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ORIGINAL RESEARCH

Unveiling the Mechanism of Liangxue Siwu Decoction in Treating Rosacea Through Network Pharmacology and in-vitro Experimental Validation

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Background: Rosacea, a recurring dermatological disorder, demands effective therapeutic approaches. Traditional Chinese medicine, particularly Liangxue Siwu Decoction (LXSWD), has shown promise in managing inflammatory skin diseases, such as rosacea. This study focuses on uncovering LXSWD's specific effects on the inflammatory symptoms of rosacea.

Objective: Our research investigates LXSWD's therapeutic effectiveness in rosacea treatment and delves into its underlying mechanisms.

Methods: Network pharmacology was utilized to identify LXSWD's key components and their targets in rosacea management, which were then validated by molecular docking. An in vivo rosacea-like model in LL-37-induced mice was developed, subdividing them into control, model, and LXSWD groups. The LXSWD group received oral administration (25.0 g/kg/day) for six days before model induction. Post-treatment evaluations included skin tissue analyses to verify our network pharmacology predictions.

Results: Key active ingredients in LXSWD, such as quercetin, kaempferol, and luteolin, were identified alongside central target proteins like TNF and MMPs. Our molecular docking study confirmed the interactions between these ingredients and targets. Analyses through GO and KEGG pathways indicated LXSWD's role in mitigating inflammation, particularly influencing the TNF and IL-17 pathways. LXSWD treatment in vivo markedly alleviated LL-37-induced symptoms in mice, showing a marked reduction in inflammatory cytokines ($p < 0.05$) and modulation of crucial genes ($p < 0.05$). These results, supported by immunofluorescence analysis and Western blot, underline the modulatory effects of LXSWD on MMPs, offering significant protection against rosacea's inflammation alterations ($p < 0.05$).

Conclusion: Integrating network pharmacology, molecular docking, and in vivo experiments, this study elucidates LXSWD's potential mechanisms in rosacea treatment. It offers a novel theoretical framework for its clinical use in managing rosacea.

Keywords: Liangxue siwu decoction, molecular docking, network pharmacology, rosacea, skin inflammation

Introduction

Rosacea is a chronic skin disorder that primarily affects the face, characterized by pronounced redness, papules, and pustules. This condition is often mistaken for other skin disorders such as acne, dermatitis, or allergic reactions, and it predominantly occurs in individuals with lighter skin tones.[1,2] Nonetheless, it can occur in people of all skin types.[3] The development of rosacea is believed to result from a combination of genetic and environmental factors.[4,5]

Rosacea's onset is often linked to an overactive immune reaction, activated by a variety of stimuli. These include exposure to certain bacteria, mites, or specific infection that cause facial blood vessels to widen.[6,7] Additionally, several external factors, such as the consumption of hot beverages, spicy foods, alcohol, exposure to extreme temperatures, and emotional stress, can exacerbate rosacea symptoms by increasing blood flow to the skin or sustaining inflammation.

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While rosacea is not a life-endangering condition, it can lead to considerable emotional and psychological discomfort, causing affected individuals to feel self-conscious about their appearance.[8–10]

For centuries, Traditional Chinese Medicine (TCM) has offered effective treatments for a variety of health issues, including skin conditions such as rosacea.[11–13] One of the notable treatments is the Liangxue Siwu Decoction (LXSWD), known for its potential therapeutic effects. According to ancient TCM principles, LXSWD is formulated to "cool the blood" and "nourish the yin", addressing the underlying imbalances believed to contribute to the development of rosacea. LXSWD's application is not limited to rosacea alone; it is also used for treating other inflammatory diseases, showcasing its broad applicability and efficacy.[14]

Among the pivotal components of LXSWD are ingredients such as licorice, scutellariae radix, and carthami flos. Licorice, known as Gancao in Traditional Chinese Medicine (TCM), is well-regarded for its anti-inflammatory properties, with its active component, glycyrrhizin, known to alleviate redness and swelling associated with skin conditions.[15] Scutellariae radix, derived from the root of Scutellaria baicalensis, is rich in flavonoids that demonstrate significant anti-inflammatory and antioxidant effects, making it effective in treating various skin disorders including eczema and psoriasis.[16] Carthami flos, the flower part of the safflower plant, plays a crucial role in promoting blood circulation and pain relief, contributing to its effectiveness in the healing of skin and reduction of inflammation.[17]

In contemporary scientific research, network pharmacology has become an effective method for uncovering the complex therapeutic mechanisms underlying Traditional Chinese Medicine (TCM) formulations like Liangxue Siwu Decoction (LXSWD).[18] This approach involves analyzing the intricate network of biological interactions between the components of LXSWD and their potential targets in the treatment of rosacea, offering a deeper understanding of the decoction's healing properties. Furthermore, molecular docking techniques complement this analysis by offering detailed insights into how the active compounds in LXSWD, such as those present in Gancao, interact with the molecular structures involved in rosacea. These methods together enhance our understanding of how LXSWD may exert its healing properties.[19]

This comprehensive study aims to investigate the potential therapeutic benefits of Liangxue Siwu Decoction in the context of treating rosacea. Utilizing the advanced techniques of network pharmacology and molecular docking, we thoroughly examine the bioactive components of LXSWD and explore how these components may act to combat rosacea and other inflammatory skin conditions. By integrating the principles of Traditional Chinese Medicine (TCM) with modern dermatological research, this study aims to provide new insights into the treatment of skin disorders. The methodology and workflow of this study are thoroughly detailed in Figure 1.

Materials and Methods

Network Pharmacology Analysis

In our research, we harnessed the Traditional Chinese Medicine Systems Pharmacology (TCMSP) database to identify the primary active components of LXSWD and their corresponding targets. Keywords used included the names of key herbs in LXSWD such as "Danggui (DG)", "Chuanxiong (CX)", "Chishao (CS)", "Shudihuang (DH)", "Huangqin (HQ)", "Fuling (FL)", "Chenpi (CP)", "Honghua (HH)", and "Gancao (GC)". We sourced rosacea-related gene targets from databases like GeneCards, DisGeNET, DrugBank, and TTD. The UniProt database facilitated the conversion of target and gene names. Using the VennDiagram package in R, we determined the drug-disease intersection targets. These targets were then visualized in a 'drug-component-target-disease' interaction network diagram using Cytoscape software. Furthermore, the String platform was employed for protein-protein interaction (PPI) analysis, utilizing Cytoscape's Cytohubba plug-in for network topology analysis. Finally, utilizing the ClusterProfiler package in R, we conducted analyses for Gene Ontology (GO) enrichment and pathways in the Kyoto Encyclopedia of Genes and Genomes (KEGG).[20]

Preparation of Liangxue Siwu Decoction (LXSWD)

Danggui (Angelicae Sinensis Radix, 3g, Cat: 220121), Chuanxiong (Chuanxiong Rhizoma, 3g, Cat: 220673), Chishao (Radix Paeoniae Rubra, 3g, Cat: 220522), Shudihuang (Rehmanniae Radix Praeparata, 3g, Cat: 220616), Huangqin (Scutellariae Radix, 3g, Cat: 220501), Fuling (Poria Cocos (Schw). Wolf., 3g, Cat: 220356), Chenpi (Citrus Reticulata, 3g, Cat: 220118), Honghua (Carthami Flos, 3g, Cat: 220237), and Gancao (Licorice, 3g, Cat: 220076) were purchased

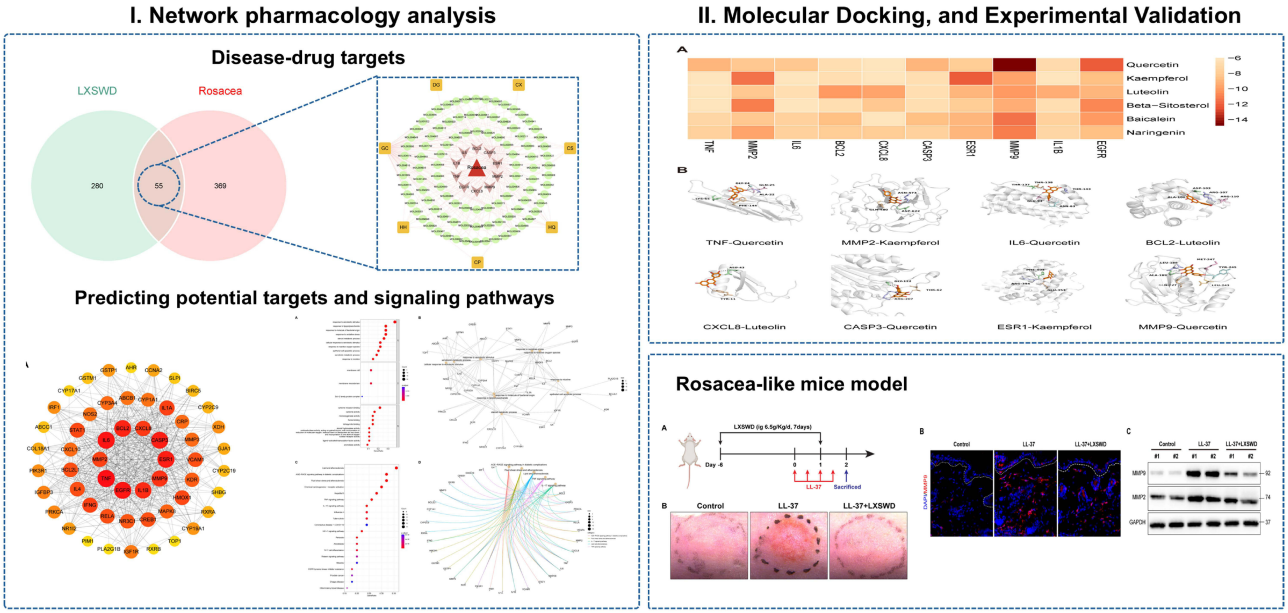

Figure 1 Flowchartchart of the research. Active components and targets of Liangxue Siwu Decoction (LXSWD) for rosacea were gathered from multiple databases. Common targets were identified at the intersection of LXSWD's ingredients and rosacea targets. Key ingredients and overlapping pathways were determined through network and pathway enrichment analyses. Hub genes in common targets were chosen using Cytohubba. LXSWD's mechanisms against rosacea were explored by analyzing these ingredients, pathways, and genes. Results were validated via molecular docking and experimental verification.

from the Guangzhou Traditional Chinese Medicine Hospital. In preparing the LXSWD, initially, the mixture was then soaked in distilled water at a ratio of ten times the total volume of the herbs for 30 minutes to ensure thorough hydration. This was followed by a reflux extraction process lasting two hours. Post-extraction, the liquid portion was separated through filtration, and the remaining solid material underwent a second reflux with eight times its volume in water for one hour. The resultant filtrates from both processes were then combined and concentrated under vacuum conditions to achieve a final extract concentration of 2.5 g/mL. This concentrated extract was preserved at −80 °C for further use.

Animal Experiments

In this study, female BALB/c mice, aged 8 weeks and weighing 18–22 g, were obtained from SLAC Laboratory Animal Co. Ltd. These experiments were conducted in a specific pathogen-free environment. The Animal Ethics Committee of The Third Affiliated Hospital at Sun Yat-sen University approved all experimental methodologies and studies under approval number KY2023-02-27. This study complies with the ARRIVE guidelines and was carried out in accordance with the UK Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments, and the National Research Council's Guide for the Care and Use of Laboratory Animals. LL-37 peptide, with over 95% purity confirmed by HPLC, was sourced from Sangon Biotech. To simulate rosacea-like skin inflammation in mice, we employed a previously established mouse model.[21] In brief, female BALB/c mice were subcutaneously injected with 320 μM LL-37 peptide into the dorsal region every 12 hours for a total of 4 injections. BALB/c mice were segregated into three distinct groups, each consisting of five mice: a control group (Control), which received only distilled water orally daily; a model group (LL-37), subjected to the same distilled water regimen; and an LXSWD group (LL-37+LXSWD), which was orally administered 25.0 g/kg of LXSWD daily. On the concluding day of the experiment, the animals were humanely euthanized, and the skin from their shaved backs was promptly excised for analysis, as illustrated in Figure 2A. We assessed skin inflammation in the mice by evaluating the severity of erythema, using methods outlined in previous studies.[22] In brief, we assessed skin redness on a scale from 1 to 5, with 5 indicating the most intense redness. We quantified the area of redness using measurements from a Leica S8AP0 stereomicroscope (Leica Biosystems). To quantify the average epidermal thickness at the inflamed skin sites, we utilized Image J software (v1.3.1).

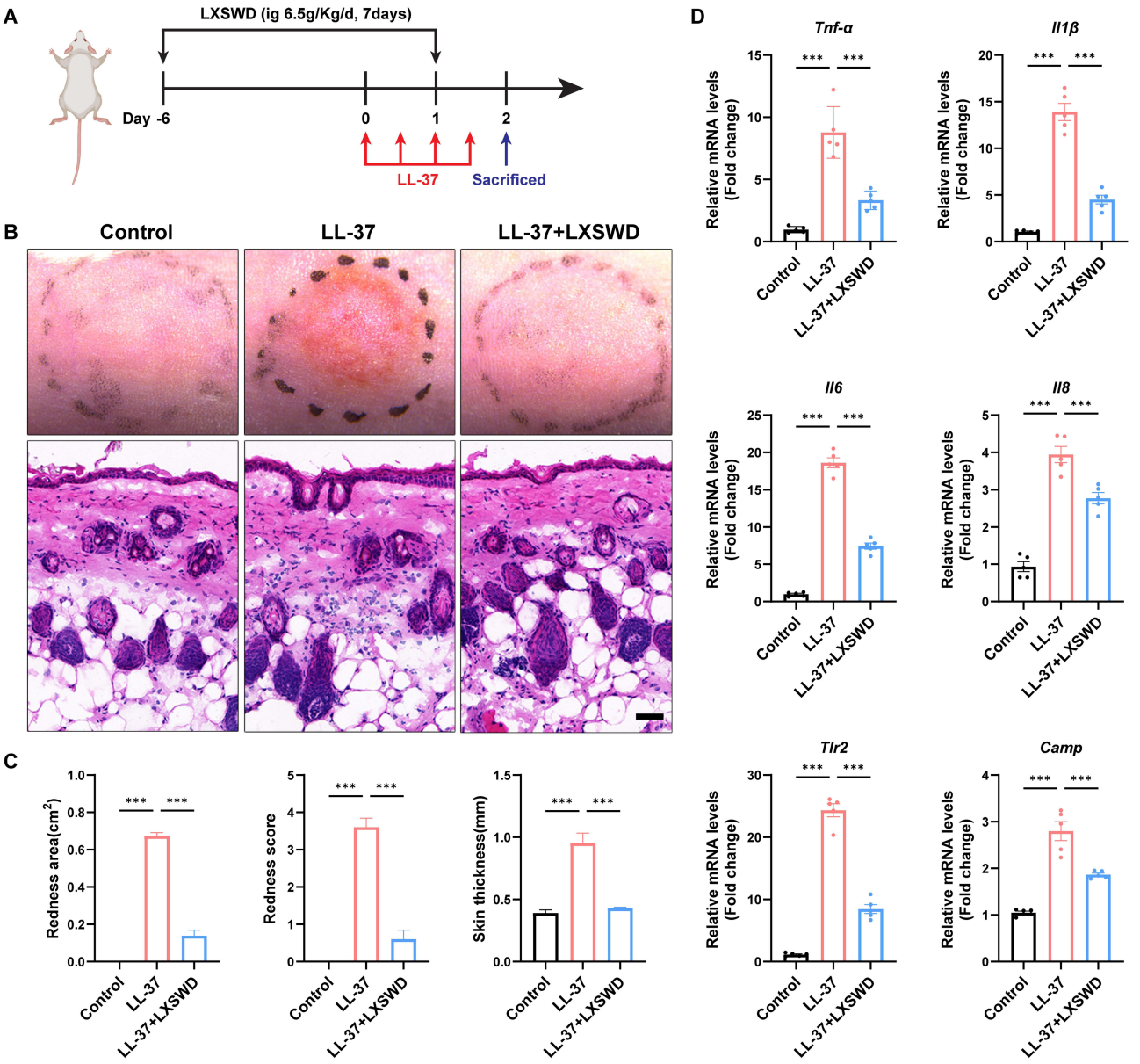

Figure 2 The therapeutic efficacy of LXSWD on rosacea-like mice induced by LL-37 was assessed. (**A**) An illustrative diagram showed continuous oral administration of LXSWD to BALB/c mice for six days before intradermal injection of LL37. (**B**) Skin conditions of mice from different treatment groups were documented. (**C**) The severity of skin inflammation was evaluated through the assessment of skin redness area, redness scoring, and quantification of dermal inflammatory cell infiltration observed in HE staining. (**D**) mRNA expression levels of *Tnf-α*, *Il1β*, *Il6*, *Il8*, *Tlr2*, and *Camp* at the sites of skin lesions were measured in each group consisting of five mice. These results represent a minimum of three independent experiments. Data are presented as mean ± SEM, analyzed using one-way ANOVA with Bonferroni's post hoc test, with significance levels marked as ***$p < 0.001$.

RNA Extraction and Real-Time Quantitative PCR (qPCR)

Using the Trizol reagent (Invitrogen), total RNA was isolated from both lesioned skin tissues and cultured cells, adhering strictly to the guidelines provided by the manufacturer. The concentration and purity of the isolated RNA were assessed using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific), ensuring an A260/A280 ratio between 1.8 and 2.0. For cDNA synthesis, 1 μg of total RNA was utilized, employing the Maxima H Minus First Strand cDNA Synthesis Kit, which includes dsDNase (Thermo Fisher Scientific). The qPCR analyses were conducted utilizing iTaq™ Universal SYBR® Green Supermix (Bio-Rad) on the CFX Connect Real-Time PCR System (Bio-Rad). The specific primers used for qPCR are detailed in Supplementary Data Table 1. Relative gene

expression levels were calculated using the 2^−ΔΔCt method, with GAPDH serving as the internal control for normalization. Each sample was analyzed in triplicate to ensure the reliability of the results.

Histological Analysis

Skin tissues from the dorsal region of mice were initially rinsed in Phosphate-Buffered Saline (PBS). Subsequently, they were fixed in a 4% formaldehyde solution for an entire night and later embedded in either paraffin or OCT compound. Thin slices of these tissues, measuring 6 μm in thickness, were then processed for staining. We utilized the traditional Haematoxylin and Eosin (H&E) staining method, as described in prior literature.[22]

For the immunofluorescence studies, both human and mouse skin sections, preserved in OCT, were cut into sections 8 μm thick. These sections underwent a series of preparatory steps, beginning with a PBS wash, followed by a 15-minute fixation in 4% frozen paraformaldehyde (PFA). Blocking of the sections was performed for an hour using a PBS solution containing 1% Bovine Serum Albumin (BSA) and 0.3% Triton X-100. We then proceeded to an overnight incubation at 4°C with primary antibodies. After the overnight incubation, the sections were thoroughly washed with PBS to remove unbound primary antibodies. They were then incubated for one hour at room temperature with fluorophore-conjugated secondary antibodies, which were also diluted in the blocking solution. DAPI staining was employed for nuclear visualization. We captured all images using a Zeiss fluorescence microscope and conducted the analysis with Zen2 software, which originates from Germany.

For these analyses, we utilized the Anti-MMP9 antibody (dilution ratio of 1:1000, Abcam, cat no. ab283575) and Alexa Fluor 594-conjugated goat anti-rabbit IgG (H+L) cross-adsorbed secondary antibody (dilution ratio of 1:500, Invitrogen, cat no. A-11037).

Immunoblotting

Skin tissue samples and harvested cells underwent lysis using RIPA buffer (sourced from Thermo Fisher Scientific) supplemented with protease inhibitors, after initial washing with chilled PBS. Subsequent to lysis, protein quantification was conducted using the BCA method, also provided by Thermo Fisher Scientific. The proteins thus isolated were then subjected to separation through SDS-polyacrylamide gel electrophoresis, followed by transfer onto PVDF membranes. Following the transfer, the membranes were soaked in a 5% non-fat milk solution at room temperature for one hour to prevent non-specific binding. Then, they were incubated first with primary antibodies, followed by HRP-conjugated secondary antibodies. We used an HRP substrate from MilliporeSigma for detecting immunoreactive bands and visualized them using a ChemiDocTM XRS+ system from Bio-Rad. For this process, anti-MMP2 (Abcam, cat no. ab92536), MMP9 (Abcam, cat no. ab283575), and GAPDH (Abcam, cat no. ab8245), each diluted at a ratio of 1:1000, were employed.

Molecular Docking

Molecular docking was conducted on specific active compounds and central genes to confirm the precision of our predicted targets. The corresponding PubChem IDs of quercetin, kaempferol, luteolin, beta-sitosterol, baicalein, and naringenin were retrieved from PubChem database. The 3D protein structures of TNF, MMP2, IL6, BCL2, CXCL8, CASP3, ESR1, MMP9, IL1B, and EGFR were obtained from the Protein Data Bank (PDB). During the process, we meticulously prepared the receptor protein by eliminating water molecules and the initial small molecule ligand. This preparation step ensured that the docking environment was free from any unnecessary interactions that might interfere with the accuracy of our results. Additionally, we introduced non-polar hydrogen atoms to the receptor protein to enhance the precision of the docking simulations. Appropriate charges were also assigned to the receptor to accurately reflect its electrostatic properties. We employed QuickVina2, an enhanced version of AutoDock Vina, for the docking studies. The exhaustiveness parameter, which controls the thoroughness of the search, was set to 24 to ensure a comprehensive exploration of the binding conformations. Additionally, the ligand binding pockets were predicted using the DeepSite web server, which provided precise identification of potential binding sites on the target proteins. Binding stability was assessed based on binding energy values, with a benchmark of −6.0 kcal/mol established as the criterion for effective

receptor-ligand interactions. The 3D and 2D representation of molecular docking results were visualized using PyMOL software and Ligplot+ software, respectively.

Statistical Analysis

Statistical analysis was conducted with GraphPad Prism (v8.0.0). Data from three independent experiments were represented as the mean ± SEM. For comparisons between two independent groups, we employed the unpaired Student's *t*-test. When assessing multiple groups, a one-way ANOVA was utilized, followed by Dunnett's test to control for multiple testing errors. We established a *p*-value of less than 0.05 as the threshold for determining statistical significance.

Results

LXSWD Mitigated Rosacea-Like Dermatitis Induced by LL-37

While it is established that LXSWD has anti-inflammatory properties in a range of illnesses, its specific mechanism in treating rosacea remain elusive. Therefore, we pretreated female BALB/c mice with LXSWD intraperitoneally for 6 days before constructing the LL-37-induced rosacea-like mouse model as the LXSWD intervention group (Figure 2A). As shown in Figure 2B, the application of LXSWD notably improved the condition of rosacea-like lesions caused by LL-37. There was a notable decline in both the extent of redness and severity scores in subjects treated with LXSWD, as opposed to the Control group. Histological examinations indicated a substantial decrease in the infiltration of immune cells in the skin's dermal layer following LXSWD treatment (Figure 2B and 2C). Meanwhile, LXSWD treatment also reduced the expression of pro-inflammatory cytokines, including *Tnf-α, Il1β, Il6, Il8, Tlr2*, and *Camp* in LL-37-induced rosacea-like lesions (Figure 2D). The findings showed LXSWD's therapeutic potential in managing dermatitis in mice that resembles rosacea.

Potential Targets of Active Ingredients

With the criteria of OB (Oral bioavailability) ≥ 30% and DL (Drug-like properties) ≥ 0.18,[23] a total of 210 active components in LXSWD were identified via TCMSP: 2 from DG, 7 from CX, 29 from CS, 2 from DH, 36 from HQ, 15 from FL, 5 from CP, 22 from HH, and 92 from GC. Comprehensive details about these active ingredients can be found in Supplementary Data Table 2. We compiled and forecasted the targets for the 210 active ingredients using the TCMSP database and Swiss Target Prediction servers. Following the elimination of duplicate targets, we identified 335 potential targets for the 210 active compounds, the details of which are available in Supplementary Data Table 3.

Common Targets–Active Ingredients Network

The pathogenesis of rosacea involves a multifaceted interplay of numerous genes and proteins, making its progression complex.[24] An analysis of GeneCards, DisGeNET, DrugBank, and TTD led to the identification of 424 targets associated with rosacea, as detailed in Supplementary Data Table 4. A comparative study between LXSWD's targets and those linked to rosacea revealed a commonality of 55 targets between rosacea and the active compounds of LXSWD, illustrated in Figure 3A. These 55 targets correlate with 112 active ingredients, enumerated in Supplementary Data Table 5. Figure 3B depicts the network of common targets and active ingredients. Our analysis identified six key active ingredients with a degree value exceeding 12, specifically quercetin (MOL000098), kaempferol (MOL000422), luteolin (MOL000006), beta-sitosterol (MOL000358), baicalein (MOL002714), and naringenin (MOL004328).

Common Targets Enrichment Analysis

Investigating the mechanism of LXSWD against rosacea, our analysis using ClusterProfiler package in R software conducted GO and KEGG enrichment analysis for the 55 shared targets. This analysis resulted in 1268 biological processes (BPs), 3 cellular components (CCs), 84 molecular functions (MFs), and 138 KEGG pathways, all with significant values ($p \leq 0.05$) (Supplementary Data Tables 6 and 7). Figure 4 displays the 10 most notable terms in

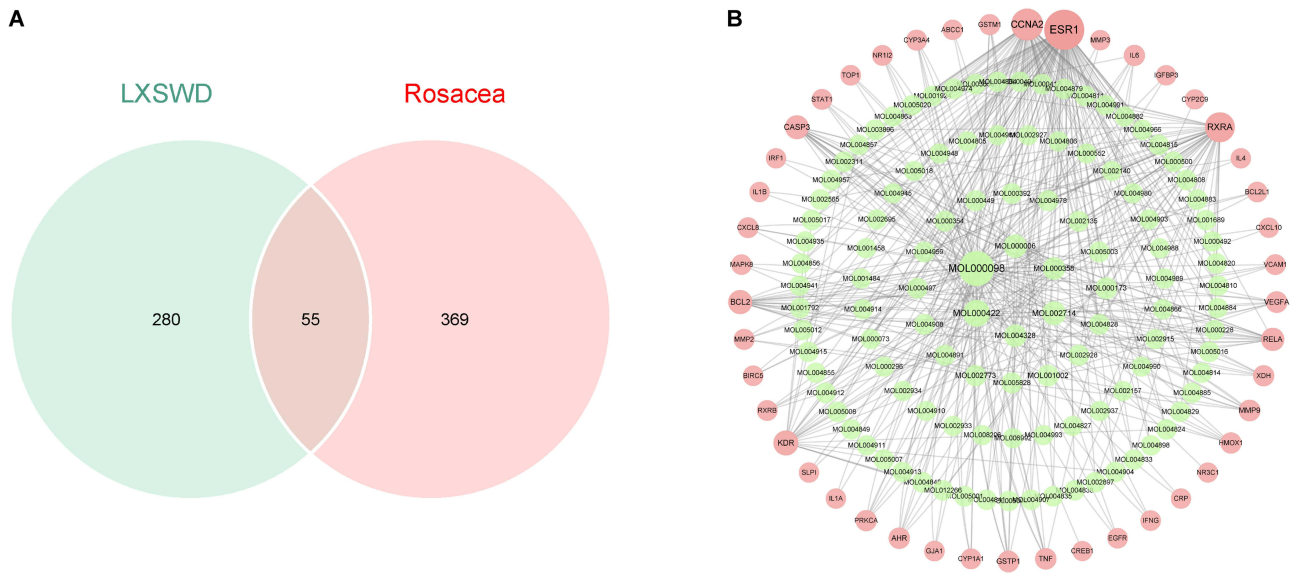

Figure 3 Common targets and common targets-active ingredients network. (**A**) Venn diagram illustrating the common targets of LXSWD and rosacea. (**B**) Common targets–active ingredients network.

BPs, MFs, CCs, and the 20 key KEGG pathways. The GO enrichment analysis revealed that prevalent targets predominantly involve processes linked to inflammation, bacterial interactions, and oxidative stress. These include reactions to oxidative stress, bacterial molecules, and the control of inflammatory responses (Figure 4A). The primary pathways identified among the common targets are those related to lipid and atherosclerosis, the TNF and IL-17 signaling pathway (Figure 4B). The 55 common targets showed significant enrichment in cytokine signaling, inflammatory-, and immune-related signaling pathways (Figure 4C). Furthermore, as depicted in Figure 4C, five distinct models demonstrated enrichment in cytokine signaling-, bacterium-, hormone-, inflammatory response- and immune-related signaling pathways. The findings suggested that pathways related to inflammation and immune functions play a vital role in the development of atopic dermatitis and rosacea.

Network of Disease Hub Genes, Active Compounds, and Herbs

For identifying key genes of LXSWD in rosacea therapy, the 55 common targets were integrated into the String database to establish a PPI network. This network was then analyzed using Cytoscape with the Cytohubba plug-in to pinpoint critical genes. Hub genes were determined based on the MCC method's top 10 nodes in this network, specifically TNF, MMP2, IL6, BCL2, CXCL8, CASP3, ESR1, MMP9, IL1B, and EGFR (Figure 5A). The network, encompassing disease targets, active compounds, and herbs, was developed in relation to the hub genes (Figure 5B). It features 117 nodes and 253 edges, encompassing 7 herbal components, 99 active compounds, 10 hub genes, and a single disease. Among the key compounds, quercetin (MOL000098), kaempferol (MOL000422), luteolin (MOL000006), beta-sitosterol (MOL000358), baicalein (MOL002714), and naringenin (MOL004328) emerged as significant within the network of common targets and active ingredients. These compounds potentially formed the foundational materials for LXSWD's effectiveness in rosacea treatment.

Validation by Molecular Docking

Based on the degree values derived from the PPI network analysis, the foremost 10 targets and 6 essential ingredients were selected for molecular docking studies. Binding stability between receptors and ligands is governed by their binding energy. A lower binding energy signifies greater stability in the receptor-ligand conformation. We have established −6.0 kcal/mol as the benchmark for assessing the efficacy of receptor-ligand binding.[25] Consequently, we identified the most effective ligand (having the lowest binding energy) for receptor binding, using binding energy as the primary criterion. The distribution of the docking score was illustrated as heat maps (Figure 6A). The outcomes of this screening are

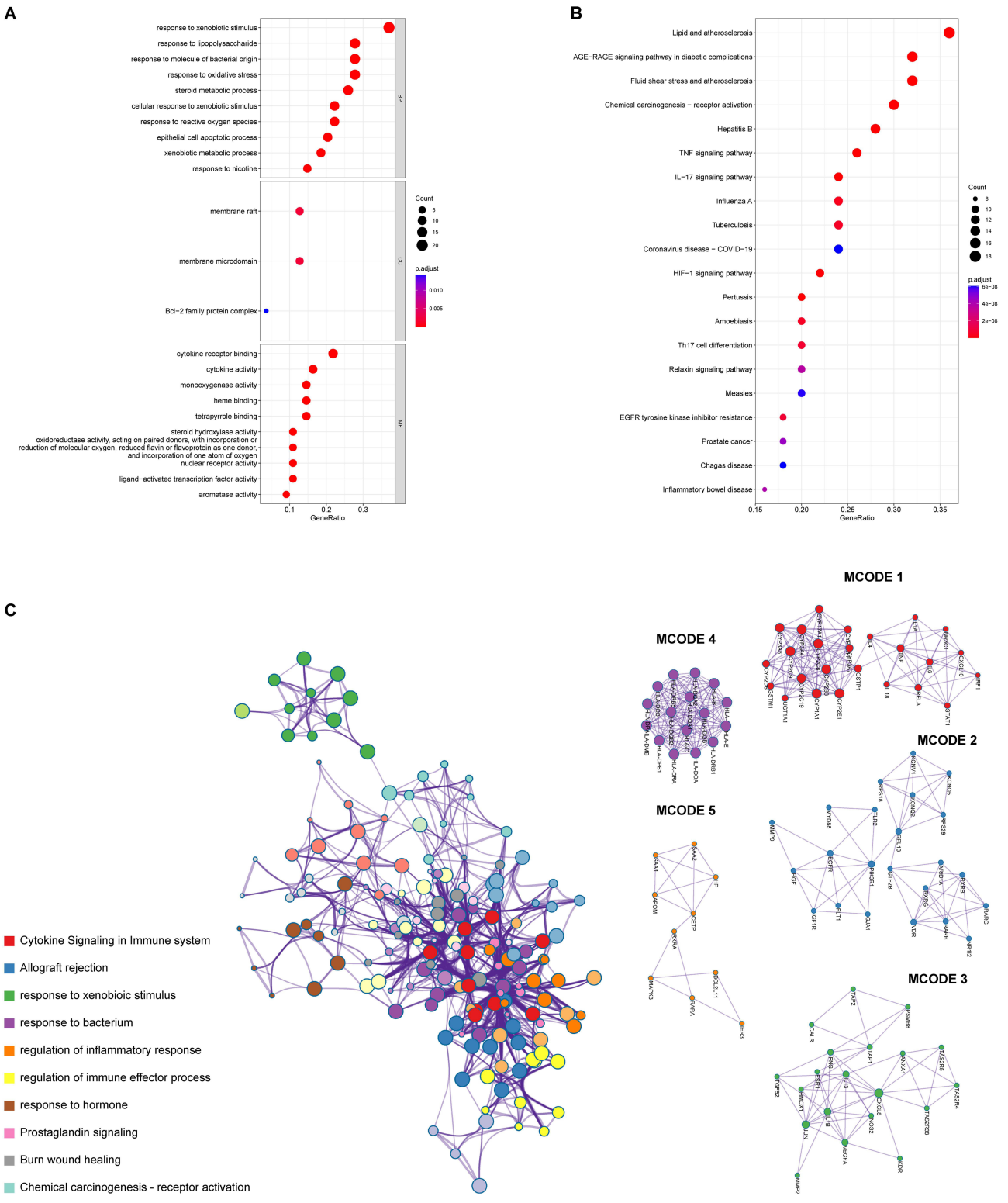

Figure 4 Analysis of Enrichment for Common Targets. (**A**) GO enrichment's top 10 significantly enriched terms. (**B**) Top 20 KEGG pathways ranked by the lowest adjusted p-values. (**C**) The enrichment analysis of common Targets using the Metascape database and MCODE components detected in the analysis of common targets.

detailed in Table 1, while Figure 6B and Supplementary Data Figure 1 illustrated the interactions between the receptors and ligands. The comprehensive results of the docking experiments can be found in Supplementary Data Table 8. The outcomes suggested enhanced affinity of the active compounds in interacting with protein targets.

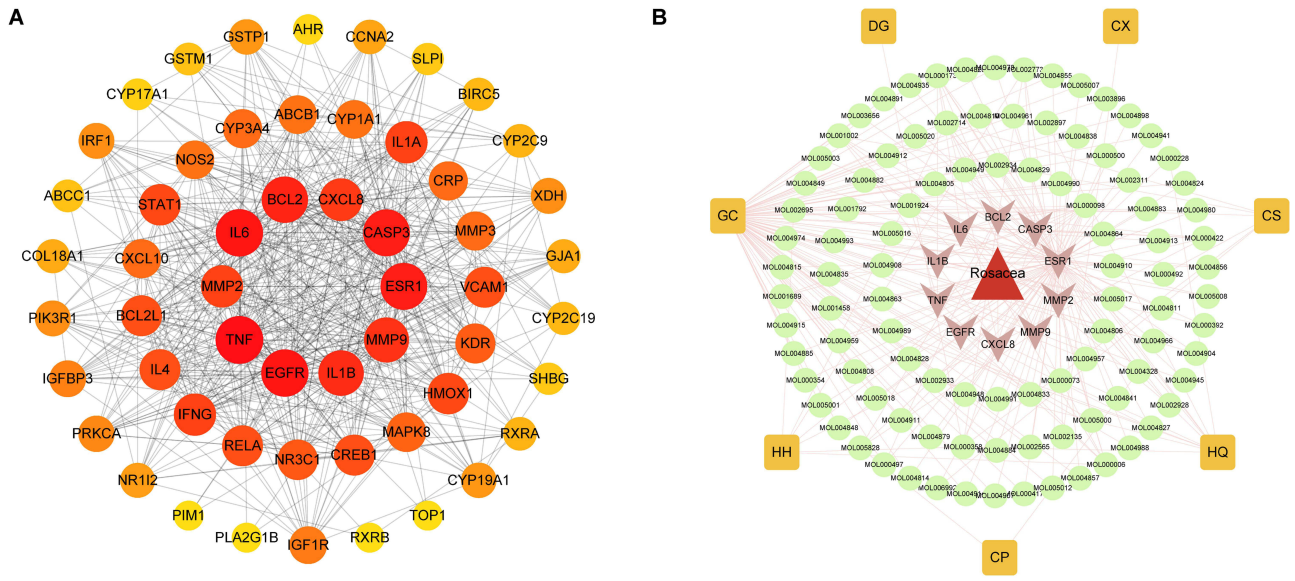

Figure 5 Network of disease-hub genes-active ingredients-key herbs. (**A**) Protein-protein interaction network featuring 55 common targets. (**B**) A network mapping disease, hub genes, active ingredients, and herbs, where triangle nodes denote disease, arrow-like nodes signify hub genes, circles represent active ingredients, and squares indicate herbs.

LXSWD Regulated the Expression of Rosacea-Characteristic Cytokines and Chemokines and Hub Genes

Skin lesions in rosacea patients and in the LL-37-induced rosacea-like mouse model exhibit heightened levels of proinflammatory cytokines and chemokines.[26] The analysis revealed that the skin lesions in mice treated with LL-37 displayed notably increased mRNA expressions of various cytokines and chemokines (*Ccl2, CCl5, Ccl20, Cxcl10*, and *Cxcl12*), which was reversed in LXSWD-treated mice (Figure 7A). Animal studies were conducted to elucidate how LXSWD affects hub gene expression. Figure 7A indicated increased levels of *Cxcl8, Esr1, Egfr, Mmp2*, and *Mmp9* in the LL-37-induced group relative to the control group, a reversal observed with LXSWD treatment. Chronic inflammation plays a critical role in rosacea, with MMPs being key elements in the inflammatory process.[27] Immunofluorescence analysis, detailed in Figure 7B, revealed marked elevation of MMP9 in the skin of mice subjected to LL-37, while those treated with LXSWD exhibited lower levels. Western blot results indicated a rise in MMP2 and MMP9 following LL-37 exposure, a trend mitigated upon LXSWD administration (Figure 7C). Conclusively, LXSWD showed potential in mitigating LL-37's impact on rosacea-like skin inflammation, notably through the downregulation of cytokines, chemokines, and hub genes.

Discussion

Rosacea is a chronic dermatological condition with significant impact on quality of life. Currently, the treatment of rosacea, particularly when triggered by environmental factors and immune response, remains challenging. As the condition progresses, it often necessitates comprehensive management strategies, emphasizing the need for effective treatments that address its multifaceted nature.[28]

Liangxue Siwu Decoction (LXSWD), derived from traditional Chinese medical formulations, has been historically utilized in the treatment of various inflammatory skin conditions. Components including Gancao (licorice) found in LXSWD, as recorded in historical manuscripts such as "Golden Mirror of Medicine" present complexities in their chemical structure. This complexity poses a significant challenge in understanding the detailed pharmacological actions of LXSWD's active ingredients, even with its extensive medicinal applications.

The synergy model of "multi-component, multi-target, multi-biological regulatory function" in traditional Chinese medicine is effectively studied using network pharmacology and molecular docking.[29] These techniques are becoming increasingly prominent in TCM research and in formulating novel therapeutic approaches. This study utilizes a blend of

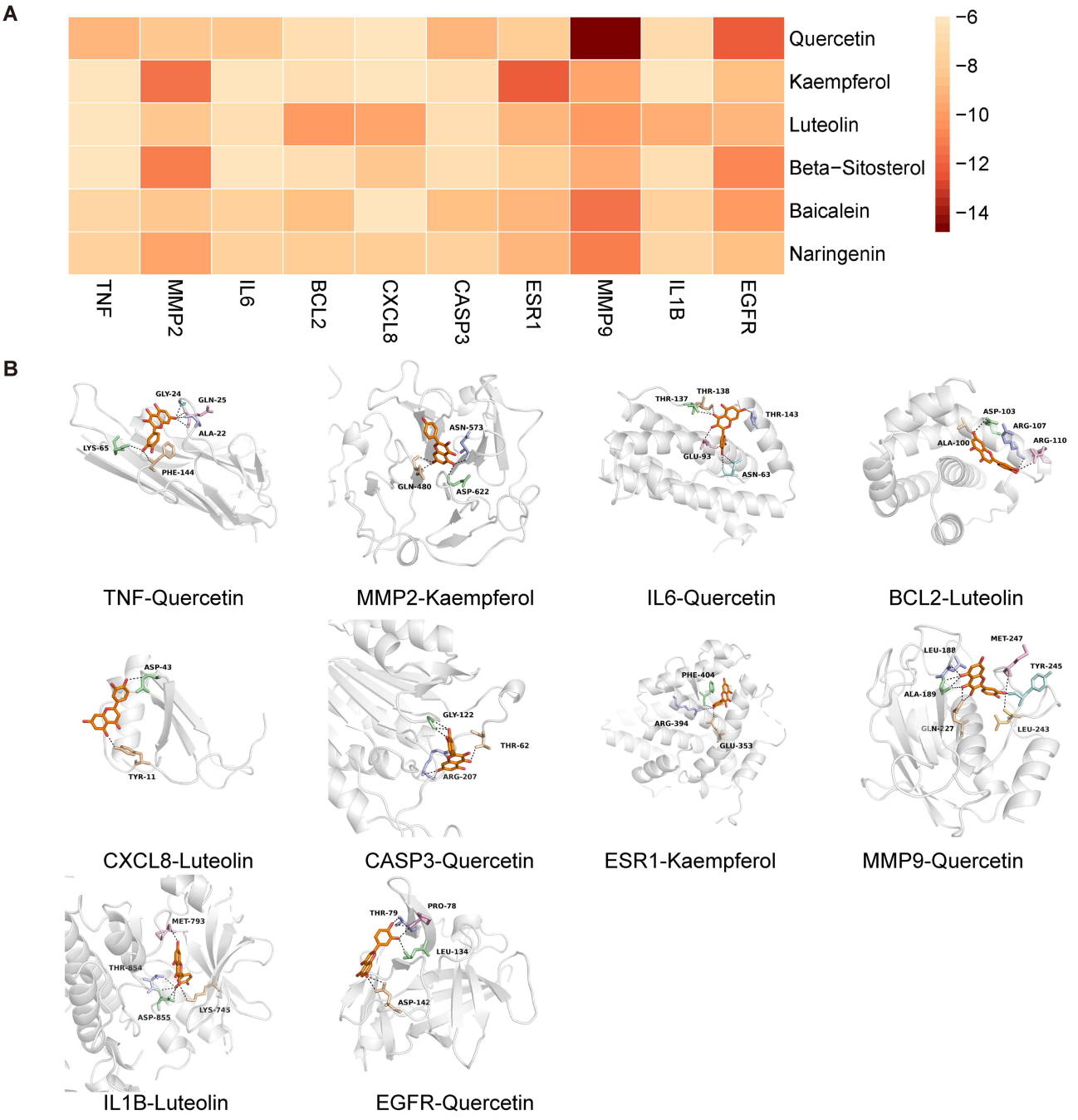

Figure 6 Results of Molecular Docking Analysis. (**A**) Docking score heatmap illustrating the interaction between LXSWD's active ingredients and hub genes. (**B**) Three-dimensional visual representation of the molecular docking interactions between active ingredients and hub targets.

network pharmacology, molecular docking, and empirical verification to methodically examine LXSWD's bioactive elements and their effectiveness in managing rosacea.

Our results indicate that LXSWD treatment significantly ameliorated LL-37-induced rosacea-like lesions in mice. The treatment resulted in a notable decline in both the extent of redness and severity scores. Histological examinations revealed a substantial decrease in the infiltration of immune cells in the dermal layer of the skin following LXSWD treatment. This was accompanied by a reduction in the expression of pro-inflammatory cytokines including Tnf-α, Il1β, Il6, Il8, Tlr2, and Camp in LL-37-induced rosacea-like lesions. These findings underline the modulatory effects of LXSWD on inflammatory cytokines and genes, supporting its potential as an effective treatment for rosacea.

Table 1 Screening Docking Results Between Ligands and Receptors

Hub targets (PDB ID)	Active ingredients	Binding energy (Kcal/mol)
TNF (2AZ5)	Quercetin	−8.9
MMP2 (1CK7)	Kaempferol	−11.3
IL6 (1ALU)	Quercetin	−8.4
BCL2 (2XA0)	Luteolin	−9.9
CXCL8 (6N2U)	Luteolin	−9.7
CASP3 (1NMS)	Quercetin	−9
ESR1 (1SJ0)	Kaempferol	−12.1
MMP9 (6ESM)	Quercetin	−14.8
IL1B (1T4Q)	Luteolin	−9.3
EGFR (1XKK)	Quercetin	−12.1

Numerous overlapping targets were identified in the active compounds of LXSWD, suggesting its therapeutic efficacy in rosacea through the synergistic action of these constituents. A total of 210 active components were identified, leading to 335 potential targets. Comparative analysis with rosacea-related targets revealed 55 common targets, involving 112 active ingredients. Key active ingredients such as quercetin, kaempferol, luteolin, beta-sitosterol, baicalein, and naringenin were highlighted for their significant roles. Flavones, key substances in Scutellariae radix, suppress IL-8 and IL-1β production, mitigating skin inflammation caused by P. acnes.[30] Hydroxysafflor yellow A (HSYA), the main component of Carthami Flos, reduces inflammatory cytokines (MDC, IL-8, IL-6) by inhibiting NF-κB pathway and p38 MAPK phosphorylation, thus decreasing inflammatory cell infiltration. HSYA also modulates downstream signaling pathways by acting on cell membranes.[31–33] Licochalcone and Dehydroglyasperin D (DHGA-D) from licorice exhibit antimicrobial and anti-inflammatory properties. Licochalcone effectively reduces IL-12p40 expression, ear thickness, and inflammation in chronic allergic contact dermatitis.[34] DHGA-D suppresses COX-2 expression by inhibiting MLK3, demonstrating its potential as an anti-inflammatory agent.[35]

Furthermore, GO and KEGG pathway analyses suggest that LXSWD predominantly impacts the development and progression of rosacea via the TNF and IL-17 signaling pathways. The abnormal activation of these pathways may accelerate rosacea's progression by promoting inflammation, cellular proliferation, and angiogenesis, exacerbating the disease. The key to rosacea treatment lies in curbing excessive cytokine production and modulating angiogenesis to alleviate inflammation.[22,36] The GO enrichment analysis revealed that the 55 shared targets are significantly involved in biological processes related to inflammation, bacterial interactions, and oxidative stress, which are critical factors in the pathophysiology of rosacea. Specifically, the identification of key pathways such as lipid and atherosclerosis, alongside the TNF and IL-17 signaling pathways, highlights the multifaceted approach of LXSWD in addressing the underlying causes of rosacea. PPI analysis identified key targets such as TNF, MMP2, IL6, BCL2, CXCL8, CASP3, ESR1, MMP9, IL1B, and EGF as central to LXSWD's efficacy in countering LL-37-induced rosacea. These hub genes, particularly TNF, MMP9, IL1B, and IL6, are critically involved in the inflammatory processes that characterize rosacea. The network's structure, consisting of 10 nodes and 45 edges with a significant p-value, underscores the importance of these targets in the therapeutic mechanism of LXSWD. Molecular docking further substantiated the binding affinity of the primary compounds in LXSWD to the core proteins identified in our study. Specifically, the docking analysis revealed that the selected active ingredients, including quercetin, kaempferol, luteolin, beta-sitosterol, baicalein, and naringenin, exhibited strong binding affinities with key targets such as TNF, MMP2, IL6, and MMP9. The detailed molecular interactions, as visualized in heat maps, highlighted that these active compounds not only interact with the proteins but also do so with high specificity and stability. The binding stability between these receptors and ligands, as indicated by binding energy values lower than −6.0 kcal/mol, underscores the potential of these compounds to modulate the biological activities of these proteins effectively.

The efficacy of network pharmacology in predicting outcomes was validated through in vivo studies, demonstrating that LXSWD decoction has potential in rosacea treatment. Its primary mechanism appears to be the modulation of both

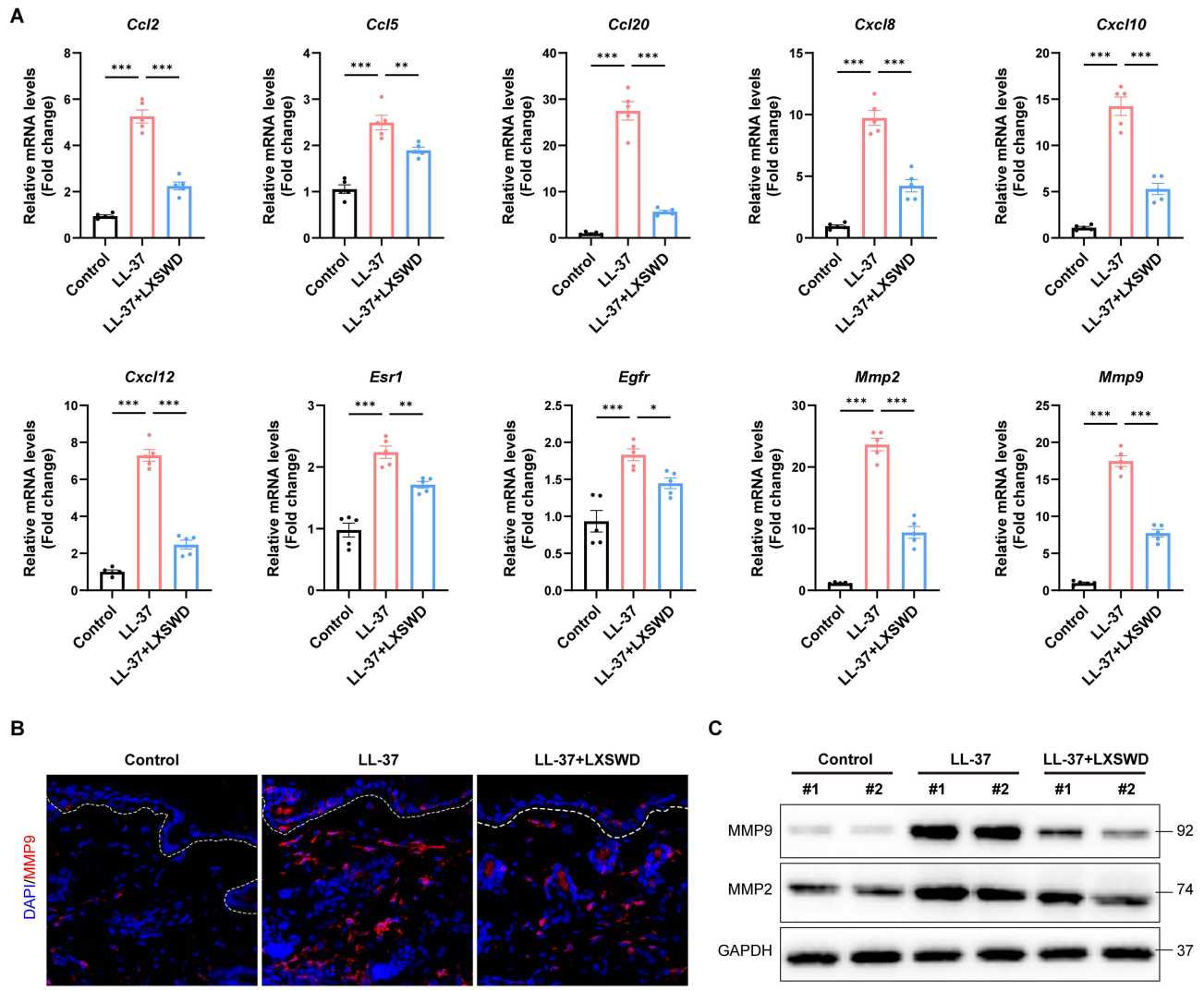

Figure 7 The efficacy of LXSWD in modulating key inflammatory markers and hub genes associated with rosacea. (**A**) Demonstrates the reversal of elevated mRNA expressions of cytokines and chemokines in LL37-induced mice by LXSWD treatment. (**B**) Depicts immunofluorescence analysis of MMP9 expression in the skin of LL-37-induced mice. (**C**) Shows Western blot analysis revealing the effects of LXSWD on MMP2 and MMP9 levels post-LL37 exposure. Data are presented as mean ± SEM, analyzed using one-way ANOVA with Bonferroni's post hoc test, with significance levels marked as *p < 0.05, **p < 0.01, and ***p < 0.001.

pro-inflammatory and anti-inflammatory cytokines, leading to an anti-inflammatory response. Recent advancements in dermatological research have shed light on the intricate relationship between matrix metalloproteinases (MMPs) and the pathogenesis of rosacea. The molecular mechanisms underlying MMPs' role in rosacea involve complex interactions with various cellular and molecular pathways.[27] For instance, recent research highlights the interplay between MMPs and the cathelicidin antimicrobial peptide (CAMP), a known factor in rosacea. CAMP, upon cleavage by MMPs, generates pro-inflammatory peptides that further stimulate the production of MMPs, creating a feedback loop that amplifies skin inflammation.[37] Additionally, oxidative stress and UV radiation, common triggers of rosacea, have been found to upregulate MMP expression, particularly MMP-2 and MMP-9. This upregulation exacerbates extracellular matrix (ECM) degradation, leading to increased skin sensitivity and the perpetuation of the inflammatory cycle.[38] The role of MMPs in angiogenesis, another critical aspect of rosacea pathophysiology, has also been explored. MMPs facilitate the release of angiogenic factors, contributing to the development of telangiectasia (visible blood vessels) seen in many rosacea patients.[39]

LXSWD's efficacy, as observed in our study, may stem from its ability to regulate MMP expression. For instance, ingredients like Gancao (licorice) contain compounds known for their anti-inflammatory properties, potentially inhibiting

MMP activity and thus reducing inflammation and ECM degradation.[40] Similarly, Huangqin (Scutellariae Radix) possesses bioactive flavonoids that can suppress inflammatory cytokines and, consequently, MMP expression.[41] Our results demonstrated that LXSWD treatment significantly ameliorated LL-37-induced rosacea-like lesions in mice, a process likely mediated through the inhibition of MMPs. By reducing MMP-induced ECM breakdown and inflammatory cell infiltration, LXSWD alleviates the typical symptoms of rosacea, such as redness and vascular instability.

Nevertheless, this study is not without limitations. First, the scope of public databases may limit the inclusivity of some compounds and target genes, a general constraint in network pharmacology research. Second, the clinical applicability of LXSWD in treating rosacea needs further validation. Third, the significance of MMPs in rosacea's pathology, as highlighted in this study, necessitates further exploration through in vitro and in vivo research.

Conclusion

In summary, this research underlines the significant anti-inflammatory effects of LXSWD, notably through the suppression of TNF and IL-17 signaling pathways and its interaction with MMPs. The findings indicate that LXSWD holds promise as an TCM treatment for rosacea. Our study pioneers a thorough analysis of LXSWD's key compounds, targets, and pathways relevant to rosacea management, utilizing network pharmacology, molecular docking, and experimental validation. These findings offer a foundation for further research into LXSWD's mechanisms and provide insights for therapy in the management of rosacea.

Abbreviations

DLm, Drug-like properties; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; LXSWD, Liangxue Siwu Decoction; MMP, Matrix metalloproteinase; OB, Oral bioavailability; PPI, Protein-protein interaction; TCM, Traditional Chinese Medicine; TNF, Tumor necrosis factor.

Data Sharing Statement

The data analyzed in this study are included in the websites mentioned above. The original contributions from this study can be found in the article and the Supplementary Material section. For further information or inquiries, please contact the corresponding author.

Ethics Approval and Consent to Participate

The data utilized in this study were sourced from previously published research, which had received approval from the respective ethics committees, eliminating the need for additional ethical clearance for this study. The animal research was duly reviewed and approved by The Animal Ethics Committee of The Third Affiliated Hospital at Sun Yat-sen University.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare no conflicts of interest.

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