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Mechanistic Study of Guan's Liushangyin for Fall Injury via Network Pharmacology and Molecular Docking

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ABSTRACT

Introduction: The study aimed to investigate the potential molecular mechanisms of Guan's Liushangyin, a classical prescription from Guan's Commonly Used Prescriptions, in the treatment of fall-related injuries and to provide mechanistic evidence supporting its therapeutic relevance.

Methods: Active compounds and their putative targets of Guan's Liushangyin (Liu Jinu, Rhizoma Drynariae, and Rhizoma Corydalis) were retrieved from the TCMSP database. Fall injury–related targets were collected from GeneCards, PharmGKB, OMIM, TTD, and DrugBank. Protein–protein interaction (PPI) networks were constructed using the STRING database and visualized with Cytoscape. Key targets were identified through topological analysis using NetworkAnalyzer and core modules were extracted via the MCODE plugin. Gene Ontology (GO) functional annotation and KEGG pathway enrichment analyses were performed using R software. Molecular docking was conducted to evaluate binding affinities between representative active compounds and core targets using Chem3D, AutoDock 1.5.6, SYBYL 2.0, and PyMOL 2.4.

Results: A total of 66 active compounds were identified, with β-sitosterol, luteolin, quercetin, corydine, curcumenol, and dehydrocorydalis bulbus alkaloids emerging as key constituents. Core therapeutic targets included PTGS2, CASP3, VEGFA, JUN, MAPK1, AKT1, and TP53. Molecular docking analysis demonstrated favorable binding interactions between β-sitosterol and CASP3/PTGS2; luteolin and quercetin with CASP3, JUN, and TP53; Corydalis-related alkaloids with PTGS2; and curcumenol and dehydrocorydalis bulbus alkaloids with MAPK1. GO enrichment analysis indicated that the therapeutic effects of Guan's Liushangyin are mainly associated with biological processes related to cell cycle regulation, inflammatory response, apoptosis, and signal transduction. KEGG pathway analysis highlighted the PI3K–Akt, TNF, IL-17, and MAPK signaling pathways as key pathways involved in its anti-injury effects.

Conclusions: Guan's Liushangyin may exert therapeutic effects on fall injuries through a multi-component, multi-target, and multi-pathway regulatory mechanism, particularly involving PTGS2, CASP3, VEGFA, JUN, MAPK1, AKT1, and TP53, and pathways related to inflammation, apoptosis, and tissue repair. These findings provide mechanistic support for its traditional use and offer a theoretical basis for future experimental validation and clinical investigation.

Keywords: Traumatic injury; Guan's prescription; Liushangyin; Mechanism; Molecular docking; Network pharmacology

Introduction

In modern medicine, traumatic injuries are generally classified as soft tissue injuries caused by external forces such as falls, sprains, contusions, or sports-related trauma. These injuries often result in damage to muscles and bones and, in severe cases, may involve injury to internal organs [1]. Clinically, traumatic injuries are characterized by symptoms including swelling, pain, hemorrhage, and ecchymosis at the affected site [2]. Conventional Western medical management primarily relies on non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids to alleviate inflammation and pain. However, long-term or repeated use of these agents is associated with adverse effects, limiting their suitability for prolonged treatment [3].

From the perspective of traditional Chinese medicine (TCM), traumatic injuries disrupt the normal flow of Qi and blood within the meridians, leading to stagnation in the skin and subcutaneous tissues and resulting in pain and swelling due to obstruction. Consequently, TCM commonly employs herbal compound formulations that promote blood circulation, remove blood stasis, reduce swelling, and relieve pain in the treatment of traumatic injuries [4].

The Guan acupuncture school originated during the Daoguang period of the Qing Dynasty and has developed over more than 150 years into an influential medical lineage in Yunnan Province and other regions of China. Although historically centered on acupuncture, the Guan school extends beyond this modality, particularly in the treatment of traumatic injuries, where acupuncture is frequently combined with herbal medicine. This integrative approach has contributed to the formation of a systematic diagnostic and therapeutic framework [5, 6]. In addition to its distinctive acupoint selection and needling techniques, the Guan school has accumulated a substantial body of empirical herbal prescriptions.

Guan's Trauma-Relief Decoction (Guan Shi Liu Shang Yin), formulated by the second-generation physician Guan Qingxin (1864–1939), is one of the most commonly used prescriptions for traumatic injuries within the Guan medical system. The formula consists of Liu Ji Nu (Artemisia anomala), Gu Sui Bu (Drynaria fortunei), and Yan Hu Suo (Corydalis yanhusuo). Clinically, this prescription, either alone or with modifications, has shown favorable therapeutic effects in the management of various traumatic injuries. However, to date, no systematic analysis has been conducted to elucidate its active compounds, molecular targets, and underlying mechanisms of action at the network or pathway level.

Network pharmacology provides an effective approach for exploring the multi-component, multi-target, and multi-pathway characteristics of traditional herbal formulations, while molecular docking offers structural insights into ligand–target interactions. Therefore, this study applies network pharmacology combined with molecular docking techniques to systematically investigate the active components, potential targets, and key signaling pathways of Guan's Trauma-Relief Decoction, based on Guan's Common Prescriptions and Medicine Guide. The findings aim to clarify its mechanistic basis and provide a scientific reference for further experimental validation and future clinical research.

Methods

First, screening of Active Ingredients and Targets for Guan's Trauma-Relief Decoction Using the criteria of oral bioavailability (OB) ≥30% and drug-likeness (DL) ≥0.18, the active ingredients and targets of Guan's Trauma-Relief Decoction (composed of Artemisia anomala, Drynaria fortune, and Corydalis yanhusuo) were screened through the Traditional Chinese Medicine System Pharmacology (TCMSP) platform (https://tcmspw.com/tcmsp.php) [7] and further validated using the PubChem database (https://pubchem.ncbi.nlm.nih.go v/). The molecular structures of the identified active compounds were retrieved and analyzed. The potential targets of these active ingredients were predicted using TCMSP and Swiss Target Prediction followed by standardization of the target names using the UniProt protein database (https://www.uniprot.org/) with R4.1.0 software.

Second, collection of Targets Related to Traumatic Injuries Using the search terms "pain," "wound," and "traumatic injury," targets related to traumatic injuries were collected from databases including GeneCards (https://www.genecards.org/), PharmGKB (https://www.pharmgkb.org/), DrugBank (https://www.drugbank.ca/),OMIM (https://omim.org/), and TTD (http://db.idrblab.net/ttd/). Using R4.1.0 software, the disease targets obtained from these databases were intersected with the effective compounds of Guan's Trauma-Relief Decoction, resulting in a set of common targets, which represent the action targets of Guan's Trauma-Relief Decoction in the treatment of traumatic injuries.

Third, construction of PPI Network for Guan's Trauma-Relief Decoction and Traumatic Injury Targets. The target names were standardized using Perl software, referencing the UniProt database for gene target name correction. Using the STRING database (https://string-db.org/cgi/input.pl) [8] with the species set to "Homo sapiens" and a minimum confidence threshold of 0.4, unconnected nodes were removed from the network. The results were then visualized using Cytoscape 3.7.2. The Network Analyzer plugin was used to calculate the connectivity values of each target, and the MCODE plugin was employed to filter and identify core modules within the PPI network of Guan's Trauma-Relief Decoction's active components.

Fourth, GO Analysis and KEGG Pathway Enrichment Analysis. GO enrichment analysis and KEGG pathway enrichment analysis were conducted using R4.1.0 software with the cluster Profiler, enrich plot, and org.Hs.eg.db packages. The filtering criteria were set at p-value<0.05, with a corrected q-value of 0.05. The most relevant pathways were visualized through graphical representations.

Fifth, Molecular Docking Validation. The three-dimensional structures of the core target proteins were retrieved from the UniProt database and further obtained from the RCSB Protein Data Bank (PDB), with selection criteria including Homo sapiens, X-ray diffraction method, and a resolution of less than 3.0 Å. The two-dimensional structures of the active compounds were downloaded from the PubChem database and subjected to energy minimization using Chem3D software.

Preliminary binding evaluation between the active compounds and target proteins was performed using SYBYL 2.0 (Surflex-Dock module), where ligand–target interactions were assessed based on the total score, a dimensionless scoring function. Active compounds with a total score greater than 3 were considered to exhibit favorable binding potential. Based on these results, the top two compounds for each core target were selected for further molecular docking analysis. Subsequently, detailed molecular docking simulations were conducted using AutoDock 1.5.6, and the binding affinities were calculated in units of kcal/mol. The docking conformations were visualized using PyMOL 2.4 to analyze ligand–target binding modes and interaction characteristics.

Results

Active Ingredients, Targets, and Disease Targets

The main components of Guan's Trauma-Relief Decoction (Artemisia anomala, Drynaria fortunei, and Corydalis yanhusuo) were screened and identified using the TCMSP database. The resulting compounds were 49 for Corydalis yanhusuo, 18 for Drynaria fortunei, and 5 for Artemisia anomala, as shown in Table 1. A total of 103 target entries were retrieved for Artemisia anomala, 303 for Drynaria fortunei, and 1096 for Corydalis yanhusuo. After de-duplication, 167 unique target entries were obtained. Using the search terms "pain," "wound," and "traumatic injury," disease-related targets were collected from databases including GeneCards (12423, 1954, 10618), TTD (198, 17, 0), OMIM (10, 200, 200), PharmGKB (135, 0, 0), and DrugBank (118, 10, 5). By removing target entries with Relevance scores below 1 and merging the results from these databases, a total of 4664 disease-related targets were obtained. Using R4.1.0 software, 114 common targets between the effective compounds of Guan's Trauma-Relief Decoction and traumatic injuries were identified, representing the therapeutic targets of Guan's Trauma-Relief Decoction in the treatment of traumatic injuries (Fig 1).

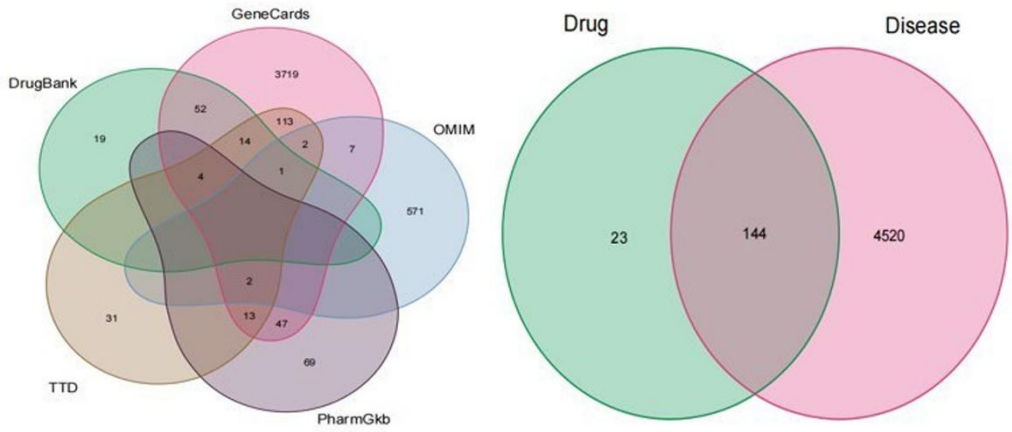


Figure 1 Venn Diagram of Guan's Trauma-Relief Decoction components and traumatic injury targets

Construction of the Guan's Trauma-Relief Decoction–Active Ingredients Traumatic Injury Network

The 66 active compounds from Artemisia anomala, Drynaria fortunei, and Corydalis yanhusuo, along with 206 traumatic injury-related targets, were imported into Cytoscape 3.7.2 to construct a network diagram (Fig 2). In this diagram, green nodes represent the therapeutic targets for traumatic injuries, while orange, red, and light blue nodes correspond to the active compounds of Artemisia anomala, Corydalis yanhusuo, and Drynaria fortunei, respectively. Edges denote the relationships between the active compounds of Guan's Trauma-Relief Decoction and disease-related targets. As illustrated in the network, a single target or intersecting gene is associated with multiple chemical compounds, and conversely, a single compound can correspond to multiple intersecting gene targets. Some chemical compounds are

unique to specific herbs, while others are shared among multiple herbs. This finding underscores the multi-component, multi-target, and multi-pathway therapeutic mechanism of Guan's Trauma-Relief Decoction in treating traumatic injuries.

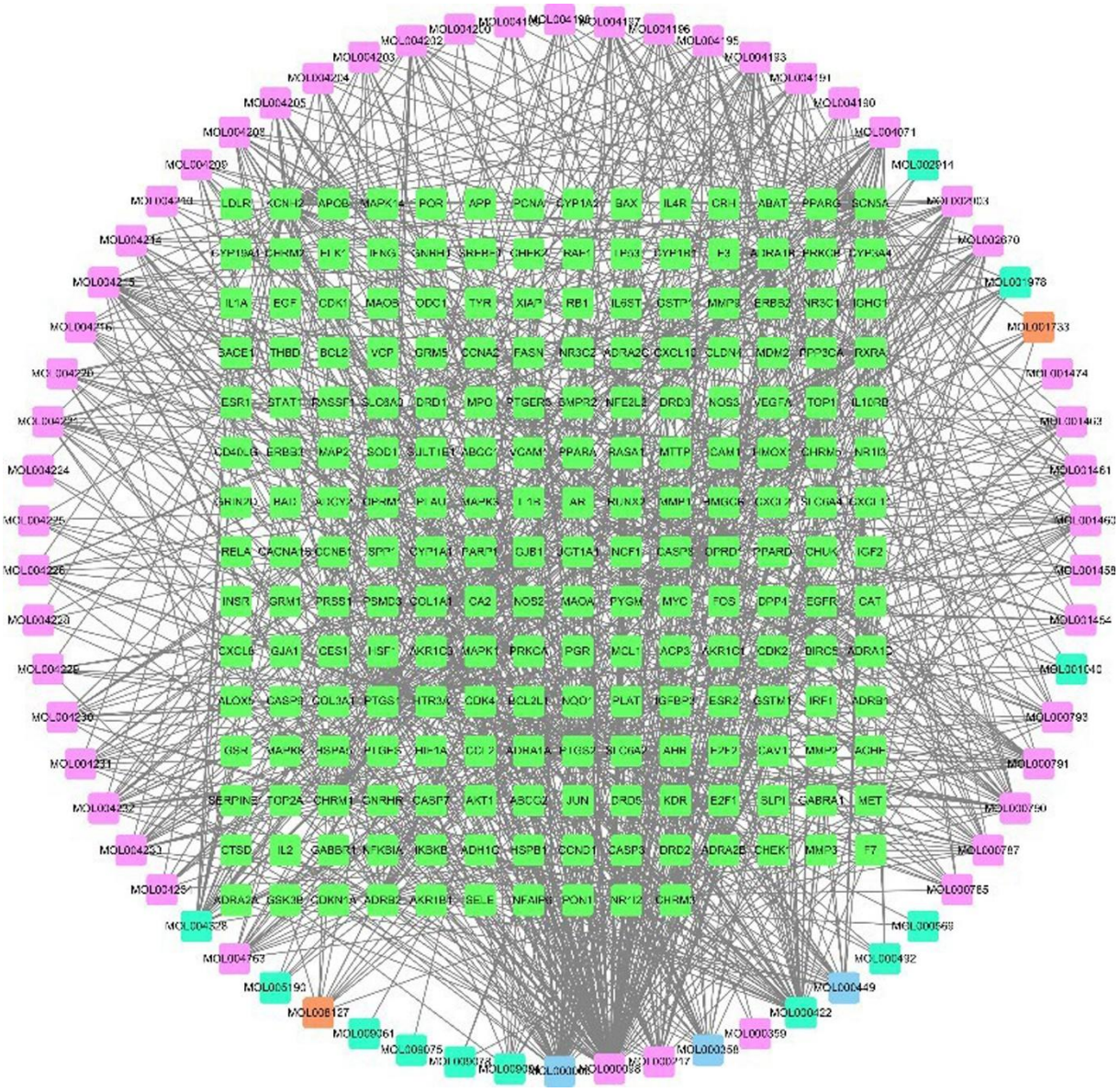


Figure 2 Network of Guan's Trauma-Relief Decoction—active ingredients—traumatic injury targets

Protein-Protein Interaction (PPI) Network of Overlapping Targets

The overlapping targets were analyzed using the STRING database to determine protein-protein interaction relationships. Cytoscape 3.7.2 software was employed to construct the PPI network of the active ingredient targets of Guan's Trauma-Relief Decoction. The resulting network consisted of 144 target nodes and 1,940 edges. The size and color of the nodes represent their degree of connectivity. Using the NetworkAnalyzer and MCODE plugins in Cytoscape 3.7.2, three rounds of screening were performed to identify the core targets. The core network was composed of 7 target nodes and 149 edges. The core genes identified were AKT1, CASP3, JUN, TP53, PTGS2, VEGFA, and MAPK1 (Table 1, Fig 3).

Table 1 Topological parameters of the core genes of the PPI network for Guanshi Liushangyin in the treatment of bruises

Core_genes	Target name	Connection	Score	Tightness
AKT1	RAC-alphaserine/threonine-proteinkinase	44	41.36508457	1
CASP3	Caspase-3	42	33.91115622	0.956521739
JUN	TranscriptionfactorAP-1	44	41.36508457	1
TP53	Cellulartumorantigenp53	44	41.36508457	1
PTGS2	ProstaglandinG/Hsynthase2	41	29.43144833	0.936170213
VEGFA	VascularendothelialgrowthfactorA	41	27.71747581	0.936170213
MAPK1	Mitogen-activatedproteinkinase1	41	31.14502615	0.936170213

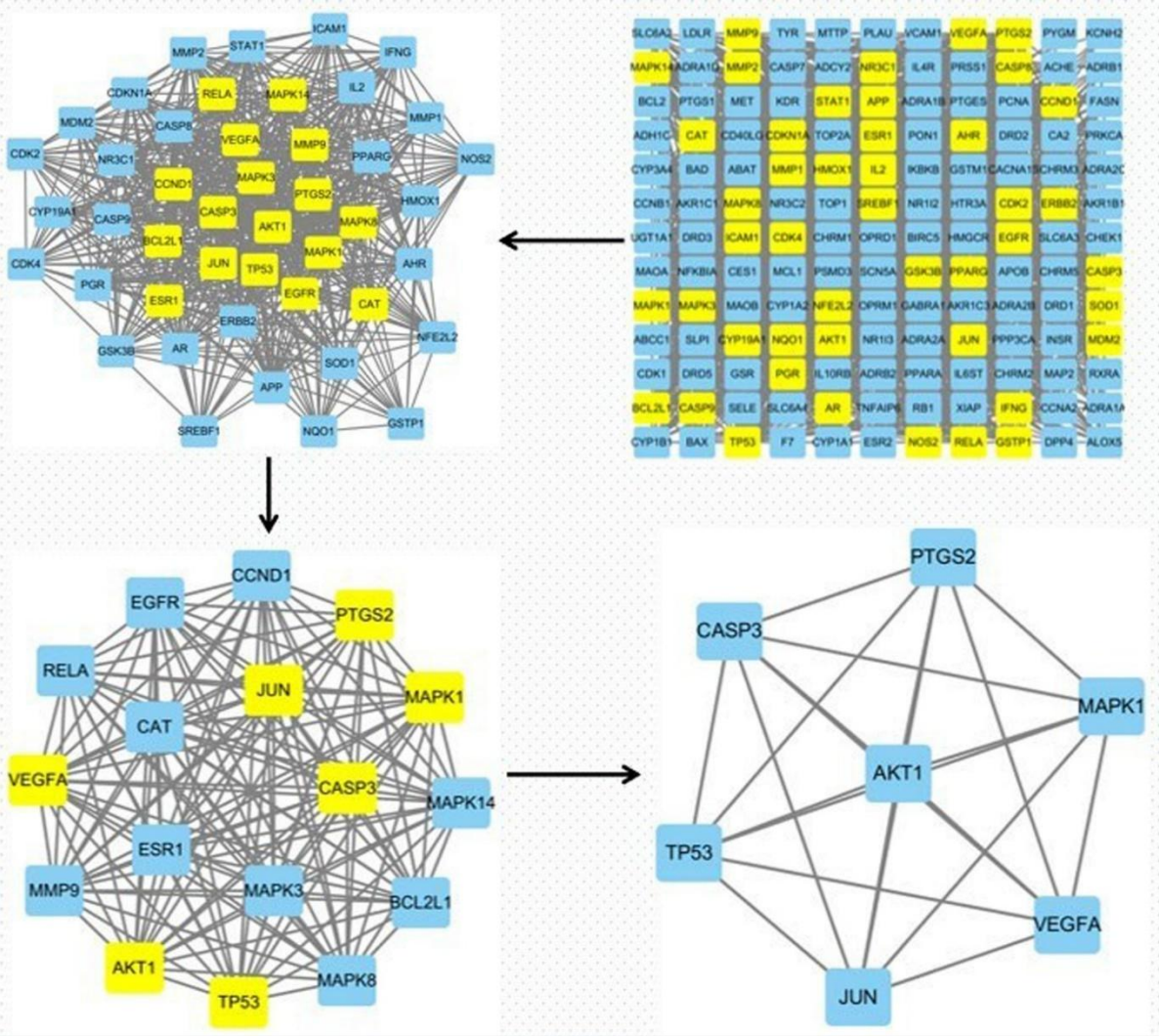


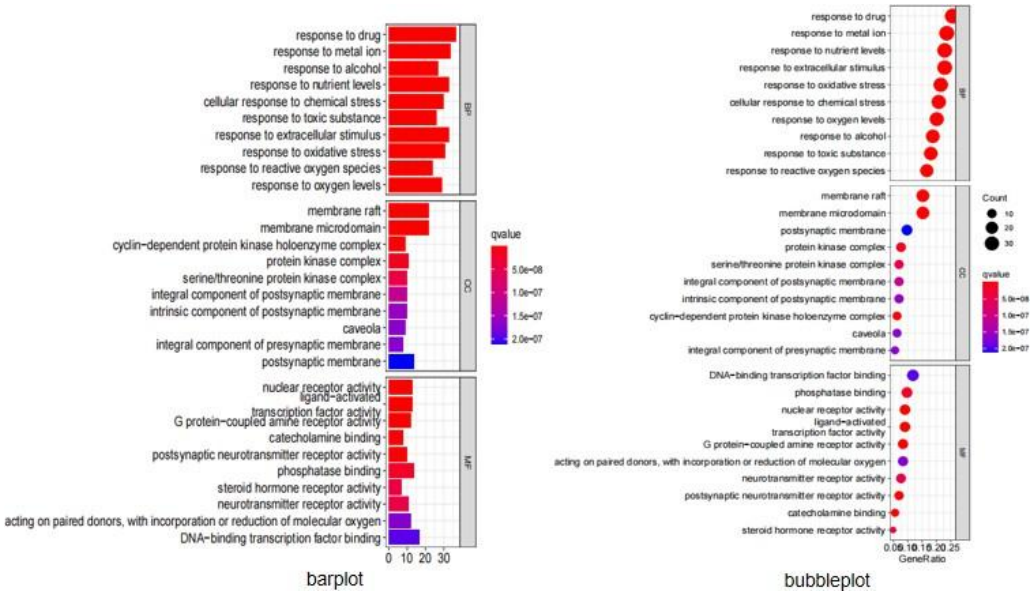
Figure 3 PPI network of overlapping targets between Guan's Trauma-Relief Decoction and traumatic injuries
Note: The size and color intensity of the circles represent the degree of connectivity (degree)

GO Biological Processes and Enrichment Analysis of Core Modules in the PPI

Network of Guan's Trauma-Relief Decoction for Traumatic Injury Targets. The overlapping targets of Guan's Trauma-Relief Decoction and traumatic injuries were subjected to Gene Ontology (GO) analysis with significance thresholds set at p-value < 0.05 and q = 0.05 as the adjusted cutoff. A total of 2,069 biological processes (BP), 97 cellular components (CC), and 174 molecular functions (MF) were identified, with the top 10 terms from each category visualized. The results indicated that the biological processes associated with the therapeutic effects of Guan's Trauma-Relief Decoction on traumatic injuries are predominantly enriched in pathways involving glycogen synthase kinase-3β

(GSK3β), cyclin-dependent kinase 1 (CDK1), caspase-3, heme oxygenase 1 (HO-1), G2/mitotic-specific cyclin-B1, and mitogen-activated protein kinase 14 (MAPK14) (Fig 4).

Figure 4 GO Analysis of potential targets of Guan's Trauma-Relief Decoction for traumatic injuries



KEGG Pathway Enrichment Analysis of Guan's Trauma-Relief Decoction for Traumatic Injuries

KEGG pathway enrichment analysis yielded a total of 170 pathways, with the top 20 pathways visualized. These include the PI3K-Akt signaling pathway, MAPK signaling pathway, tumor necrosis factor signaling pathway, IL-17 signaling pathway, lipid and atherosclerosis, chemical carcinogenesis-receptor activation, multiple neurodegenerative disease pathways, hepatitis B, and Kaposi's sarcoma-associated herpesvirus infection (Fig 5). Literature and PubMed database searches confirm that the PI3K- Akt signaling pathway is associated with traumatic injuries (Fig 6).

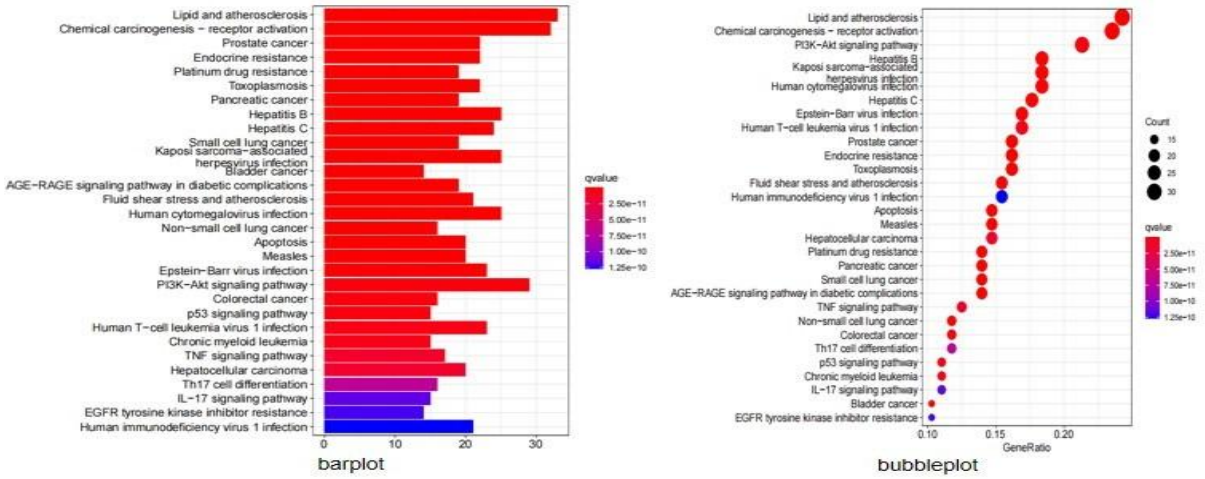


Figure 5 KEGG Pathway Analysis of Guan's Trauma-Relief Decoction for traumatic injuries

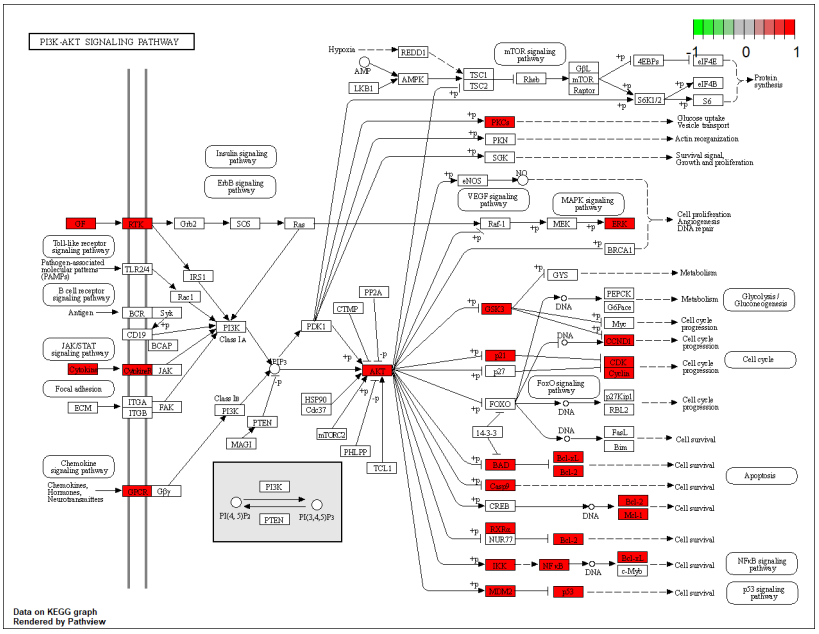


Figure 6 PI3K-Akt Signaling Pathway Diagram

Molecular docking results

The three-dimensional structures of the core target proteins were retrieved from the UniProt and Protein Data Bank databases, including CASP3 (PDB ID: 1NME), JUN (6Y3V), TP53 (6WQX), PTGS2 (5F19), and MAPK1 (4ZZN). Molecular docking analyses were performed to evaluate the binding affinity and interaction stability between the core active compounds and their corresponding targets. Preliminary ligand–target screening was performed using SYBYL 2.0 based on total score ranking. Detailed binding affinities were subsequently calculated using AutoDock 1.5.6 and are reported in kcal/mol (Table 2).

Subsequently, the molecular structures of the key active compounds were docked into the active sites of the core target proteins using AutoDock 1.5.6, and the docking conformations were visualized with PyMOL 2.4. The resulting docking models are presented in Figures 7–11, illustrating the binding modes and interaction characteristics between representative active compounds and their respective targets.

Table 2 Binding energies of key active ingredients of Guan's Liushangyin docked with core target proteins.

Ingredients	Chemical formula	Relative molecular weight	Target point	Molecular docking bond energy (kcal/mol)
beta-sitosterol	C29H50O	414.7g/mol	CASP3	-6.2
Luteolin	C15H10O6	286.24g/mol		-7.1
Luteolin	C15H10O6	286.24g/mol	JUN	-6.1
Quercetin	C15H10O7	302.23g/mol		-5.9
Luteolin	C15H10O6	286.24g/mol	TP53	-8.5
Quercetin	C15H10O7	302.23g/mol		-8.0
beta-sitosterol	C29H50O	414.7g/mol	PTGS2	-7.3
Corydine	C20H23NO4	341.4g/mol		-6.2
xanthogalenol	C21H22O5	354.4g/mol	MAPK1	-5.9
Dehydrocorybulbine	C21H22NO4	352.4g/mol		-5.9

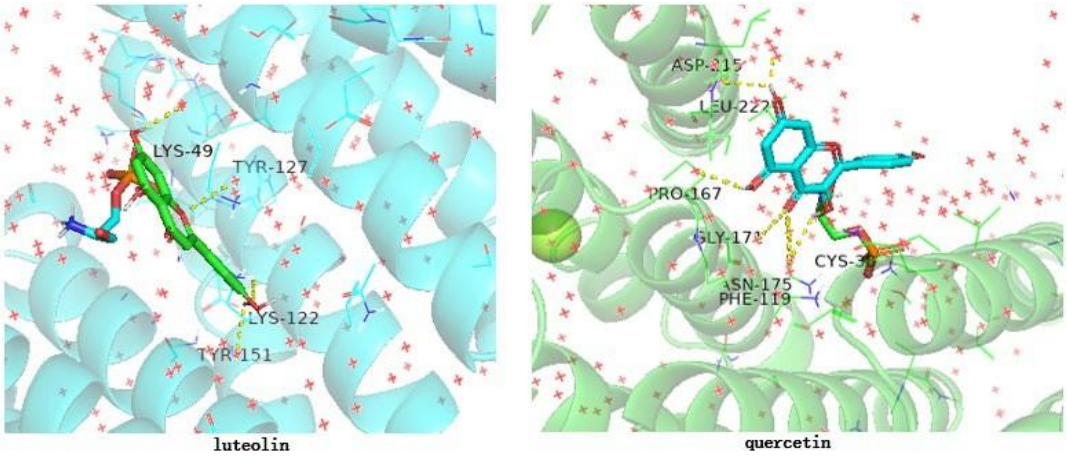


Figure 7 JUN Target Docking

Docking conformations of luteolin and quercetin with JUN, showing the binding modes and key interactions within the JUN binding pocket (Fig 7).

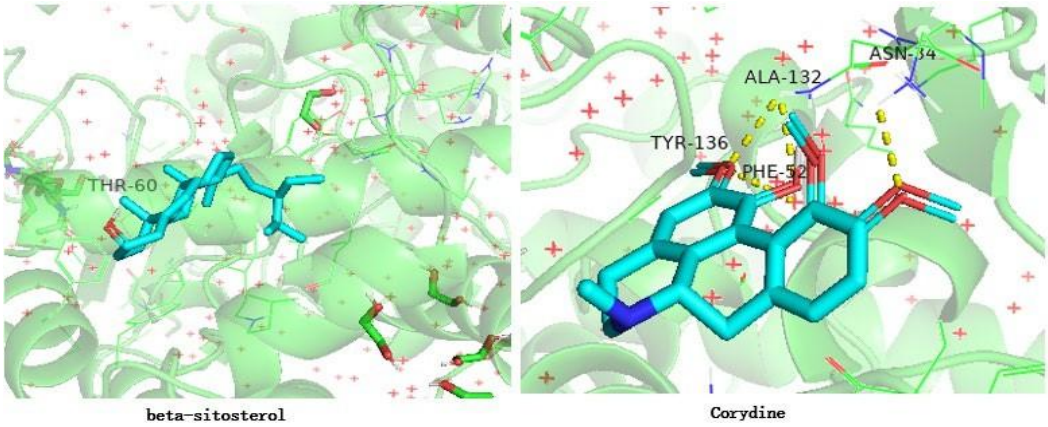


Figure 8 MAPK1 Target Docking

Molecular docking models of β-sitosterol and corydine with MAPK1, illustrating stable ligand–target interactions in the kinase domain (Fig 8).

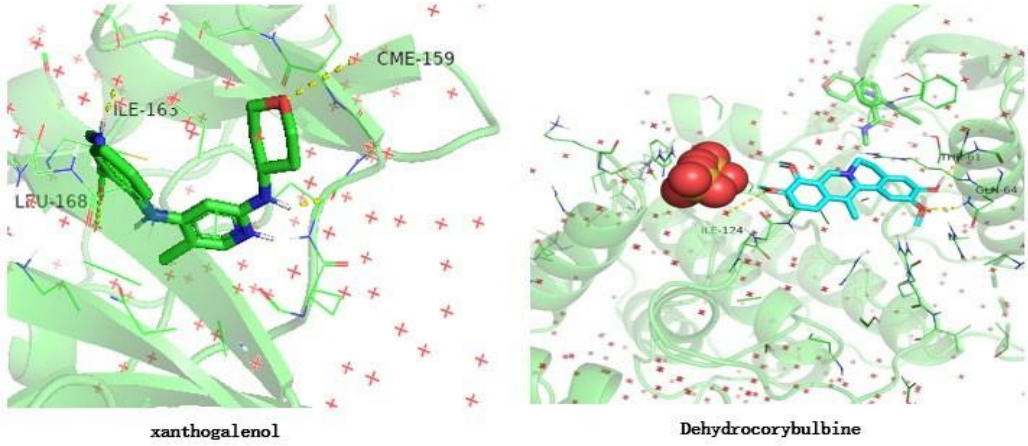


Figure 9 PTGS2 Target Docking

Docking results of xanthogenol and dehydrocorybulbine with PTGS2, highlighting ligand binding within the active site region (Fig 9).

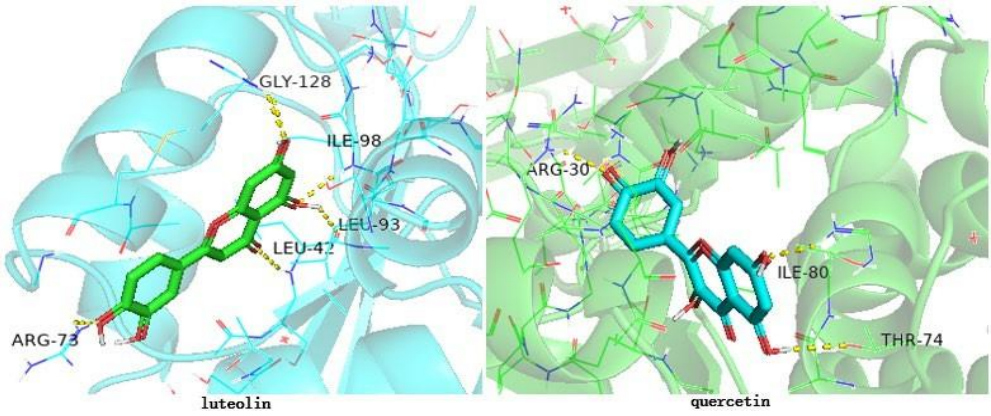


Figure 10 TP53 Target Docking

Docking conformations of luteolin and quercetin with TP53, demonstrating representative ligand–target interactions (Fig 10).

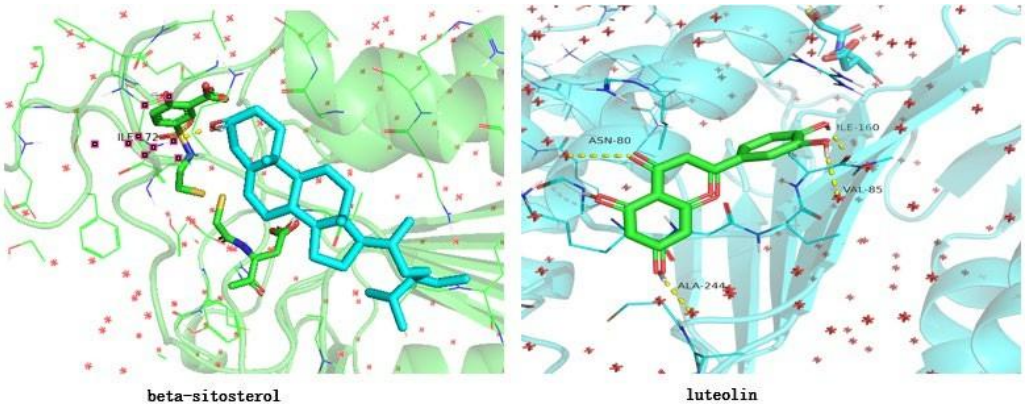


Figure 11 CASP3 Target Docking

Molecular docking models of β-sitosterol and luteolin with CASP3, showing ligand positioning within the catalytic pocket (fig 11).

Discussion

The Guan acupuncture school has had a substantial academic influence in the treatment of traumatic injuries; however, existing studies on Guan medicine have largely focused on acupuncture-based therapies rather than herbal prescriptions [9]. According to Guan's Common Chinese Medicine Formulas and Dispensing Principles, 71 out of 117 formulas developed by Guan Qingxin are indicated for traumatic injuries, highlighting the central role of trauma management in his clinical practice [10]. In traditional Chinese medicine (TCM), traumatic injuries are understood to cause stagnation of qi and blood due to meridian obstruction, leading to pain and swelling [11]. Accordingly, therapeutic strategies emphasize promoting blood circulation, resolving blood stasis, and alleviating pain.

In this study, network pharmacology analysis identified 66 active compounds in Guan's Trauma-Relief Decoction, among which β-sitosterol, flavonoids (e.g., quercetin and luteolin), and representative alkaloids exhibited high network connectivity. Rather than acting independently, these compounds converged on a shared set of therapeutic targets, suggesting a coordinated pharmacological profile consistent with the multi-component nature of TCM formulations. This integrative pattern is further supported by the compound–target network (Figure 2), in which multiple compounds were linked to overlapping targets involved in inflammation, apoptosis, and tissue repair. Protein–protein interaction (PPI) analysis identified PTGS2, CASP3, VEGFA, JUN, MAPK1, AKT1, and TP53 as core hub targets. These targets represent key regulatory nodes in inflammatory signaling, apoptotic control, angiogenesis, and cellular stress responses. Importantly, the identification of PTGS2 and CASP3 as central hubs provides a mechanistic link between inflammatory regulation and apoptosis in traumatic injury, which is consistent with the clinical manifestations of pain, swelling, and tissue damage. Molecular docking analysis further demonstrated moderate and favorable binding affinities (−5.9 to −8.5 kcal/mol) between representative active compounds and these core targets, supporting the structural

feasibility of the predicted interactions rather than implying exceptionally strong binding.

Gene Ontology (GO) enrichment analysis revealed that the overlapping targets were mainly associated with biological processes related to cell cycle regulation, apoptosis, inflammatory response, and signal transduction, including processes involving CDK1, CCNB1, CASP3, and MAPK family members. These findings suggest that Guan's Trauma-Relief Decoction may contribute to tissue recovery by modulating the balance between inflammation, cell proliferation, and programmed cell death, rather than through a single isolated mechanism.

KEGG pathway enrichment analysis further highlighted the PI3K–Akt, MAPK, TNF, and IL-17 signaling pathways as key pathways involved in the therapeutic action of the formula. These pathways are widely recognized for their roles in regulating inflammatory responses, immune activation, angiogenesis, and tissue repair following injury. The convergence of multiple core targets on these pathways provides a systems-level explanation for how Guan's Trauma-Relief Decoction may intervene in traumatic injury through coordinated pathway modulation. Additional enriched pathways related to lipid metabolism, viral infection, and neurodegeneration may reflect broader stress-response and repair mechanisms activated during tissue injury, although their specific roles require further investigation.

Overall, by integrating network pharmacology, PPI analysis, GO and KEGG enrichment, and molecular docking results, this study demonstrates that Guan's Trauma-Relief Decoction exerts its therapeutic potential through a multi-target and multi-pathway regulatory mechanism that aligns with TCM principles of promoting blood circulation and resolving stasis. While the present findings provide computational and network-based mechanistic support, further experimental and clinical studies are needed to validate these predicted interactions and biological effects.

Conclusions

Guanshi Liushang Decoction treats traumatic injuries through a multi-component, multi-target, and multi-pathway approach to regulate disease progression. Its active ingredients likely modulate the expression of key targets such as PTGS2, CASP3, VEGFA, JUN, MAPK1, AKT1, and TP53, and may modulate key targets and signaling pathways associated with inflammation, apoptosis, and tissue repair. This study provides a theoretical basis for the clinical application of Guanshi Liushang Decoction and offers a foundation for future research.

However, there are some limitations. First, due to the limitations of databases, this study did not include all active ingredients of the components of Guanshi Liushang Decoction. Second, the study results are virtual predictions and require subsequent experimental validation. Last, the complexity of traditional Chinese medicine formulas has not considered the interactions between active ingredients within the human body, pharmacokinetic changes, and the regulatory strength on various targets. To address these limitations, future directions include continuing clinical and basic experimental research based on this network pharmacology study to provide new insights into the clinical treatment of traumatic injuries; conducting targeted foundational research on the core components of Guanshi Liushang Decoction, such as serum pharmacology and the extraction of active ingredients in compound preparations; , and regarding the core targets of this study, which are approached through anti-inflammatory, apoptosis regulation, and immune system enhancement, there is currently no basic research directly related to traumatic injuries. Future research can explore the biological regulation of soft tissues and muscles, providing new directions for drug development or novel formulation strategies.

Acknowledgements

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Conflict of interest

The authors declare that they have no conflicts of interest.

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Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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