RESEARCH ARTICLE

Construction and functional analysis of a protein-protein interaction network for Gancao Xiexin Decoction in the treatment of oral ulcer based on data mining

Xizhen Li[1], Wenjuan Li[2, *]

[1]Department of Traditional Chinese Medicine, [2]Dental Department, Cangzhou Medical College, Cangzhou, Hebei, China.

Received: January 8, 2026; **accepted:** May 16, 2026.

Oral ulcers have a high incidence and are prone to recurrence. Current treatments are primarily symptomatic, and there is no curative approach. As a classic formula, Gancao Xiexin Decoction (GCXXT) is commonly used in clinical practice to treat oral ulcers, but its mechanism of action remains unclear. This research clarified active components (ACs), potential targets, and molecular mechanisms of GCXXT in oral ulcer therapy using data mining and network pharmacology and experimentally validated its anti-inflammatory and mucosal repair effects. The study screened the ACs of GCXXT through TCMSP and PubChem databases and predicted their targets. Common targets associated with oral ulcers were obtained from GeneCards, OMIM, and other databases. A "drug-component-target-disease" network and a protein-protein interaction (PPI) network were constructed to identify core targets, and their biological processes and pathways were explored. The binding affinity between core components and targets was validated. A composite cell model was established using H_2O_2-induced injury of human oral keratinocytes combined with lipopolysaccharide-stimulated THP-1 cell inflammatory conditioned medium. Drug-containing serum of GCXXT was prepared, and the expression of inflammatory cytokines and pathway proteins was detected by enzyme-linked immunosorbent assay (ELISA) and Western blot. The results identified 66 ACs and 47 common targets. Core components included berberine, baicalein, and liquiritin, while key targets involved tumor necrosis factor (TNF), interleukin 6 (IL-6), AKT serine/threonine kinase 1 (AKT1), and nuclear factor kappa B subunit 1 (NF-κB1). Enrichment analysis revealed involvement in inflammatory response, oxidative stress, and epithelial proliferation, as well as signaling pathways including NF-κB and Toll-like receptors (TLRs). Molecular docking demonstrated stable binding between core components and targets with binding energy ≤ -7.0 kcal/mol. Experimental validation results showed that GCXXT-containing serum dose-dependently increased human oral keratinocyte (HOK) cell viability, reduced TNF-α level, suppressed NF-κB, cyclooxygenase-2 (COX-2), and inducible nitric oxide synthase (iNOS) protein expression. GCXXT exerted therapeutic effects on oral ulcer through multiple ACs of liquiritin, baicalin, berberine acting on core targets of TNF, IL-6, AKT1, NF-κB, synergistically regulating NF-κB, TLR, and hypoxia-inducible factor-1 (HIF-1) signaling pathways to alleviate inflammation, reduce oxidative stress, and promote mucosal repair.

Keywords: Gancao Xiexin Decoction; oral ulcer; network pharmacology; protein-protein interaction network; molecular docking; inflammatory cytokines.

[*]**Corresponding author:** Wenjuan Li, Dental Department, Cangzhou Medical College, Cangzhou, Hebei 061000, China. Email: 18031711827@163.com.

Introduction

Oral ulcers refer to oral mucosal disorders characterized by recurrent, round or oval ulcers accompanied by pain, burning sensations, and difficulty in eating [1]. It affects approximately 5 – 25% of the global population, showcasing higher prevalence among young adults and a recurrence rate exceeding 50% [2, 3]. Etiology involves complex factors such as immune dysregulation, genetic predisposition, micronutrient deficiencies, and environmental triggers [4]. Current treatments primarily focus on symptomatic relief and lack curative efficacy, resulting in limited therapeutic outcomes [5].

Traditional Chinese medicine (TCM) demonstrates distinct advantages in dealing with oral ulcers. This condition falls under the category of "oral aphthae" in TCM with pathogenesis that often related to spleen and stomach damp-heat, hyperactivity of heart fire, and deficiency of "Qi" and "Yin" [6]. Gancao Xiexin Decoction (GCXXT) derived from "Shang Han Lun" functions to harmonize the spleen and stomach, clear heat and detoxify, and resolve masses and dissipate stagnation [7]. GCXXT is a compound preparation composed of multiple Chinese medicinal herbs that work synergistically. It mainly includes Huanglian (*Coptidis Rhizoma*) that possesses anti-inflammatory and antibacterial activities, Huangqin (*Scutellariae Radix*) with significant anti-inflammatory and antioxidant effects, Banxia (*Pinelliae Rhizoma*) that exhibits anti-inflammatory and immunomodulatory activities, Ganjiang (*Zingiberis Rhizoma*) with anti-inflammatory and gastroprotective effects, Renshen (*Ginseng Radix*) that enhances immune function and tissue regeneration, Dazao (*Jujubae Fructus*) with immunomodulatory and antioxidant activities, and the principal herb Gancao (*Glycyrrhizae Radix et Rhizoma*) that exerts broad anti-inflammatory, immunomodulatory, and mucosal protective effects. Modern pharmacological studies have shown that components such as glycyrrhizic acid, baicalin, and gingerols in this formula possess anti-inflammatory and antioxidant activities [8, 9], while ginsenosides like ginsenoside Rc, panaxadiol play important roles in mucosal repair by regulating intestinal inflammation and improving barrier function [10, 11]. Although clinical studies suggest the efficacy of GCXXT in treating oral ulcers, its specific molecular mechanisms and multi-target actions remain insufficiently systematically elucidated. With the advancement of bioinformatics, data mining techniques are applied in TCM mechanism studies. By integrating TCM components, targets, and disease-causing genes, the construction of compound-target-disease networks enables systematic elucidation of the multi-target actions and potential pathways of TCM [12]. Combined with protein-protein interaction networks (PPINs), this approach further facilitates the identification of key targets and the analysis of their regulatory networks. Research in TCM syndrome metabolomics (Chinmedomics) has demonstrated that the classical formula Kaixin San improves cognitive function, remodels lipid and amino acid metabolism, regulates 20 biomarkers, and identifies active components (ACs) such as ginsenoside Rf and poricoic acid E as highly correlated with efficacy [13]. As a complex herbal formula, GCXXT has not been systematically deciphered in terms of its mechanisms of action, underscoring the urgent need for research on network pharmacology methodologies.

This research employed data mining and network pharmacology approaches to systematically investigate the molecular mechanisms of GCXXT in oral ulcer therapy. The study screened ACs of GCXXT and their targets, integrated oral ulcer-related targets to identify common drug-disease targets, constructed a PPIN to collect key targets and pathways, validated binding affinity between core components and key targets through molecular docking to enhance predictive reliability [14], and utilized *in vitro* cell models to experimentally verify the effects of GCXXT. The findings of this study elucidated the mechanism of GCXXT at a systems level, providing a basis for new drug development.

Materials and methods

Active ingredients and potential targets of GCXXT

Chemical constituents of each herb in GCXXT were systematically collected from TCMSP (https://tcmsp-e.com/), TCMID (https://www.cloudtcm.com/), HERB (https://herb.ac.cn/), and PubChem (https://pubchem.ncbi.nlm.nih.gov/) databases. Initial screening was based on well-defined chemical structures and reported pharmacological activities. Further selection of ACs incorporated pharmacokinetic parameters with criteria of oral bioavailability (OB) ≥ 30% and drug-likeness (DL) ≥ 0.18. Gastrointestinal (GI) absorption was evaluated by using SwissADME (https://www.swissadme.ch/), prioritizing components with "high" GI absorption. Key compounds supported by literature evidence for anti-inflammatory or immunomodulatory effects such as glycyrrhizic acid and baicalin were retained even if partial parameters did not meet thresholds. All selected components underwent structural standardization *via* ChemDraw (https://www.perkinelmer.com/category/chemdraw) and were converted into simplified molecular input line entry system (SMILES) format for subsequent target prediction, network construction, and molecular docking analysis.

Acquisition of targets related to oral ulcers

Potential targets of ACs in GCXXT were predicted by using TCMSP and STITCH (https://stitch.embl.de/) databases with the confidence score ≥ 0.7 and uniformly converted to standard HUGO gene nomenclature committee (HGNC) gene symbols. Oral ulcer-related targets were retrieved from GeneCards (https://www.genecards.org/) with the relevance score ≥ 10, DisGeNET (https://www.disgenet.com/) with the score ≥ 0.2, and online mendelian inheritance in man (OMIM) (https://www.omim.org/) databases using the keywords "oral ulcer" and "recurrent aphthous stomatitis". After deduplication, these targets were also converted to HGNC symbols. Intersection of drug-predicted and disease targets was identified as the core candidate target set for GCXXT in oral ulcer treatment, providing a foundation for subsequent PPIN construction and functional enrichment analysis.

Intersection analysis of drug-disease targets

Target intersection for ACs of GCXXT and oral ulcer-related targets was obtained to generate a common target set. A Venn diagram was plotted using the Venny 2.1 online tool (https://bioinfogp.cnb.csic.es/tools/venny/). A multi-layer network of "herb-compound-target-disease" was then constructed with Cytoscape 3.9.1 (https://cytoscape.org/). Topological analysis of the network was performed to screen core ACs and key targets with high degree values, providing a basis for subsequent PPIN fabrication and functional enrichment analysis.

PPIN fabrication and analysis

The obtained list of intersecting genes was uploaded to the STRING version 12.0 (https://string-db.org/) to construct a high-confidence PPIN with the species specified as "homo sapiens" to ensure biological relevance. A minimum interaction confidence score of 0.7 was set to filter for highly supported associations. Disconnected nodes were hidden to simplify the network and improve visualization clarity. The filtered interaction data was exported for subsequent topological analysis and core module identification, providing a structural basis for uncovering key targets. Cytoscape 3.9.1 was employed to implement network visualization. NetworkAnalyzer (https://apps.cytoscape.org/apps/networkanalyzer) was used to calculate node degree, betweenness centrality, and closeness centrality. CytoHubba plugin (https://apps.cytoscape.org/apps/cytohubba) was applied to identify core genes based on maximal clique centrality (MCC) and degree algorithms with the top ten key targets ultimately selected.

Functional enrichment

To further elucidate the biological functions and potential pathways of common targets, systematic bioinformatic enrichment analysis was performed *via* clusterProfiler in R 4.2.0,

which included gene ontology (GO) (https://geneontology.org/) functional annotation and Kyoto encyclopedia of genes and genomes (KEGG) (https://www.kegg.jp/) pathway analysis, to reveal the major functional involvements of the target genes across three domains of biological process (BP), molecular function (MF), and cellular component (CC). A dual-threshold criterion was applied for statistical significance with a $P < 0.05$ and a false discovery rate < 0.05 after multiple testing correction, ensuring the robustness and reliability of the results. Biological processes and signaling pathways related to immune-inflammatory response, mucosal barrier repair, and apoptosis regulation were prioritized. To further identify key functional modules and potential core subnetworks within the PPIN, cluster analysis was performed using MCODE in Cytoscape to detect highly interconnected clusters of nodes, facilitating the identification of functional modules that might participate in shared biological processes or form protein complexes with the parameters of Degree Cutoff = 2, Node Score Cutoff = 0.2, K-core = 2, and Max Depth = 100. These parameters collectively controlled the sensitivity and compactness of module detection, enabling effective screening of core network regions with potential functional importance and providing structural support for subsequent key target selection and mechanistic investigation. Independent KEGG analysis was performed on targets within identified significant modules to elucidate the functional characteristics of the subnetworks.

Molecular docking verification

The 3D structures of high-degree components in GCXXT were retrieved from PubChem. Core targets' crystal structures were downloaded from research collaboratory for structural bioinformatics protein data bank (RCSB PDB) (https://www.rcsb.org/) prioritizing conformations with resolution ≤ 2.5 Å. Hydrogenation, charge assignment, and format conversion (PDBQT) were performed using AutoDock Tools (https://www.rcsb.org/). Semi-flexible docking was carried out by using AutoDock Vina with the grid box covering the active pocket and exhaustiveness set to 100. In the evaluation of molecular docking results, the binding energy value served as a key indicator for assessing the interaction degree between ligand and target protein. A docking score (i.e., binding free energy) ≤ –5.0 kcal/mol was generally considered indicative of moderate binding activity between the small molecule and the target, suggesting potentially stable physicochemical interactions. A binding energy ≤ –7.0 kcal/mol was defined as a strong binding capability, reflecting higher affinity and potentially significant bioactivity. Hydrogen bonds, hydrophobic interactions, *etc*. were visualized by using PyMOL (https://pymol.org/) and LigPlot+ (https:// www.ebi.ac.uk/thornton-srv/software/LigPlus/).

Experimental verification

1. Experimental animal and the preparation of drug-containing serum

36 male SPF-grade SD rats weighted 200 – 220 g were obtained from Beijing Vital River (Beijing, China) and housed in the Laboratory Animal Center, Health Science Center, Peking University, Beijing, China at 22 ± 2°C, relative humidity 50 ± 10%, and a 12 h light/dark cycle. All animals were housed in a standard barrier environment and had free access to autoclaved pellet feed and sterile drinking water with daily monitoring of dietary intake to guarantee adequate nutrition and maintain normal physiological conditions. After a 7-day acclimatization period, animals were randomly assigned to three groups with twelve animals in each group including blank control group without intervention, GCXXT treatment group, and compound glycyrrhizin zinc granule treatment group. Body weight, mental state, and dietary intake were monitored throughout the study period. The GCXXT group and the compound glycyrrhizin zinc granules group were administered the corresponding drugs *via* oral gavage at a clinical equivalent dose of 10 g/kg·d daily, while the control group received distilled water for seven days. GCXXT was prepared from Chinese herbal decoction pieces (Beijing Tongrentang, Beijing, China).

Compound glycyrrhizic acid zinc granules were obtained from Darentang Group Co., Ltd. (Tianjin, China). 1 h after last administration, animals were anesthetized with isoflurane (RWD Life Science, Shenzhen, Guangdong, China) for whole blood collection. The collected blood sample was left standing at 25°C for 30 min before centrifugation at 3,000 rpm for 15 min using Eppendorf 5810R low temperature high speed centrifuge (Eppendorf, Hamburg, Germany). The supernatant serum was collected, inactivated, sterilized, aliquoted, and stored at −20°C. All experimental procedures were approved by the Institutional Animal Ethics Committee (Peking University, Beijing, China).

2. Experimental cells and model construction

Human oral keratinocyte cell line (HOK) (ScienCell Research Laboratories, Carlsbad, CA, USA) and human THP-1 (ATCC, Manassas, VA, USA) were employed to establish an inflammatory response model. HOK cells were used to simulate oral mucosal epithelial injury and repair, while THP-1 cells were used to investigate inflammatory cytokine secretion and immune responses. HOK cells were cultured in Keratinocyte medium (ScienCell Research Laboratories, Carlsbad, CA, USA) with growth factors and 1% penicillin-streptomycin (Gibco, Grand Island, NY, USA) at 37°C and 5% CO_2. Subculture was performed at 70 – 80% confluence to ensure optimal proliferation. THP-1 cells were grown in suspension in RPMI-1640 with 10% fetal bovine serum and 1% penicillin-streptomycin (Gibco, Grand Island, NY, USA) under the same cultural conditions with medium refreshed every 2 – 3 days. To induce pathological conditions, HOK cells were treated with 200 μM H_2O_2 (Sigma-Aldrich, St. Louis, MO, USA) for 30 minutes to establish an oxidative stress-induced epithelial injury model. Meanwhile, THP-1 cells were differentiated into macrophage-like cells using 100 ng/mL phorbol myristate acetate (PMA) (Sigma-Aldrich, St. Louis, MO, USA) for 48 hours followed by 1 μg/mL lipopolysaccharide (LPS) (Sigma-Aldrich, St. Louis, MO, USA) stimulation for 24 hours to activate inflammatory pathways. Conditioned medium from LPS-stimulated THP-1 cells was then applied to oxidatively injured HOK cells to establish a composite *in vitro* model of "epithelial damage + inflammatory microenvironment". This system simulated the complex pathological interaction between epithelial barrier disruption and immune inflammatory response in oral ulcers, providing a powerful platform for subsequent pharmacological evaluation and mechanistic studies.

3. Cell grouping and processing

The cells in control group included untreated HOK cells + 5% blank serum, while the cells in model group included H_2O_2-injured HOK cells + THP-1 inflammatory conditioned medium + equal volume blank serum). The positive control group (PC) was the model group + compound glycyrrhizin zinc granules serum. The low-dose GCXXT-containing serum group (L-GCXXT) was the model cells + 5% medicated serum, while the medium-dose group (M-GCXXT) contained model cells + 10% medicated serum, and the high-dose group (H-GCXXT) formed by model cells + 15% medicated serum. All experimental cell groups were intervened for 24 – 48 h before subsequent assays.

4. Cell viability (CV) assay

CV of HOK cells was assessed *via* cell counting kit-8 (CCK-8) (Dojindo, Kumamoto, Japan) following manufacturer's instructions. After 24 h model treatment, CCK-8 reagent was applied for 1 – 4 h. The absorbance was measured at 450 nm using BioTek ELx800 microplate reader (BioTek, Winooski, VT, USA) to calculate the CV.

5. Inflammatory cytokine level detection

Tumor necrosis factor (TNF-α), interleukin 6 (IL-6), and IL-1β were measured *via* enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN, USA) following manufacturer's instructions. The optical density (OD) values were measured at the appropriate wavelength using a microplate reader, and the concentrations of inflammatory cytokines were computed accordingly.

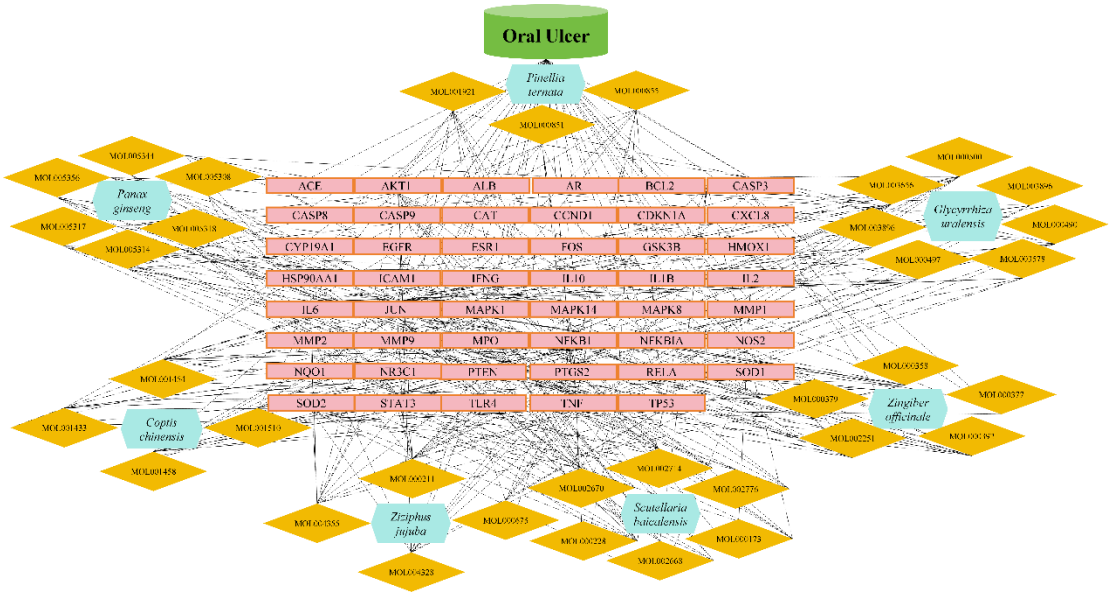

Figure 1. Multi-layer network diagram of GCXXT - compounds - targets - oral ulcer. **Hexagon**: Chinese herbal medicine. **Diamond**: active compound. **Rectangle**: common target. **Cylinder**: disease.

6. Western blot detection of inflammation-related pathway expression

The expression levels of key inflammation-related proteins were determined by using Western blot analysis. HOK cells were seeded at 6×10^5 cells/well and allowed to adhere before treatment. After interventions, cells were harvested using RIPA lysis buffer (Beyotime, Shanghai, China) containing protease inhibitors. Lysates were centrifuged, and supernatants were collected as total protein samples. The BCA protein assay kit (Beyotime, Shanghai, China) was used to quantify protein concentrations, and equal amounts of protein were separated by Mini-PROTEAN Tetra SDS-PAGE (Bio-Rad, Hercules, CA, USA) according to molecular weight. The proteins were transferred to polyvinylidene fluoride (PVDF) or nitrocellulose membranes *via* the wet transfer method followed by rapid blocking at 25℃ for 1 h. The membranes were incubated overnight at 4℃ with primary antibodies against NF-κB, cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), and GAPDH as an internal reference (Abcam, Cambridge, UK). After rinsing and incubating with secondary antibodies at 25°C for 1 h, protein bands were visualized *via* ChemiDoc MP enhanced chemiluminescence (ECL) (Bio-Rad, Hercules, CA, USA). ImageLab (Bio-Rad, Hercules, CA, USA) was applied for protein band grayscale analysis to calculate the relative expression levels.

Statistical analysis

SPSS 26.0 (IBM, Armonk, NY, USA) or GraphPad Prism9.0 (GraphPad Software, San Diego, CA, USA) were employed for statistical analysis of this research. Experimental data recorded as mean ± SD were compared between *in vitro* groups by ANOVA. When homogeneity of variance was satisfied, LSD or Tukey's multiple comparison test was applied. Otherwise, Welch's corrected ANOVA or non-parametric tests were adopted. *P* value less than 0.05 was defined as statistically significant difference.

Results

Active ingredients and potential target prediction in GCXXT

156 chemical components were initially screened from GCXXT using the TCMSP, TCMID, HERB, and PubChem databases. Based on pharmacokinetic

Table 1. Top 10 core components by degree value and their corresponding targets.

No.	Active compound	Degree value	Potential target	Herb source
MOL001454	Berberine	20	TNF, IL6, AKT1, NFκB1, JUN, CASP3, EGFR	*Coptis chinensis*
MOL002714	Baicalein	19	TNF, IL6, AKT1, PTGS2, TP53, NFκB1, JUN	*Scutellaria baicalensis*
MOL000500	Liquiritin	18	TNF, IL6, AKT1, PTGS2, TP53, CASP3,	*Glycyrrhiza uralensis*
MOL002776	Baicalin	17	TNF, IL6, AKT1, PTGS2, STAT3, CASP3	*Scutellaria baicalensis*
MOL000490	Glycyrrhizic acid	16	TNF, IL6, PTGS2, CASP3, NFκB1	*Glycyrrhiza uralensis*
MOL000173	Wogonin	16	TNF, IL6, AKT1, PTGS2, IL1B	*Scutellaria baicalensis*
MOL005344	Ginsenoside Rg1	16	TNF, IL6, AKT1, NFκB1, CASP3	*Panax ginseng*
MOL003896	Isoliquiritin	15	TNF, IL6, PTGS2, EGFR, JUN	*Glycyrrhiza uralensis*
MOL005308	Ginsenoside Rb1	15	TNF, IL6, AKT1, JUN	*Panax ginseng*
MOL000497	Liquiritigenin	14	TNF, IL6, AKT1, PTGS2, CASP3	*Glycyrrhiza uralensis*

parameters (OB ≥ 30%, DL ≥ 0.18) and gastrointestinal absorption capacity evaluation, 66 potential ACs were ultimately identified. Among them, compounds of liquiritin, baicalein, berberine, and glycyrrhizic acid were retained despite partially failing to meet the thresholds, as their anti-inflammatory or immunomodulatory effects were supported by literature evidence. Preliminary predictions identified 216 potential targets of the ACs. After HGNC standardization and integration of data from TCMSP and STITCH databases with synonymous targets removed, 482 potential targets were obtained for subsequent drug-disease intersection analysis.

Analysis of oral ulcer-related targets and drug disease intersection

A total of 1,238 oral ulcer-related targets were harvested after deduplication. Integration of predicted targets for the ACs of GCXXT yielded 482 targets. Venny 2.1 analysis identified 47 common drug-disease targets.

Analysis of potential components and key targets of oral ulcer in GCXXT

The multilayer network of "GCXXT – compound – target – oral ulcer" showed that this network contained 85 nodes including 7 Chinese medicinal herbs, 35 active compounds that interacted with the common targets, 47 common targets, 1 disease node and 312 edges, visually presenting the multi-component, multi-target synergistic relationship of GCXXT in the treatment of oral ulcers (Figure 1). Topological analysis showed that compounds of berberine, baicalein, and liquiritin had the highest degree values, serving as hub nodes connecting multiple targets in the network, whereas targets of TNF, IL6, and AKT serine/threonine kinase 1 (AKT1) exhibited extensive interactions with various compounds. Based on topological analysis, the top 10 core components with the highest degree values and their corresponding targets demonstrated that the top three active compounds ranked by degree value were berberine from Huanglian (*Coptidis Rhizoma*) with degree of 20, baicalein from Huangqin (*Scutellariae Radix*) with degree of 19, and liquiritin from Gancao (*Glycyrrhizae Radix et Rhizoma*) with degree of 18. The corresponding targets of the above compounds mainly included TNF, IL6, AKT1, and NFκB1 (Table 1).

PPIN analysis

The network was visualized using Cytoscape 3.9.1 with topological parameter calculation and core gene identification performed *via* the NetworkAnalyzer and CytoHubba plugins. The results indicated that the top ten key hub genes were TNF, IL6, AKT1, NFκB1, MAPK1, JUN, TP53, CASP3, STAT3, and PTGS2 (Figure 2).

Enrichment analysis

Enrichment in biological processes was noted including inflammatory response (P = 3.2e-18), response to oxidative stress (P = 5.6e-15), and

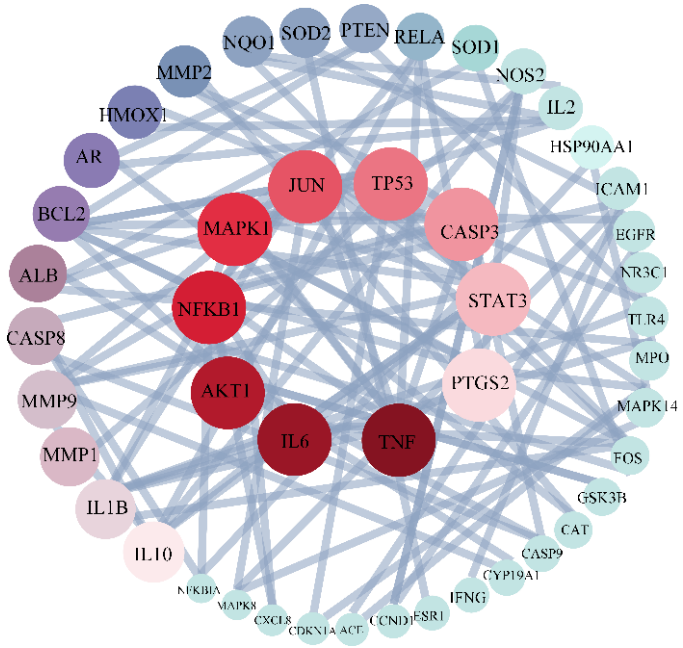

Figure 2. PPIN of GCXXT and oral ulcer.

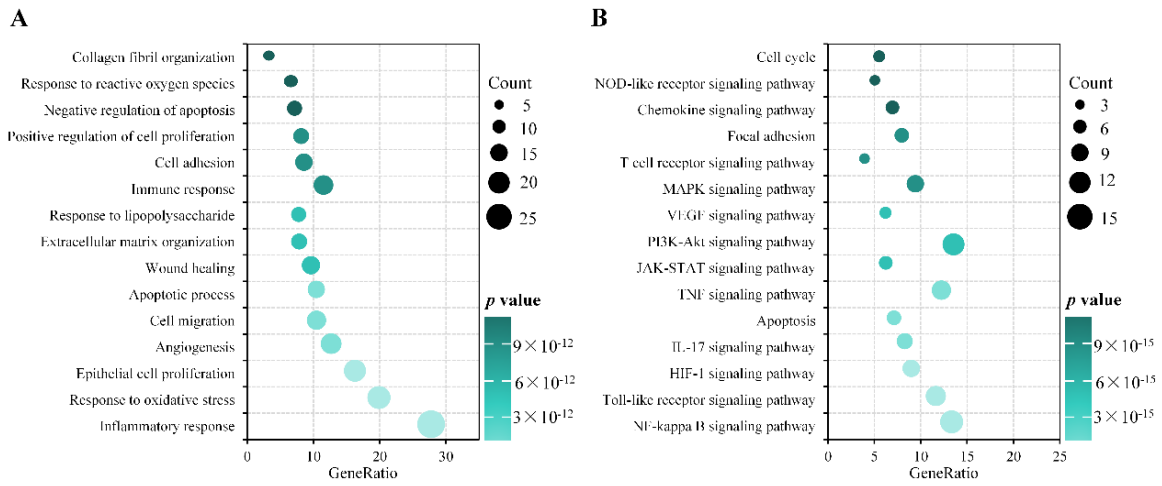

Figure 3. Bubble chart of GO and KEGG enrichment analysis. **A.** GO biological process. **B.** KEGG pathway.

epithelial cell proliferation (*P* = 7.8e-12) (Figure 3A). KEGG pathway analysis indicated predominant involvement in the NF-κB signaling pathway (*P* = 4.5e-14), Toll-like receptor signaling pathway (*P* = 6.2e-11), HIF-1 signaling (*P* = 8.1e-10), IL-17 signaling (*P* = 2.3e-9), and apoptosis pathway (*P* = 5.4e-9) (Figure 3B). MCODE module analysis further demonstrated highly synergistic interactions between the NF-κB and HIF-1 pathways within subnetworks.

Molecular docking verification

The top three components of liquiritin, baicalin, berberine by degree value were taken for molecular docking with core targets of TNF, IL-6, AKT1. The results showed that all ligand-receptor binding energies were ≤ -7.0 kcal/mol, indicating stable binding. Glycyrrhizic acid exhibited the lowest binding energy with TNF (-9.8 kcal/mol). Baicalin formed four hydrogen bonds with AKT1. Berberine enhanced binding with IL-6 (Figure 4).

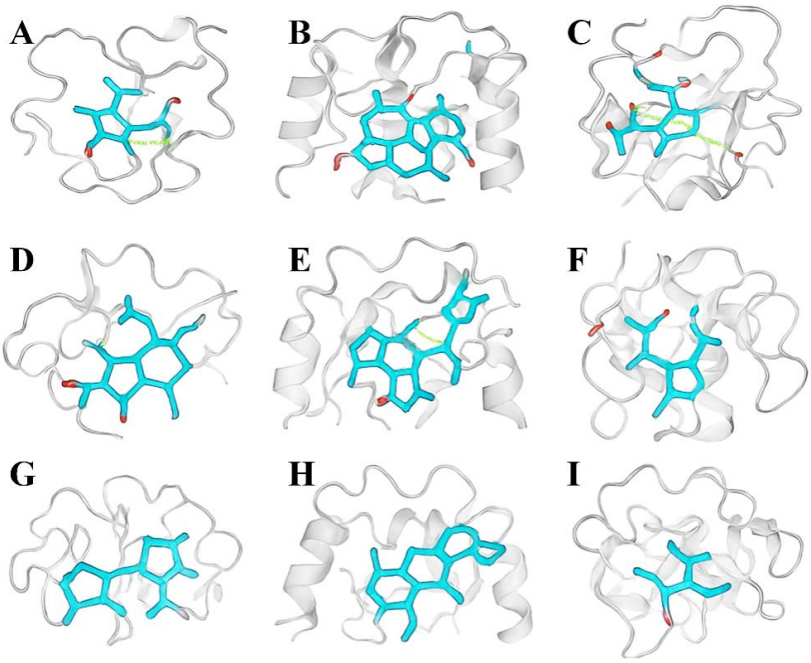

Figure 4. Molecular docking diagrams. **A.** liquiritin-TNF. **B.** liquiritin-IL-6. **C.** liquiritin-AKT1. **D.** baicalin-TNF. **E.** baicalin-IL-6. **F.** baicalin-AKT1. **G.** berberine-TNF. **H.** berberine-IL-6. **I.** berberine-AKT1.

Comparison of cell survival rates

The results of CCK-8 assay demonstrated that the CV of HOK cells in the model group dramatically decreased compared to that in the normal control (NC) group ($P < 0.01$), indicating successful fabricated model. L-GCXXT, M-GCXXT, and H-GCXXT groups exhibited progressively increased HOK CV compared to the model group ($P < 0.05$). There was no statistically significant difference between H-GCXXT group and PC group ($P > 0.05$) (Figure 5).

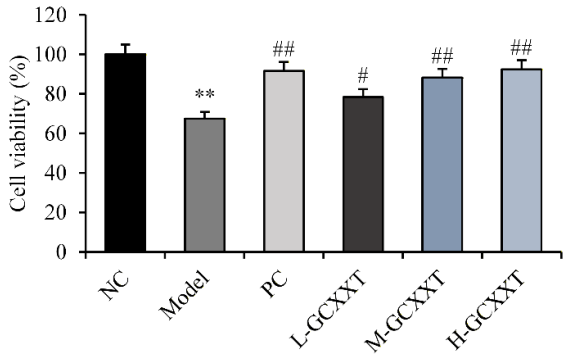

Figure 5. Comparison of CV among groups. **: $P < 0.01$ compared to NC group. #: $P < 0.05$. ##: $P < 0.01$ compared to model groups in Figures 6 and 7.

Inflammatory cytokine levels

ELISA analysis of inflammatory cytokines in the supernatant revealed that the secretion levels of TNF-α, IL-6, and IL-1β were substantially elevated in the model group compared to that in NC group ($P < 0.01$), indicating successful establishment of the inflammatory model. Further analysis demonstrated that intervention with different doses of L-GCXXT, M-GCXXT, H-GCXXT resulted in a marked downregulation of these inflammatory cytokines. The inhibitory effect strengthened with increasing drug concentrations, exhibiting a typical dose-dependent response. All treatment groups showed significantly reduced cytokine levels compared to the model group ($P < 0.05$), while M-GCXXT and H-GCXXT groups showed no significant difference with PC group (Figure 6).

Protein expression analysis

In HOK cells, the relative protein expression levels of NF-κB, COX-2, and iNOS were markedly elevated in the model group compared to that in the NC group ($P < 0.01$). The upregulation of these proteins was markedly suppressed in L-GCXXT, M-GCXXT, and H-GCXXT groups dose-

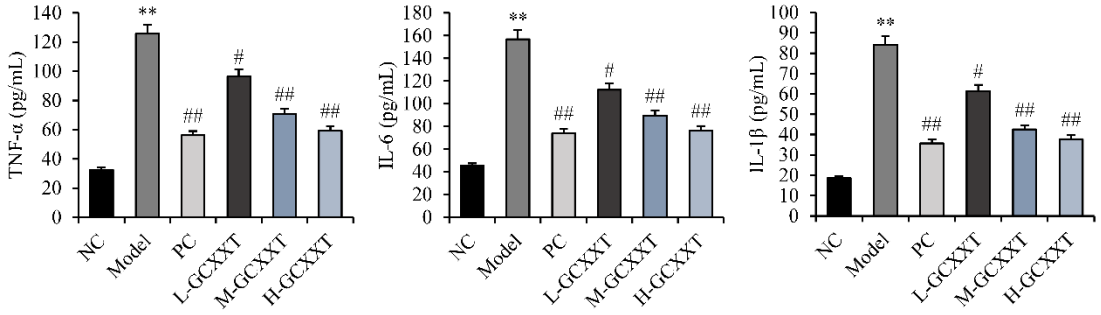

Figure 6. Comparison of inflammatory cytokine levels among groups.

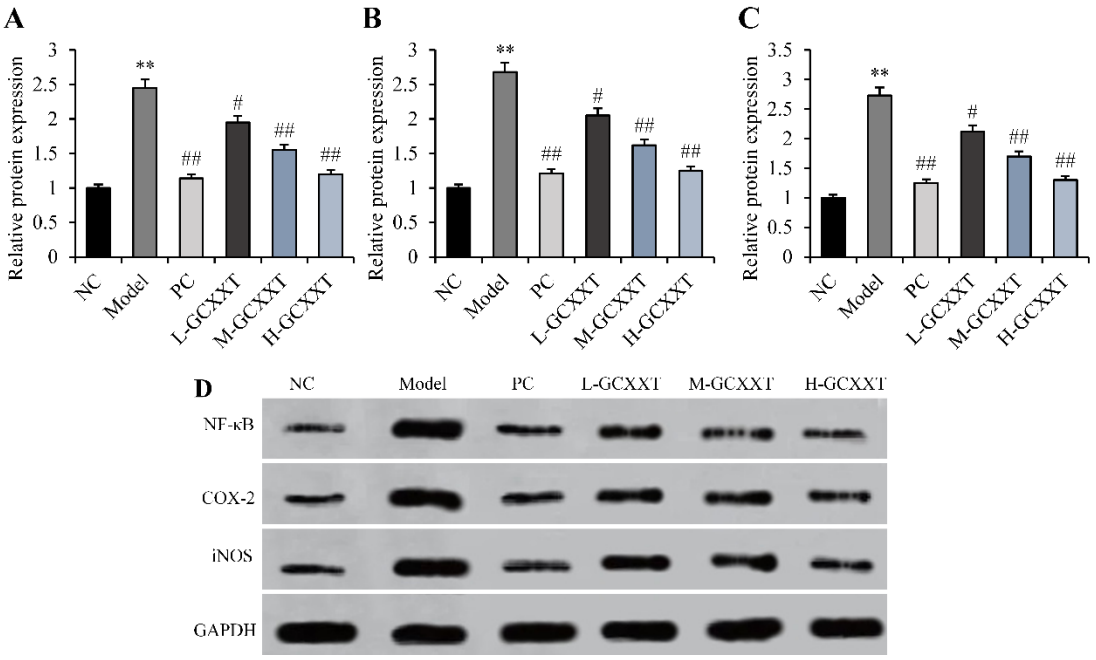

Figure 7. Comparison of relative protein expression levels of NF-κB, COX-2, and iNOS in each group. **A.** NF-κB. **B.** COX-2. **C.** iNOS. **D.** Western blot band images.

dependently compared to that in the model group ($P < 0.05$), consistent with the trends observed for inflammatory cytokines (Figure 7).

Discussion

This study, based on data mining and network pharmacology approaches, systematically constructed a "compound-target-pathway" network for GCXXT in oral ulcer therapy. With molecular docking and *in vitro* validation, it preliminarily revealed the mechanism by which this formula exerted anti-inflammatory, antioxidant, and mucosal repair effects. Inflammation is a core mechanism in the onset and progression of oral ulcers. Local immune cell activation and inflammatory factor release are critical contributors to epithelial injury and ulcer formation [15]. Vitamin C plus vitamin E significantly accelerates the proliferation of oral epithelial cells and promotes ulcer healing [16]. In the present study, GO enrichment analysis revealed that targets of GCXXT were enriched in inflammatory response, suggesting its potential therapeutic role in oral ulcer by modulating these

key processes. As frontline sensors of innate immunity, Toll-like receptors (TLRs) are upregulated during oral mucosal inflammation and activate NF-κB *via* myeloid differentiation primary response 88 (MyD88), inducing TNF-α production. This mechanism explains how microbial or LPS stimulation exacerbates local inflammation in oral ulcers. HIF-1, traditionally recognized as a core regulator of hypoxic response, modulates mucosal (including oral) repair and metabolic adaptation under hypoxia/oxidative stress, exerting both protective and pro-inflammatory effects. The IL-17/T helper 17 (Th17) axis helps maintain mucosal barrier integrity and induce antimicrobial peptides, but its overactivation can aggravate tissue damage and chronic inflammation. Additionally, apoptosis pathways are closely associated with epithelial shedding and ulcer formation in oral ulcers. TNF-α or oxidative stress can trigger epithelial cell apoptosis *via* Caspase-3, thereby delaying healing [17]. This study confirmed that the targets of GCXXT were significantly enriched in NF-κB, Toll-like receptor, HIF-1, IL-17, and apoptosis pathways. MCODE module analysis further highlighted the high synergy between NF-κB and HIF-1 within the subnetwork. NF-κB and HIF-1 collaboratively regulate transforming growth factor-β-activated kinase 1 (TAK1)/IκB kinase (IKK)-NF-κB activity in epithelial and immune cells to mitigate inflammatory damage and promote repair [18]. Thus, the coordinated modulation of NF-κB and HIF-1 might represent a key mechanism by which GCXXT suppressed inflammation, reduced oxidative stress, inhibited epithelial apoptosis, and promoted mucosal repair. This study simulated the oxidative stress and inflammatory microenvironment of oral ulcer using an H_2O_2-induced HOK cell injury model and an LPS-stimulated THP-1 inflammatory model. The results demonstrated that intervention with GCXXT-containing serum progressively enhanced the CV of HOK cells in a dose-dependent manner. Concurrently, it significantly reduced the secretion level of the inflammatory cytokine TNF-α, indicating potent anti-inflammatory effects. Western blot analysis further revealed that key inflammation-related proteins, NF-κB, COX-2, and iNOS, were markedly suppressed with the degree of inhibition increasing alongside higher drug concentrations. Modern studies have clearly established that NF-κB directly activates the transcription of COX-2 and iNOS genes. Upregulation of COX-2 enhances the synthesis of prostaglandins like PGE2, promoting vasodilation and immune cell recruitment, thereby driving the inflammatory process. Simultaneously, increased iNOS expression elevates NO production, which not only regulates the functions of inflammatory cells such as chemotaxis and adhesion but may also further amplify the scope of inflammation [19]. This study confirmed that GCXXT alleviated inflammatory responses by inhibiting the NF-κB pathway, echoing predictions from network pharmacology. Molecular docking results demonstrated that liquiritin, baicalin, and berberine exhibited strong binding affinities to the core targets TNF, IL-6, and AKT1. Notably, liquiritin showed the lowest binding energy with TNF (-9.8 kcal/mol), suggesting its potential as a key anti-inflammatory component in GCXXT. Biologically, licorice and its glycosides exhibit anti-inflammatory activity, downregulating TNF-α and IL-6 expressions, supporting their role as critical anti-inflammatory agents [20]. Baicalin modulates TLR4-PI3K/AKT/NF-κB, enhancing mucosal barrier integrity and repair capacity, thereby exerting anti-inflammatory and antioxidant effects. Numerous recent network pharmacology and experimental studies indicate that berberine targets inflammation-related molecules including IL-6, TLR4, and AKT-associated pathways and frequently demonstrates high affinity for kinases/cytokines in molecular docking analyses, supporting its potential as a synergistic anti-inflammatory component.

This study integrated data mining, network construction, molecular docking, and *in vitro* experimental validation to preliminarily elucidate that GCXXT exerted therapeutic effects on oral ulcer through multiple ACs including liquiritin, baicalin, berberine, acting on core targets of TNF,

IL-6, AKT1, and NF-κB. These components synergistically regulated signaling pathways including NF-κB, TLR, and HIF-1, thereby achieving anti-inflammatory effects, alleviating oxidative stress, and promoting mucosal repair. The findings provided a basis for the therapy utilization of GCXXT and demonstrated the utility of network pharmacology in deciphering mechanisms of complex TCM formulations.

References

1. D'Amario M, Foffo G, Grilli F, Capogreco M, Pizzolante T, Rastelli S. 2025. Treatments for recurrent aphthous stomatitis: A literature review. Dent J (Basel). 13(2):66.
2. Conejero Del Mazo R, García Forcén L, Navarro Aguilar ME. 2023. Recurrent aphthous stomatitis. Estomatitis aftosa recurrente. Med Clin (Barc).161(6):251-259.
3. Yang X, Zhang G, Shan Q. 2025. Nutritional intake and health status of populations and the relationship between diet and oral ulcers: A cross-sectional study based on NHANES data and machine learning predictions. Medicine (Baltimore). 104(29):e43383.
4. Bethlehem RAI, Seidlitz J, White SR, Vogel JW, Anderson KM, Adamson C, *et al*. 2022. Brain charts for the human lifespan. Nature. 604(7906):525-533.
5. Mashrah MA, Fang Y, Song W, Al-Maweri SA, Lan Y, Linhu G, *et al*. 2023. Topical medications for the treatment of recurrent aphthous stomatitis: A network meta-analysis. J Oral Pathol Med. 52(9):811-825.
6. Aldehlawi H, Jazzar A. 2023. The power of Licorice (*Radix glycyrrhizae*) to improve oral health: A comprehensive review of its pharmacological properties and clinical implications. Healthcare (Basel). 11(21):2887.
7. Shaukat M, Nazir A, Fallico B. 2023. Ginger bioactives: A comprehensive review of health benefits and potential food applications. Antioxidants (Basel). 12(11):2015.
8. Ping J, Hao HZ, Wu ZQ, Yang YJ, Yu HS. 2024. Integrating data mining and network pharmacology for traditional Chinese medicine for the drug discovery of diabetic peripheral neuropathy. SLAS Technol. 29(6):100228.
9. Yu Y, Chen J, Zhang X, Wang Y, Wang S, Zhao L, *et al*. 2021. Identification of anti-inflammatory compounds from Zhongjing formulae by knowledge mining and high-content screening in a zebrafish model of inflammatory bowel diseases. Chin Med. 16(1):42.
10. Tang K, Kong D, Peng Y, Guo J, Zhong Y, Yu H, *et al*. 2022. Ginsenoside Rc attenuates DSS-induced ulcerative colitis, intestinal inflammation, and barrier function by activating the farnesoid X receptor. Front Pharmacol. 13:1000444.
11. Qin Y, Zhang RY, Zhang Y, Zhao YQ, Hao HF, Wang JP. 2025. Network pharmacology and *in vivo* study: Unraveling the therapeutic mechanisms of Panax ginseng in potentially treating ulcerative colitis. World J Gastroenterol. 31(9):100271.
12. Novak T, Hamedi M, Bergmeier LA, Fortune F, Hagi-Pavli E. 2021. Saliva and serum cytokine profiles during oral ulceration in Behçet's disease. Front Immunol. 12:724900.
13. Motimath A, Shaik S, Udaniya H, Choudhary P, Shah J, Pattnaik N, *et al*. 2025. *In vitro* study on the effect of antioxidants on oral mucosal healing. J Pharm Bioallied Sci. 17(Suppl 2):S1487-S1489.
14. Pan Z, Zhang X, Xie W, Cui J, Wang Y, Zhang B, *et al*. 2024. Revisited and innovative perspectives of oral ulcer: From biological specificity to local treatment. Front Bioeng Biotechnol. 12:1335377.
15. Liu W, Fan X, Jian B, Wen D, Wang H, Liu Z, *et al*. 2023. The signaling pathway of hypoxia-inducible factor in regulating gut homeostasis. Front Microbiol. 14:1289102.
16. Mao H, Zhao X, Sun SC. 2025. NF-κB in inflammation and cancer. Cell Mol Immunol. 22(8):811-839.
17. Kim Me, Lee JS. 2025. Advances in the regulation of inflammatory mediators in nitric oxide synthase: Implications for disease modulation and therapeutic approaches. Int J Mol Sci. 26(3):1204.
18. Fatima I, Sahar A, Tariq A, Naz T, Usman M. 2024. Exploring the role of licorice and its derivatives in cell signaling pathway NF-κB and MAPK. J Nutr Metab. 2024:9988167.
19. Sun A, Yang H, Li T, Luo J, Zhou L, Chen R, *et al*. 2024. Molecular mechanisms, targets and clinical potential of berberine in regulating metabolism: A review focusing on databases and molecular docking studies. Front Pharmacol. 15:1368950.
20. Luo G, Gao M, Lin Q. 2024. Integration of bioinformatics analysis, molecular docking, and animal experiments to study the therapeutic mechanisms of berberine against allergic rhinitis. Sci Rep. 14(1):11999.