water and adjusted to a final volume of 100 mL to obtain a 1.5% (w/v) stock solution, which was stored at 4 °C in sealed containers. Given the complex composition of the extract, its concentration was normalized to a theoretical molar equivalent using an estimated average molecular weight of approximately 800 g/mol to enable comparison with single-compound treatments.

Identification of hub targets

The overlapping genes between predicted targets of ginseng constituents and IS-associated genes were defined as potential therapeutic targets and visualized using Venn diagram analysis. These intersecting genes were subsequently imported into the STRING database (https://cn.string-db.org/) to construct a protein–protein interaction (PPI) network, applying a high-confidence interaction threshold (confidence score > 0.9) [25].

Key targets within the PPI network were identified based on network topology analysis, which reflects their regulatory importance in signaling pathways. Degree centrality was employed to identify nodes with extensive direct interactions, suggesting essential functional roles in IS-related biological processes. Betweenness centrality was used to detect nodes acting as critical connectors or bottlenecks that regulate information flow across the network. While additional measures such as closeness and eigenvector centrality can provide complementary insights, degree and betweenness centrality were selected due to their interpretability and frequent application in similar studies.

To further refine hub target identification, Maximum Clique Centrality (MCC) analysis was conducted, as higher MCC values indicate stronger connectivity and greater biological relevance [26]. Additionally, the MCODE plugin was applied to detect densely connected sub-networks within the PPI structure. Network visualization, optimization, and hub target screening were performed using Cytoscape software, integrating MCC analysis via the CytoHubba plugin and cluster detection via MCODE.

Functional enrichment analysis based on GO and KEGG

To investigate the biological significance and molecular mechanisms underlying the identified targets, Gene Ontology (GO) functional annotation and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were conducted using the Metascape platform (www.metascape.org). These analyses were applied to the differentially expressed and intersecting genes to determine their involvement in biological processes, molecular functions, cellular components, and signaling pathways relevant to IS. Enrichment results meeting the criteria of a minimum overlap of ≥ 3 genes and a statistical significance threshold of $P \leq 0.01$ were considered meaningful.

Molecular docking between core compounds and hub targets

Three-dimensional structures of the hub proteins PTPN11, JAK2, PIK3R1, EGFR, PIK3CA, SRC, and PTK2 were obtained from the Protein Data Bank (PDB), while the structure of PIK3CB was retrieved from the AlphaFold database [27]. Structural data for the corresponding small-molecule ligands were downloaded from the PubChem database.

Protein structures were preprocessed using AutoDock Tools, including hydrogen atom addition, charge calculation, and format conversion to pdbqt files. Ligand molecules underwent identical preprocessing procedures. Binding pockets were predicted using Autosite software, and a docking grid box measuring 40 × 40 × 40 Å³ was centered on the predicted binding site. Docking conformations were clustered using the maximum neighborhood clustering algorithm, with an RMSD threshold of < 0.5 Å.

To enhance the reliability of docking predictions, each protein–ligand pair was docked independently three times using AutoDock Vina. The most favorable docking poses from the top three clusters were selected for subsequent interaction and binding analysis.

Quantitative Real-Time PCR (qRT-PCR)

Alterations in the expression levels of core genes were quantified using qRT-PCR. Total RNA was extracted using Trizol reagent and reverse-transcribed into cDNA according to the instructions provided in the PrimeScript RT kit (TaKaRa, Japan). Quantitative PCR amplification was then performed using the SYBR Premix Ex Taq Kit (TaKaRa, Japan) following the manufacturer's protocol. Specific primer sequences used for amplification are listed in **Table 1.**

Table 1. Quantitative real-time PCR primer information

Target Gene	Full Gene Description	Forward (5′–3′) Primer Sequence	Reverse (5′–3′) Primer Sequence
PTPN11	Protein Tyrosine Phosphatase, Non-Receptor Type 11	5′-AGCTGGAGGCTGACAGAAAC-3′	5′-GGGTTGTAGGACCTGCTGCT-3′
JAK2	Janus Kinase 2	5′-GGGAGCCCATCAGTTCATTC-3′	5′-GTAGGTCAGGTCAGGTGGGA-3′
PIK3R1	Phosphoinositide 3-Kinase Regulatory Subunit 1	5′-CAGAGGGAGGAGGAGGAGAA-3′	5′-TGGAGGGAGGAGGAAGGAAG-3′
EGFR	Epidermal Growth Factor Receptor	5′-CTTCCAGGGAGGAGGAGAAA-3′	5′-CTGGGAGGAGGAGGAAGGAG-3′
PIK3CA	Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha	5′-GAGGAGGAGGAGGAGGAGGA-3′	5′-CTAGGGTAGGAGGAGGAGGA-3′
PIK3CB	Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Beta	5′-GAGGAGGAGGAGGAGGAGGA-3′	5′-TCTGGTAGGAGGAGGAGGAG-3′
SRC	Proto-Oncogene Tyrosine-Protein Kinase Src	5′-TCCAGGTGCTGAGGAGGAGA-3′	5′-AGAGGGAGGAGGAGGAGGAG-3′
PTK2	Protein Tyrosine Kinase 2	5′-GAGGAGGAGGAGGAGGAGGA-3′	5′-TCTGGAGGAGGAGGAGGAGG-3′
GAPDH	Glyceraldehyde-3-Phosphate Dehydrogenase (Internal Control)	5′-TGCACCACCAACTGCTTAGC-3′	5′-GGCATGGACTGTGGTCATGAG-3′

Western blot analysis

Following the designated treatments, cells were harvested and lysed using RIPA buffer supplemented with protease and phosphatase inhibitors. Cell lysates were clarified by centrifugation at 12,000 × g for 15 min at 4 °C, and the resulting supernatants were collected for protein analysis. Total protein concentrations were quantified using a bicinchoninic acid (BCA) assay.

Equivalent amounts of protein (30–50 μg per sample) were resolved by 10% SDS–polyacrylamide gel electrophoresis and subsequently transferred onto polyvinylidene fluoride (PVDF) membranes. The membranes were blocked with 5% skim milk prepared in TBST (Tris-buffered saline containing 0.1% Tween-20) for 1 h at room temperature to minimize nonspecific binding.

Membranes were then incubated overnight at 4 °C with primary antibodies directed against the selected core target proteins. After thorough washing, membranes were exposed to horseradish peroxidase (HRP)-linked secondary antibodies for 1 h at room temperature. Protein bands were detected using an enhanced chemiluminescence (ECL) system and captured with a digital imaging platform. Densitometric analysis was performed using ImageJ software, and all experiments were independently repeated three times to ensure data reliability.

SRC gene silencing

bEnd.3 and PC12 cells in the exponential growth phase were subjected to SRC knockdown using validated SRC-targeting small interfering RNA (siRNA). Transfections were carried out with Lipofectamine 3000 (Invitrogen) according to the manufacturer's protocol, while a scrambled siRNA sequence served as a negative control. Cells were seeded into appropriate culture vessels and transfected upon reaching approximately 60–70% confluence with SRC siRNA at a final concentration of 50 nM. Following transfection, cells were incubated for 48 h to allow sufficient suppression of SRC expression. Subsequently, SRC-silenced cells were exposed to either the OGD/R injury protocol or the glutamate-induced neuronal injury model, as previously established.

Evaluation of SRC–compound interactions by Surface Plasmon Resonance (SPR)

Surface plasmon resonance assays were conducted at 25 °C using a Biacore T200 system (GE Healthcare). PBS-P running buffer supplemented with 1% DMSO was filtered through a 0.22 μm membrane and degassed prior to use. Recombinant human SRC protein (UniProt ID: P12931; amino acids 2–536) was obtained from Abcam (catalog no. ab79635; expressed in Sf9 cells; N-terminal His-tag; purity >85%). Prior to immobilization, the protein was buffer-exchanged into 10 mM sodium acetate (pH 4.5).

SRC protein was diluted to 20 μg/mL in sodium acetate buffer (pH 4.5) and immobilized onto a CM5 sensor chip via standard amine coupling chemistry using an immobilization kit (BR-1000-50, GE Healthcare), following the manufacturer's instructions. Approximately 6500 response units (RU) of SRC were captured on the chip surface. Reference flow cells without protein served as background controls, and competitive binding assays were initially performed to verify the specificity of core compound–SRC interactions. Residual active sites were quenched with 1 M ethanolamine (pH 8.5).

Core compounds were prepared in PBS-P buffer containing 1% DMSO and injected at five concentrations (0.1, 1, 10, 50, and 100 μM) at a flow rate of 30 μL/min. Association was monitored for 120 s, followed by a 180 s dissociation phase. After each binding cycle, the sensor surface was regenerated using 10 mM glycine–HCl (pH 2.5) for 30 s at the same flow rate. Sensorgrams were double-referenced and analyzed using a 1:1 Langmuir binding model.

Quantification of inflammatory mediator secretion by ELISA

Core bioactive compounds of ginseng identified through network pharmacology were applied to cells following OGD/R induction and drug treatment protocols described earlier. The secretion of inflammatory mediators was subsequently quantified using enzyme-linked immunosorbent assay (ELISA).

At the end of the incubation period, culture plates were collected and centrifuged at 300 × g for 5 min. Supernatants were carefully harvested and transferred into EP tubes. Samples were either analyzed immediately or stored at −80 °C to prevent degradation due to repeated freeze–thaw cycles.

The concentrations of VEGF and Ang-1 in cell culture supernatants were determined using corresponding ELISA kits according to the manufacturers' instructions. In PC12 cells subjected to glutamate-induced injury, inflammatory responses were primarily assessed by measuring IL-1β and TNF-α levels. All procedures for cell modeling, compound treatment, and ELISA measurements followed the standardized protocols described above.

Statistical analysis

All statistical analyses were performed using two-tailed tests. Differences were considered statistically significant when the p-value was less than 0.05.

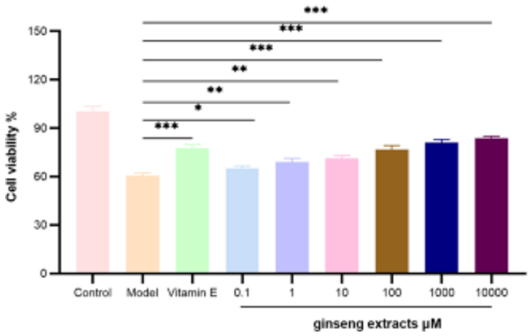
Results and Discussion

Ginseng extracts promote cell survival in in vitro ischemic stroke models

To evaluate the protective potential of ginseng in ischemic stroke, bEnd.3 cells exposed to OGD/R injury were treated with increasing concentrations of ginseng

extract, and cell viability was assessed using the CCK-8 assay. The results demonstrated a concentration-dependent enhancement in endothelial cell survival following ginseng treatment **(Figure 2a).**

Similarly, PC12 cells subjected to glutamate-induced excitotoxic injury were treated with graded concentrations of ginseng extract. CCK-8 analysis revealed a marked, dose-dependent increase in PC12 cell viability, indicating a protective effect of ginseng against neuronal injury **(Figure 2b).**



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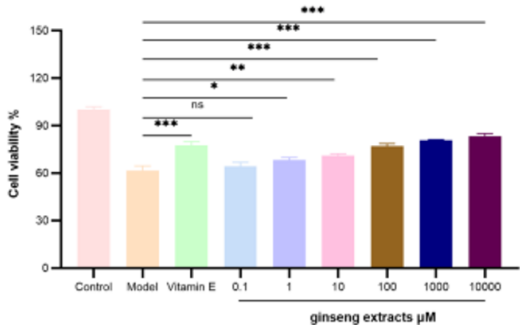


b)

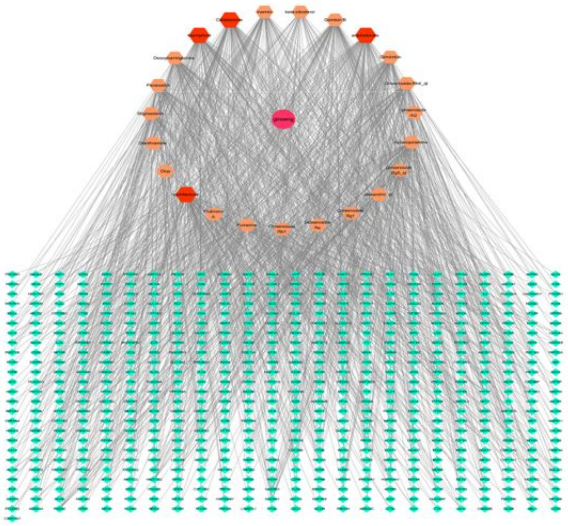
Figure 2. Effects of ginseng extract on cellular viability in in vitro ischemic stroke models. (a) bEnd.3 endothelial cells subjected to oxygen–glucose deprivation/reperfusion (OGD/R). (b) PC12 cells exposed to glutamate-induced excitotoxic injury. Cells were treated with ginseng extract for 48 h. All experiments were conducted in triplicate or more. Statistical differences between groups were evaluated based on p-values (*p < 0.05; **p < 0.01; ***p < 0.001).

Identification of core bioactive constituents of ginseng

To determine the principal bioactive constituents responsible for the pharmacological effects of ginseng, active compounds were first retrieved from the Traditional Chinese Medicine Systems Pharmacology (TCMSP) database and further supplemented using the Pharmacopoeia of the People's Republic of China, yielding a total of 23 candidate components. The putative molecular targets of these compounds were subsequently predicted using the SwissTargetPrediction online platform, resulting in the identification of 601 potential targets.

The compound–target interaction data were then imported into Cytoscape software to generate a comprehensive drug–active ingredient–target interaction network consisting of 625 nodes and 1399 edges **(Figure 3a)**. Network topology analysis was performed to screen for key bioactive constituents, revealing arachidonate, kaempferol, celabenzine, and suchilactone as the most influential components within the network **(Figure 3b).** The chemical structures of these four compounds are illustrated in **Figures 3c–3f.**

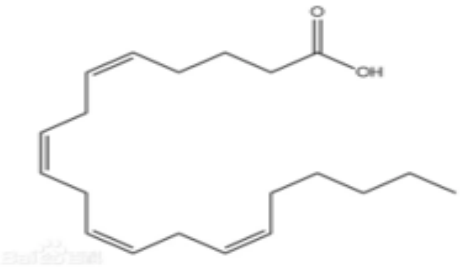
In addition, ginsenosides are widely regarded as the primary material basis underlying the biological activity of ginseng. Accordingly, ginsenoside Re and ginsenoside Rg1 were also designated as core active ingredients and included in subsequent analyses.



a)

Compound name	Degree score	Betweenness Centrality	Compound class
Arachidonate	101	0.17277296	Long-chain fatty acid
Kaempferol	101	0.15270673	Tetrahydroxyflavone
Celabenzine	101	0.14185075	Cyclic spermidine alkaloid
Suchilactone	101	0.13864861	Flax Lignans

b)



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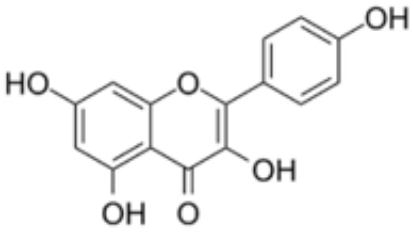

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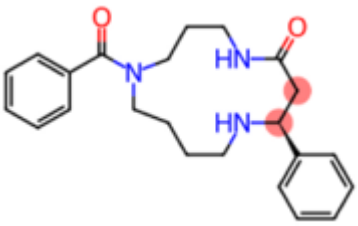

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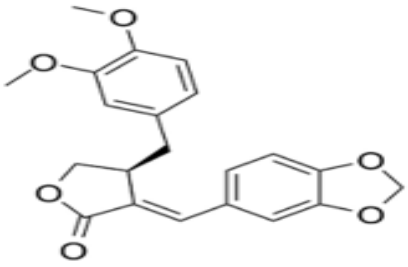
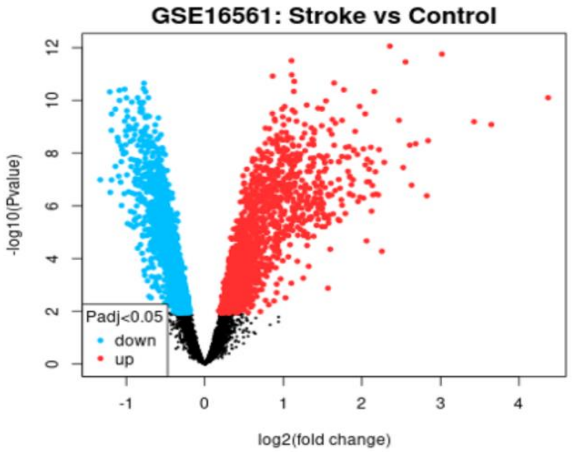


f)

Figure 3. Screening key ginseng components. (a) A network illustrating the interplay between ginseng, its bioactive compounds, and corresponding targets, where circles denote ginseng, square hexagons indicate active compounds, and diamonds represent potential targets; connecting lines reflect interactions among them. (b) Analysis using Cytoscape to evaluate core ginseng constituents, highlighting each node's Degree score and Betweenness Centrality, which guided the selection of principal active compounds. (c–f) Molecular structures of the primary ginseng components: (c) Arachidonate, (d) Kaempferol, (e) Celabenzine, and (f) Suchilactone.

Compilation of IS-Associated targets

To gather targets relevant to ischemic stroke (IS), the transcriptomic dataset GSE130823 was examined, identifying 296 differentially expressed genes, including 24 downregulated and 272 upregulated **(Figure 4a).** Additional targets were retrieved from databases—GeneCards (3493), TTD (17), OMIM (43), and DrugBank (22). After removing redundancies, a comprehensive set of 3790 targets was established as IS-associated targets **(Figure 4b).**



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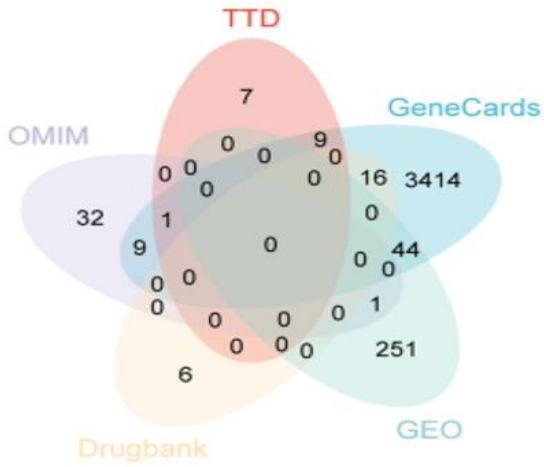


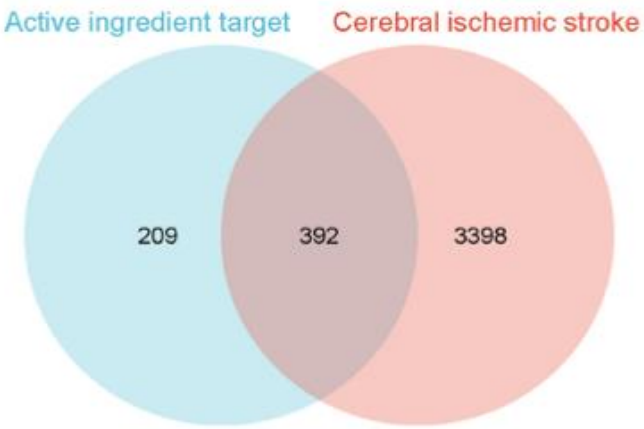
b)

Figure 4. Construction of IS-associated gene sets. (a) Differential gene expression in GSE16561, containing peripheral blood samples from thirty nine ischemic stroke patients and twenty four healthy controls, is visualized in a volcano plot; genes upregulated in stroke are shown in red, and

downregulated genes in blue (adjusted $P < 0.05$, $|\log_2 FC| \geq 1$). (b) A five-set Venn diagram demonstrates the overlap of stroke-related genes collected from TTD, OMIM, DrugBank, GEO (GSE16561), and GeneCards, with the central intersection of 392 genes selected for downstream PPI network analysis.

Screening hub targets for ginseng intervention in IS

To systematically identify IS-related targets, transcriptomic data from GSE16561 were analyzed, revealing 296 genes with differential expression, including 272 upregulated and 24 downregulated **(Figure 5a)**. These overlapping targets were used to construct a PPI network through STRING, which was further refined in Cytoscape. Functional analysis of genes shared between ginseng targets and IS-associated targets was performed using GO and KEGG enrichment based on the PPI network **(Figure 5b).** Hub nodes were then prioritized using the CytoHubba plugin with the MCC algorithm, identifying the top ten key genes: PTPN11, JAK2, PIK3R1, EGFR, PIK3CA, PIK3CB, SRC, PTK2, ERBB2, and PDGFRB **(Figure 5c).** Module-level network analysis with the MCODE plugin highlighted critical nodes in the PPI network, including PTPN11, JAK2, PIK3R1, EGFR, PIK3CA, PIK3CB, SRC, and PTK2 **(Figure 5d)**. By intersecting the MCC-prioritized nodes with MCODE-derived functional modules, eight central hub targets were ultimately identified: PTPN11, JAK2, PIK3R1, EGFR, PIK3CA, PIK3CB, SRC, and PTK2 **(Table 2).**



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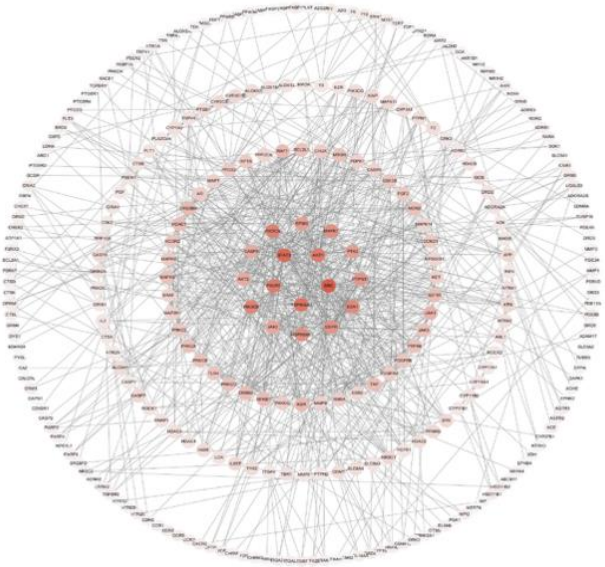

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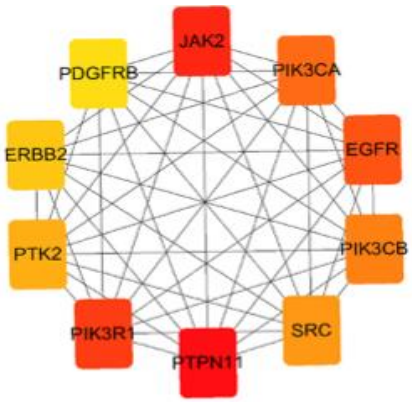
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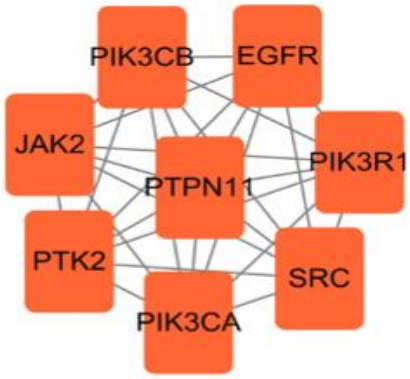
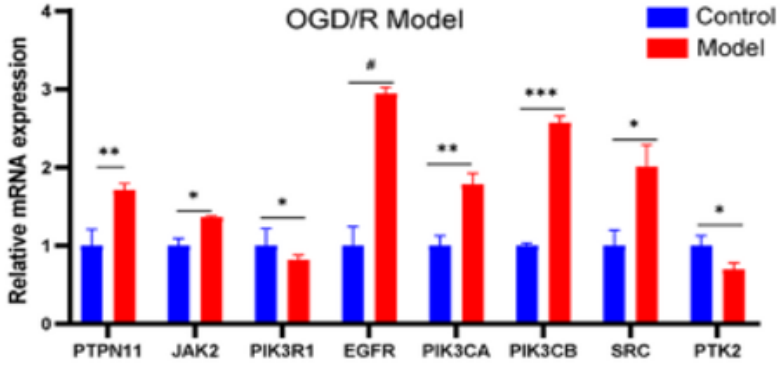
PDGFRB
PIK3CA
ERBB2
EGFR
PTK2
PIK3CB
PIK3R1
SRC
PTPN11

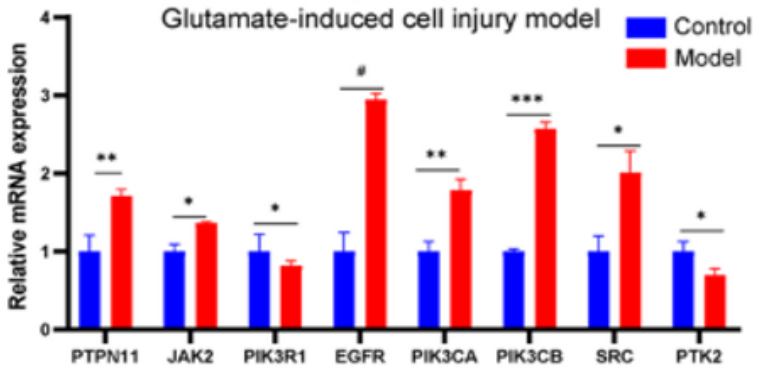
c)


PIK3CB
EGFR
JAK2
PIK3R1
PTPN11
PTK2
SRC
PIK3CA

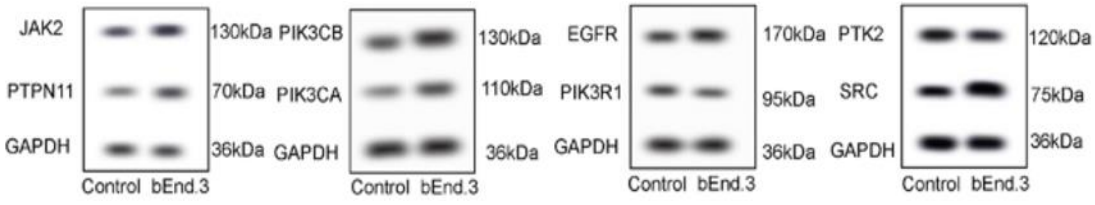
d)


OGD/R Model
Control
Model
Relative mRNA expression
PTPN11
JAK2
PIK3R1
EGFR
PIK3CA
PIK3CB
SRC
PTK2

e)


Glutamate-induced cell injury model
Control
Model
Relative mRNA expression
PTPN11
JAK2
PIK3R1
EGFR
PIK3CA
PIK3CB
SRC
PTK2

f)


JAK2
130kDa
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130kDa
EGFR
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PTK2
120kDa
PTPN11
70kDa
PIK3CA
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PIK3R1
95kDa
SRC
75kDa
GAPDH
36kDa
GAPDH
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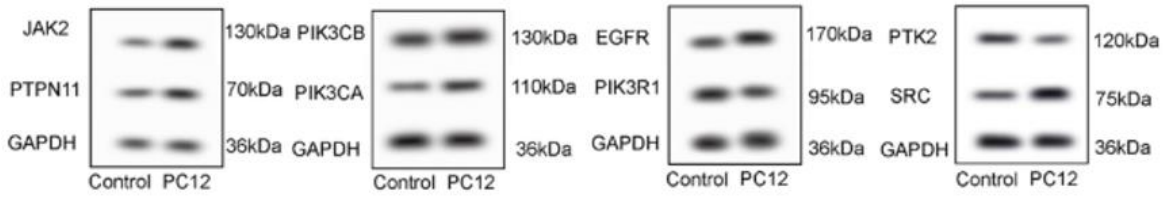
g)



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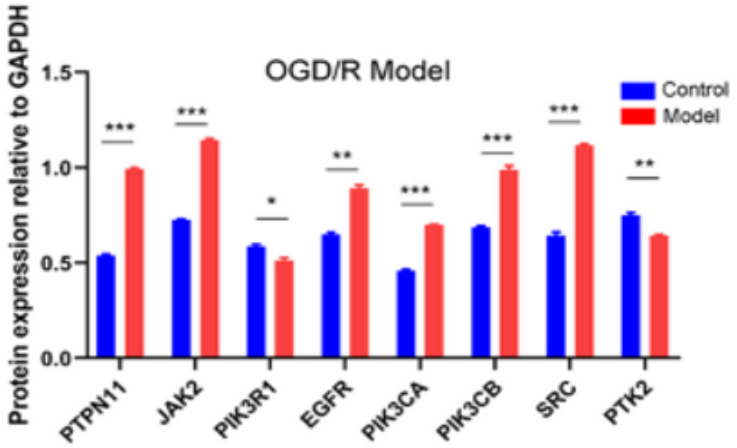

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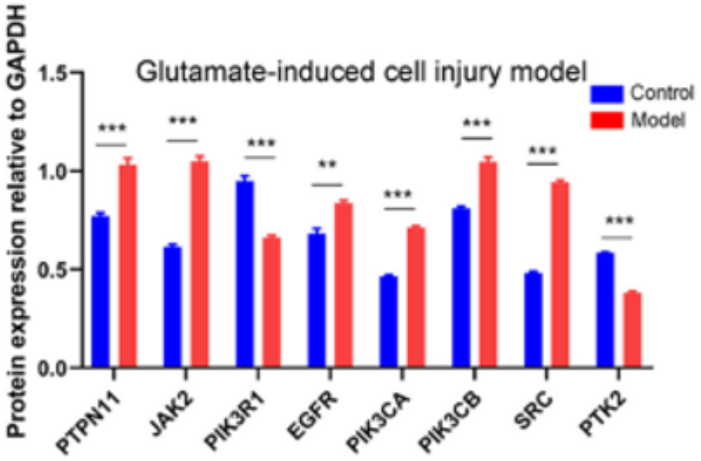


j)

Figure 5. Identification of ginseng compounds targeting IS Hub genes. (a) Venn diagram showing the overlap between potential targets of ginseng bioactive compounds and ischemic stroke-associated genes. (b) Protein-protein interaction (PPI) network of the shared targets. (c) Hub genes within the PPI network identified using the CytoHubba plugin. (d) Key network modules detected through MCODE analysis of the PPI network. (e–f) Evaluation of mRNA expression changes of core targets under experimental injury conditions: (e) oxygen-glucose deprivation/reperfusion (OGD/R) models and (f) glutamate-induced injury models. (g–h) Assessment of core target protein expression in injury models: (g) OGD/R and (h) glutamate-induced injury. (i–j) Quantitative analysis of core target protein levels: (i) OGD/R and (j) glutamate-induced injury models. In all experiments, the blue group represents controls, and the red group represents model conditions. Each experiment was repeated at least three times. Statistical significance is indicated as *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$, denoting significant differences between groups.

Table 2. Hub targets of ginseng regulation in ischemic stroke (IS)

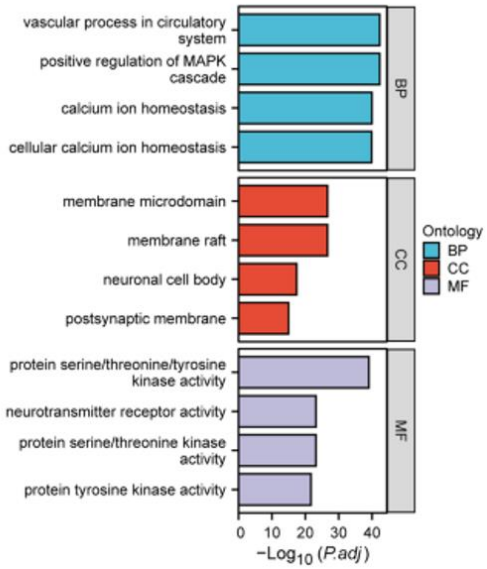
Target	MCODE score	MCC Score
PTPN11	10.03296703	1,057,631
JAK2	10.03296703	1,056,865
PIK3R1	10.03296703	1,052,918
EGFR	10.03296703	996,429
PIK3CA	9.230769231	961,388
PIK3CB	9.230769231	960,666
SRC	9.230769231	866,251
PTK2	9.230769231	852,644

Alterations in mRNA expression of hub genes in OGD/R and glutamate-induced injury models

To validate the network pharmacology predictions, the mRNA expression of core hub genes was examined using qRT-PCR. Significant changes were observed in both OGD/R and glutamate-induced injury cell models **(Figures 5e and 5f).** Protein expression levels were further confirmed through Western blot analysis **(Figure 5g–5j)**. Notably, PTPN11, JAK2, EGFR, PIK3CA, PIK3CB, and SRC exhibited marked upregulation, whereas PIK3R1 and PTK2 showed decreased expression.

Functional enrichment of PPI network genes

To investigate the biological roles of genes within the PPI network, GO and KEGG enrichment analyses were conducted. GO analysis indicated that these genes were involved in biological processes such as vascular processes in the circulatory system, positive regulation of the MAPK cascade, and calcium ion homeostasis at both the cellular and overall levels. Enriched cellular components included membrane microdomains, membrane rafts, neuronal cell bodies, and postsynaptic membranes. Key molecular functions encompassed protein serine/threonine/tyrosine kinase activity, neurotransmitter receptor activity, and protein kinase activities specific to serine/threonine and tyrosine residues **(Figure 6a).**



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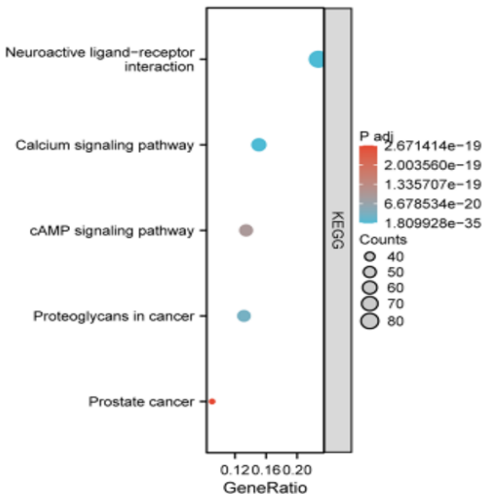


b)

Figure 6. GO and KEGG Enrichment Analyses of Intersecting Targets. (a) Gene Ontology (GO) enrichment of ginseng-associated IS targets, showing the top terms across three categories: biological process (BP, teal), cellular component (CC, red), and molecular function (MF, purple), represented as –log10 adjusted P values. Terms were considered significant at adjusted $P \leq 0.01$ with a minimum overlap of three genes. (b) KEGG pathway analysis highlighting the top five signaling pathways influenced by ginseng in IS, ranked by gene ratio. Dot size reflects the number of overlapping genes,

while color corresponds to adjusted P values (scale provided).

KEGG enrichment results indicate that ginseng's active compounds likely modulate IS through pathways including neuroactive ligand–receptor interaction, calcium signaling, cAMP signaling, proteoglycans in cancer, and prostate cancer **(Figure 6b).**

Molecular docking of core compounds with hub targets

To explore interactions between the eight hub targets (PTPN11, JAK2, PIK3R1, EGFR, PIK3CA, PIK3CB, SRC, PTK2) and ginseng's core compounds, molecular docking analyses were performed. The results demonstrated that most hub targets displayed moderate (Vina score > −5 kcal·mol⁻¹) or strong (Vina score > −7 kcal·mol⁻¹) predicted binding affinities with the compounds **(Figure 7)**. In general, a Vina score below −5 kcal·mol⁻¹ indicates a favorable binding interaction. Remarkably, ginsenoside Rg1 showed a Vina score of −10.44 kcal·mol⁻¹ with SRC, surpassing many established SRC inhibitors, suggesting that it may effectively modulate SRC activity in the context of ischemic stroke.

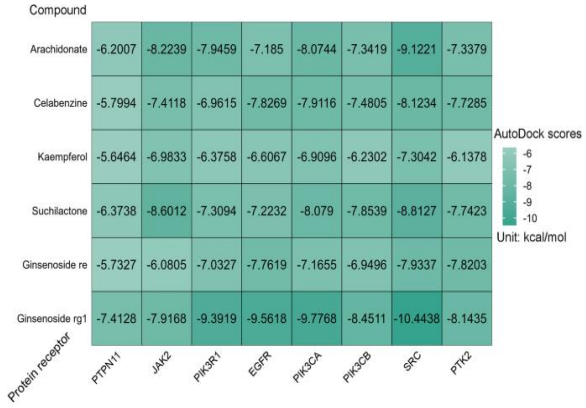
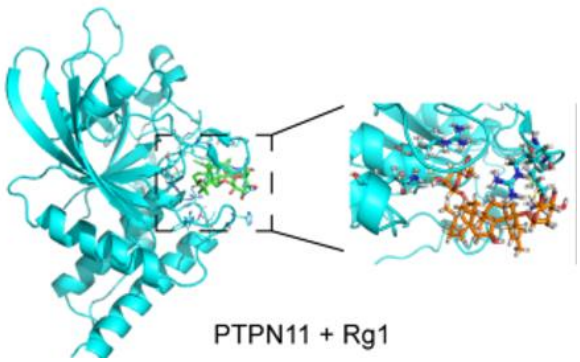


Figure 7. Binding affinity analysis of ginseng core compounds with hub targets. A heatmap summarizes the average AutoDock binding energies (kcal·mol⁻¹) obtained from three separate docking simulations for each compound–target pair. Darker green shades represent stronger predicted interactions, corresponding to more negative binding energies.

High-affinity complexes were further examined using PyMOL 2.5.5 to explore the molecular interactions. Visualization revealed that the compounds engage with key residues at the active sites through a combination of hydrogen bonding, π–π stacking, and van der Waals interactions, highlighting the structural basis for their potential regulatory effects on the targets **(Figures 8a–8h).**



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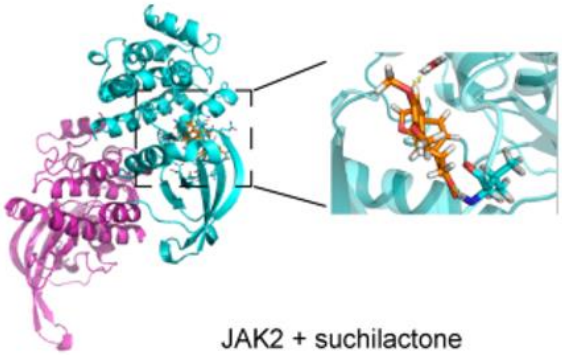

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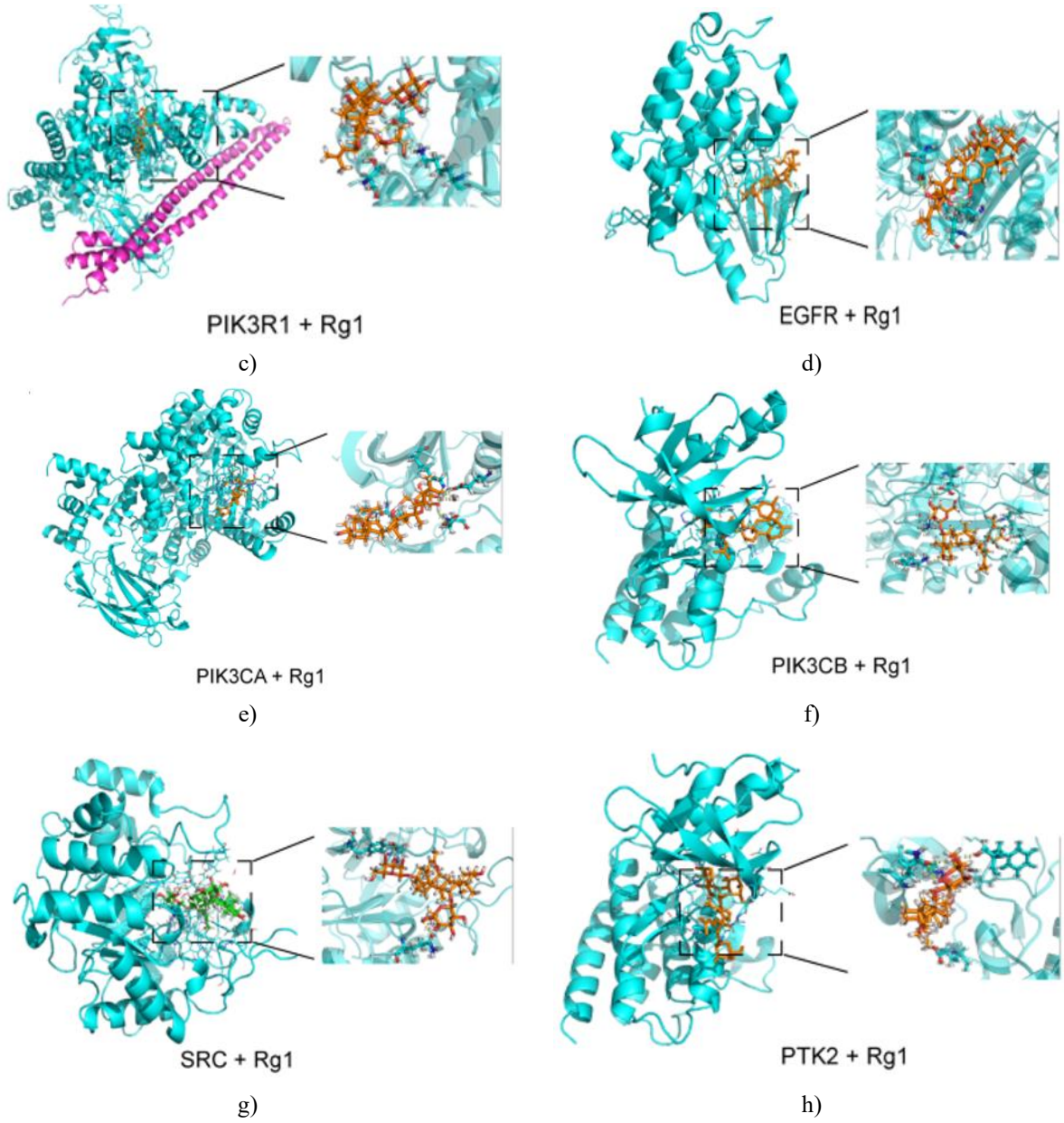
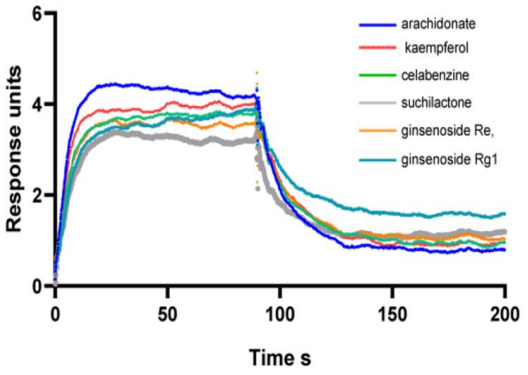


Figure 8. A molecular docking overview depicts how ginseng's core bioactive compounds interact with hub target proteins. (a) PTPN11 bound to ginsenoside Rg1. (b) JAK2 interacting with suchilactone, with the JH1 kinase domain highlighted in green and the JH2 pseudokinase domain in cyan. (c) PIK3R1 complexed with ginsenoside Rg1, showing the N-SH2 domain in dark blue and the C-SH2 domain in light blue. (d–h) Interactions of EGFR, PIK3CA, PIK3CB, SRC, and PTK2 with ginsenoside Rg1 are illustrated. Proteins are represented by colored ribbons, ligands by stick models, and in-panel labels indicate each protein-ligand pair.

Validation of SRC binding via SPR

Molecular docking suggested a strong affinity between SRC and ginseng's core compounds. To experimentally confirm this interaction, surface plasmon resonance (SPR) assays were performed, demonstrating that all core active ingredients of ginseng effectively bind to SRC **(Figure 9c).**



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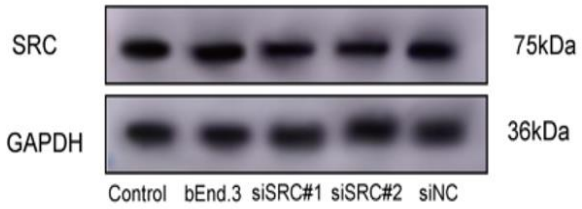

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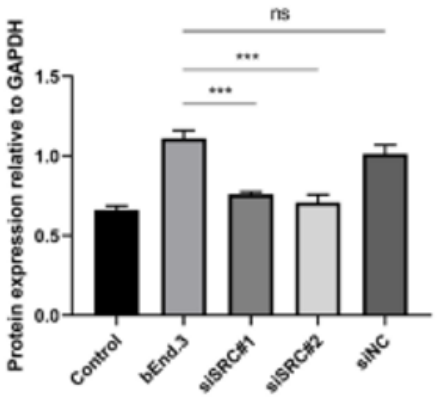

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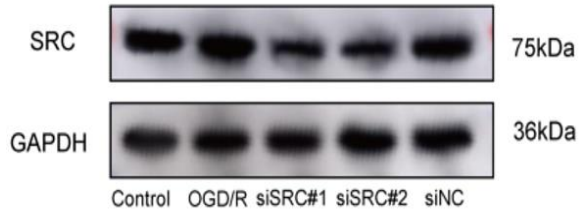

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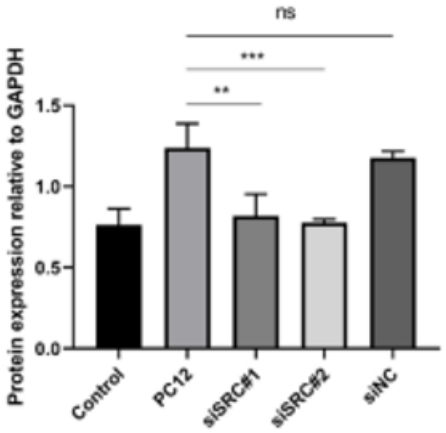

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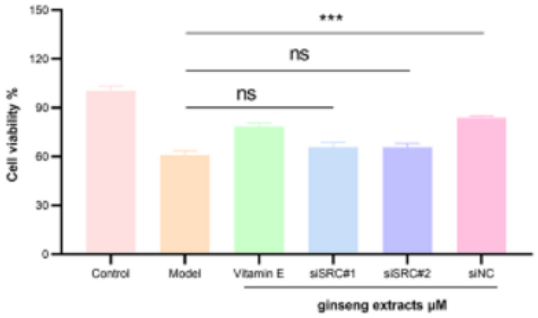

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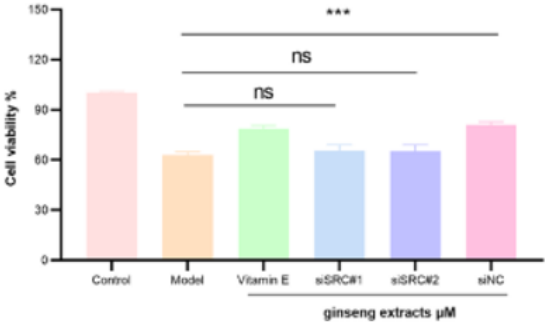


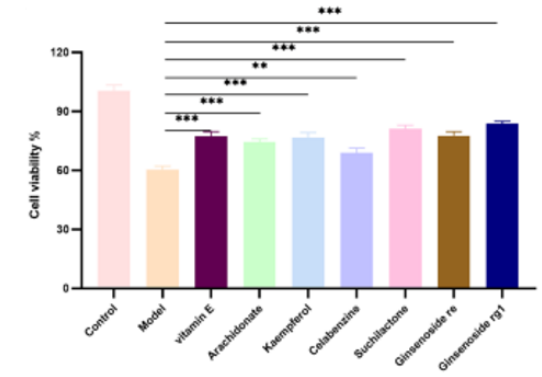
g)

Figure 9. Experimental confirmation of network pharmacology findings. Surface plasmon resonance (SPR) assays verified that SRC interacts with the core active compounds of ginseng, with each compound represented by a unique color (a). Protein expression was then evaluated in both OGD/R (b, c) and glutamate-induced injury models (d, e) following SRC knockdown, with quantitative analyses confirming the changes. Additionally, cell viability under SRC knockdown conditions was assessed using the CCK8 assay after 48 hours of treatment

with 10,000 μM ginseng extract in both OGD/R (f) and glutamate-induced injury models (g). All experiments were repeated at least three times, and statistical significance is indicated as *p < 0.05, **p < 0.01, ***p < 0.001.

Therapeutic effects of ginseng core compounds in IS cell models

The protective effects of the core ginseng ingredients were tested in ischemic stroke-relevant cell models. In OGD/R-treated bEnd.3 cells, treatment with arachidonate, kaempferol, celabenzine, suchilactone, ginsenoside Re, and ginsenoside Rg1 significantly enhanced cell survival relative to untreated controls **(Figure 10a)**. Parallel experiments in glutamate-challenged PC12 cells showed that the same compounds markedly improved cell viability compared with the model group, confirming their cytoprotective potential in multiple IS cell models **(Figure 10b).**



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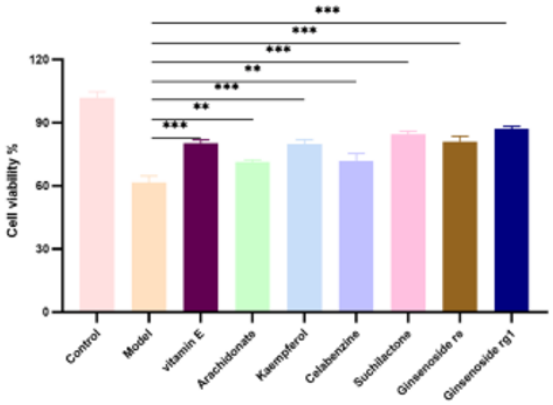

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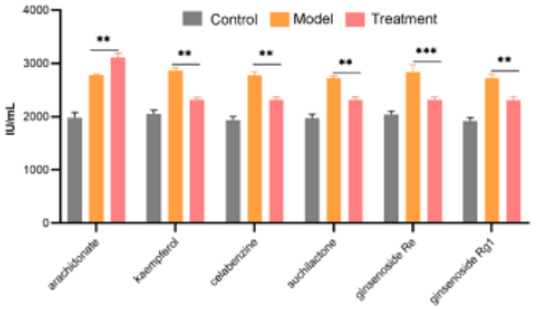

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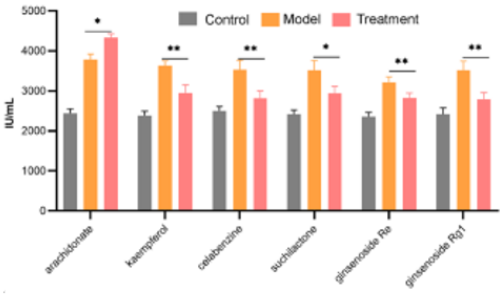

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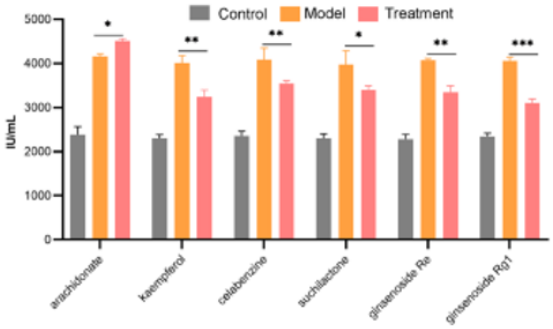

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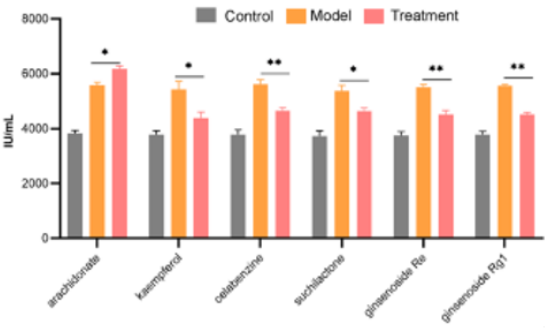

f)

Figure 10. Mechanistic insights into ginseng core compounds in IS. (a–b) The core active ingredients of ginseng enhanced cell viability in ischemic stroke-

relevant models: (a) bEnd.3 cells subjected to OGD/R and (b) glutamate-challenged PC12 cells. (c–f) Effects of ginseng compounds on inflammatory factor release were measured via ELISA. In bEnd.3 cells, VEGF (c) and Ang-1 (d) levels increased with arachidonate but decreased upon treatment with other core compounds. Similarly, in PC12 cells, TNF-α (e) and IL-1β (f) secretion was elevated by arachidonate but suppressed by the remaining active ingredients. All compounds were applied at 10 μM, experiments were repeated three times, and significance is indicated by *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Anti-inflammatory effects of core ginseng ingredients

The regulatory pathways influenced by ginseng in ischemic stroke were closely linked to inflammatory responses. ELISA assays confirmed that most core ginseng compounds effectively reduced the release of pro-inflammatory factors in both OGD/R-treated bEnd.3 cells and glutamate-induced PC12 cells, while arachidonate uniquely increased inflammatory factor release. These results suggest that ginseng's neuroprotective effects involve modulation of cellular inflammation.

Effective interventions are essential for ischemic stroke (IS), a major cerebrovascular disorder [28]. Although ginseng is widely employed in traditional Chinese medicine for cardiovascular and cerebrovascular health, its precise mechanisms in IS remain incompletely understood [29–31]. In this study, we combined network pharmacology, molecular docking, and experimental validation to demonstrate that ginseng acts on IS by targeting core proteins (PTPN11, JAK2, PIK3R1, EGFR, PIK3CA, PIK3CB, SRC, PTK2) with its active compounds (arachidonate, kaempferol, celabenzine, suchilactone, ginsenoside Re, ginsenoside Rg1). These interactions modulate key signaling pathways, including Neuroactive ligand–receptor interaction, Calcium signaling, and cAMP signaling.

We employed two complementary cell models—OGD/R-treated bEnd.3 cells and glutamate-injured PC12 cells—to generate robust and mutually validating experimental data. The observed improvements in cell viability across both models indicate the therapeutic potential of ginseng's active ingredients in mitigating ischemic injury.

Screening and identifying the core bioactive compounds of traditional Chinese medicines enhances research focus and efficiency. In this study, ginsenoside Re, ginsenoside Rg1, arachidonate, kaempferol, celabenzine, and suchilactone were highlighted as key constituents. While ginsenoside Re and Rg1 have been extensively studied for IS [32–36] and kaempferol is known to exert neuroprotective effects via anti-apoptotic, anti-inflammatory, and iron-mediated mechanisms [37–41], the therapeutic roles of arachidonate, celabenzine, and suchilactone in IS remain largely unexplored. Collectively, these findings suggest that ginseng's efficacy in IS arises from the synergistic activity of multiple bioactive compounds, each contributing to its anti-ischemic stroke properties.

The findings of this study indicate that PTPN11, JAK2, PIK3R1, EGFR, PIK3CA, PIK3CB, SRC, and PTK2 may act as central targets through which ginseng exerts therapeutic effects in ischemic stroke (IS). Gene Ontology analysis suggests that ginseng's bioactive compounds can modulate vascular functions in the circulatory system, enhance MAPK cascade signaling, and maintain both cellular and systemic calcium ion homeostasis, collectively contributing to its neuroprotective activity. KEGG pathway enrichment further revealed that 392 targets are significantly involved in multiple pathways, including neuroactive ligand–receptor interactions, calcium signaling, cAMP signaling, proteoglycans in cancer, and prostate cancer, highlighting ginseng's capacity to influence diverse biological processes simultaneously. Notably, the neuroactive ligand–receptor interaction pathway, which is critical in early neural development, has been linked to neuronal death regulation and may influence conditions such as hypertension and IS [42, 43]. Although several of these pathways are also affected by other neuroprotective herbs, ginseng's multi-component composition may allow for synergistic modulation across vascular, apoptotic, and inflammatory pathways, potentially producing a stronger neuroprotective effect than single-target herbs. Further comparative studies are warranted to clarify whether such synergistic effects are unique to ginseng or represent a general feature of neuroprotective herbal therapies.

Molecular docking analysis indicated that ginseng's core bioactive compounds bind strongly to key targets, with SRC showing the highest predicted affinity for ginsenoside Rg1 (Vina score −10.44 kcal/mol). Previous studies have shown that activation of the SRC signaling pathway promotes angiogenesis and supports neural repair [44–46]. Since inflammation plays a central role in IS pathology, the pathways modulated by ginseng appear

to influence inflammatory processes that are essential for both vascular and neuronal recovery. Ginseng may contribute to maintaining blood–brain barrier integrity by regulating factors such as VEGF and Ang-1, which control vascular permeability and stability. Additionally, modulation of pro-inflammatory cytokines like TNF-α and IL-1β suggests that ginseng can reduce harmful inflammatory responses after ischemic injury. bEnd.3 cells were employed as a model of brain microvascular endothelial cells, where VEGF and Ang-1 levels serve as indicators of vascular integrity, while PC12 cells modeled neuronal responses, with TNF-α and IL-1β reflecting excitotoxicity and inflammatory stress under ischemic conditions.

The signaling pathways enriched in this study predominantly govern apoptosis, cell proliferation, and inflammatory responses. Accordingly, we examined the effects of ginseng's core compounds on cell viability and cytokine release in these two IS cell models. Among all compounds tested, ginsenoside Rg1 demonstrated the strongest protective effect, consistent with its high predicted SRC binding affinity. Nevertheless, further functional studies are required to establish causality. Collectively, our network pharmacology and experimental data suggest that the therapeutic action of ginseng in IS may largely arise from interactions between its core compounds—especially ginsenoside Rg1—and key targets, affecting major pathways related to cell survival, apoptosis, and inflammation [47].

Previous reports indicate that ginsenoside Rg1 is present in standardized ginseng extracts at 0.5–2.0 mg/g, while ginsenoside Re and kaempferol are found at lower concentrations (0.3–1.5 mg/g) [48]. Considering our in vitro experiments using 0.5% w/v extracts, ginsenoside Rg1 likely represents the primary contributor to ginseng's therapeutic effects. SRC activation promotes angiogenesis, stabilizes the blood–brain barrier, and reduces neuronal apoptosis, thereby facilitating neurovascular repair in IS. Consistently, SRC knockdown experiments abolished the protective effects of ginseng extracts, highlighting SRC's essential role in mediating these benefits. Interestingly, arachidonic acid increased inflammatory factor release, which appears contrary to ginseng's overall anti-inflammatory action. As a known pro-inflammatory mediator, arachidonic acid can activate intracellular signaling cascades leading to cytokine production [49, 50]; however, its low abundance in the extract likely results in only a minor pro-inflammatory effect, which is offset by the broader anti-inflammatory and neuroprotective actions of the other ginseng components. Further studies are warranted to explore dose-dependent effects, in vivo regulatory mechanisms, and interactions among multiple active compounds. Additionally, computational docking predictions, while indicative of strong binding, require experimental confirmation through enzyme activity assays, mutagenesis, or co-crystallography to validate true inhibitory effects.

While our study primarily employed in vitro models and computational simulations to investigate the neuroprotective potential of ginseng and its principal bioactive compounds, existing literature supports the relevance of these findings in vivo and potentially in clinical contexts. Evidence indicates that ginsenoside Rg1 can improve neurological outcomes following ischemic stroke by promoting angiogenesis, reducing neuronal apoptosis, and reinforcing blood–brain barrier integrity [47, 51]. Additionally, studies in rodent stroke models have shown that administration of ginseng extracts reduces infarct volume and enhances functional recovery [52, 53]. These observations align with our experimental results and suggest that the identified core constituents, particularly ginsenoside Rg1, may have translational significance. Nevertheless, despite these promising in vivo outcomes, rigorous clinical trials are needed to fully establish both the efficacy and safety of ginseng for ischemic stroke therapy. Future research should utilize established in vivo IS models, such as the MCAO rat model, to substantiate our findings and extend their translational relevance.

This work represents the first integrative analysis combining network pharmacology, molecular docking, and experimental validation to clarify the therapeutic mechanisms of ginseng in ischemic stroke. Our results demonstrate that ginseng's neuroprotective effects arise from the combined action of multiple compounds, targets, and pathways. Importantly, SRC knockdown experiments confirmed the essential role of SRC in mediating these effects; inhibition of SRC eliminated the protective benefits of the extract, highlighting ginsenoside Rg1 as a key modulator of SRC activity. While our findings provide mechanistic insights and identify promising therapeutic targets and compounds for IS, the study is limited to in vitro and computational approaches. Additional in vivo studies and clinical investigations are required to confirm and translate these therapeutic effects into practical applications.

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