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ORIGINAL RESEARCH

Combining Network Pharmacology, Molecular Docking, Molecular Dynamics Simulation, and Experimental Validation to Uncover the Efficacy and Mechanisms of Si-Miao-Yong-An Decoction in Diabetic Wound Healing

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Purpose: Si-Miao-Yong-An (SMYA) Decoction, a traditional Chinese herbal mixture, shows promise for managing diabetic complications. Up to this point, no reports have explored the effects of SMYA on diabetic wounds or the underlying mechanisms. This study aimed to investigate the therapeutic potential of SMYA in promoting diabetic wound healing and to elucidate the underlying molecular mechanisms.

Methods: The wound healing effects of SMYA were evaluated in db/db diabetic mice by measuring wound closure rates and histological characteristics, including epidermal thickness and collagen deposition. Network pharmacology was utilized to identify active ingredients and corresponding therapeutic targets of SMYA, followed by validation through molecular docking and molecular dynamics simulations. KEGG and GO enrichment analyses were conducted to elucidate the relevant biological processes and pathways. In vitro studies involving high-glucose-treated HUVECs assessed the effects of SMYA-containing serum on cellular migration and angiogenesis. Finally, the expression of inflammatory factors and RAGE in the wound tissue was detected by qRT-PCR.

Results: SMYA significantly accelerated wound closure in db/db mice, as evidenced by improved epidermal thickness, tissue morphology, and collagen deposition. Network pharmacology identified 140 overlapping genes involved in angiogenesis and inflammation, with the AGE-RAGE signaling pathway playing a central role. Molecular docking and dynamics simulations revealed strong binding stability of quercetin and kaempferol to inflammation-related hub targets, including IL-6, TNF, and IL-1β. In vitro, SMYA-containing serum alleviated high-glucose-induced impairments in HUVEC migration and angiogenesis. Furthermore, qRT-PCR analysis showed that SMYA significantly downregulated Tnf, Il1b, Il6, and Rage expression in wound tissues, supporting its anti-inflammatory effect.

Conclusion: SMYA promotes diabetic wound healing by modulating the inflammatory microenvironment and inhibiting the AGE-RAGE signaling pathway. These findings provide robust evidence for SMYA's therapeutic potential and lay a foundation for its future clinical application in treating diabetic wounds.

Keywords: Si-Miao-Yong-An decoction, diabetic wound, network pharmacology, molecular docking, molecular dynamics simulation, inflammation

Introduction

Diabetes mellitus is a chronic metabolic disorder of the endocrine system, primarily characterized by elevated blood glucose levels. It often leads to a range of complications, including retinopathy, neuropathy, renal failure, cardiovascular disease, and diabetic foot ulcers.[1] The healing of skin wounds in diabetic patients is notably impaired due to

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several factors, including increased oxidative stress, disrupted energy metabolism, chronic inflammation, impaired angiogenesis, epidermal neural injury, wound hypoxia, and reduced secretion of growth factors.[2,3] Recent studies have emphasized the critical roles of inflammation, extracellular matrix remodeling, and other underlying factors in wound repair, further highlighting the complexity of the healing process in diabetic patients.[4] Additionally, some biomaterials show promise in promoting wound healing by modulating inflammatory responses and supporting tissue regeneration.[5] These findings highlight that optimizing wound healing requires addressing both the inflammatory phase and tissue regeneration. However, the complex nature of wound healing presents significant challenges in the development of effective therapies.

Numerous studies have suggested that traditional Chinese medicine could provide novel targets for addressing chronic conditions and metabolic syndrome.[6,7] Si-Miao-Yong-An (SMYA) decoction comprises four herbs: *Lonicera japonica* (Jinyinhua), *Scrophularia ningpoensis* (Xuanshen), *Angelica sinensis* (Danggui), and *Glycyrrhiza uralensis* (Gancao). SMYA has shown promising effects in improving insulin sensitivity and alleviating diabetes-related complications, including diabetic retinopathy and peripheral vascular disease.[8,9] These studies indicate that SMYA enhances insulin sensitivity and mitigates symptoms such as increased water and food consumption, elevated blood glucose levels, and higher serum glucagon levels in diabetic mice.[8] Additionally, SMYA has been shown to attenuate the progression of diabetic retinopathy by inhibiting retinal inflammation and angiogenesis through the modulation of the NFκB-TNFα and HIF1α-VEGF signaling pathways.[9] These findings underscore the potential of SMYA in treating diabetic complications. However, limited studies have specifically investigated the effects of SMYA on diabetic wound healing. Its direct role in this process, along with the molecular pathways involved, remains incompletely explored.

This gap in the literature highlights the novelty of our study, which provides a systematic investigation of SMYA's effects on wound healing in diabetic mice. By using a combination of in vivo, in vitro, and bioinformatics approaches, we aim to clarify the mechanistic pathways involved and establish SMYA as a potential therapeutic agent for diabetic wound management. The protocol of our study is shown in Figure 1.

Materials and Methods

Preparation of SMYA

The herbs utilized in this research were obtained from Sinopharm Group Co., Ltd. (China). Specifically, Danggui was procured from Gansu, China (batch No. 201010); Gancao from Xinjiang, China (batch No. 210302); Jinyinhua from

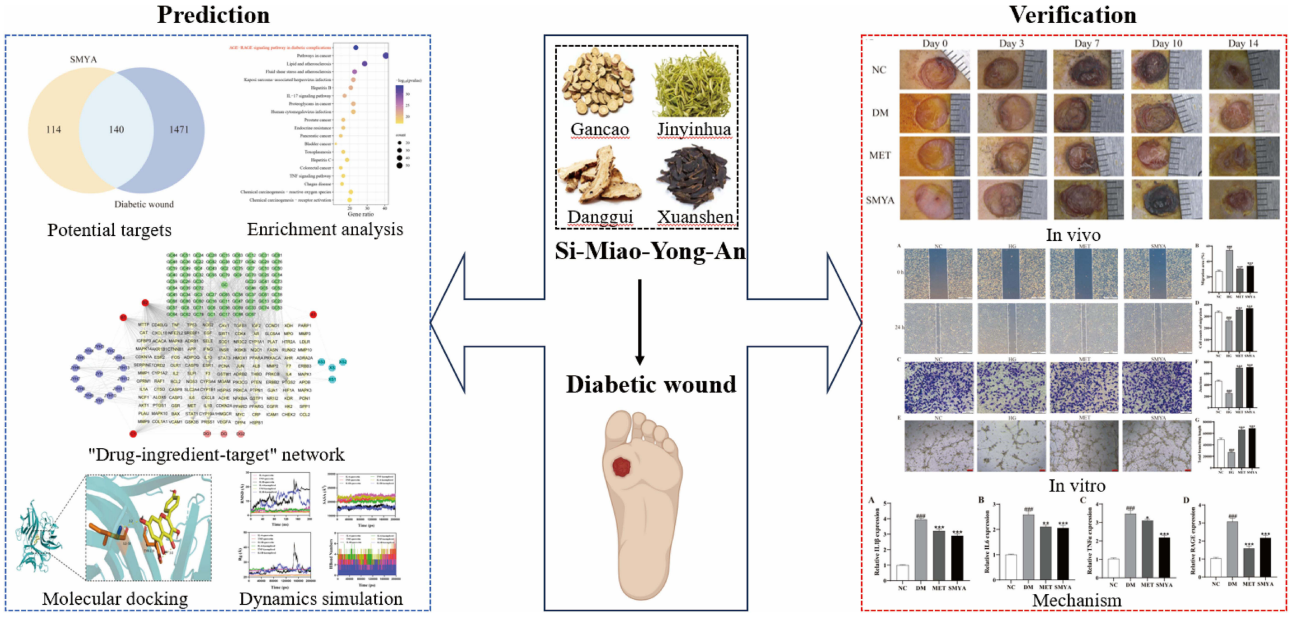

Figure 1 The schematic diagram of this study.

Henan, China (batch No. 20200201); and Xuanshen from Zhejiang, China (batch No. 210518). Jinyinhua, Xuanshen, Danggui, and Gancao were measured in ratios of 3:3:2:1, respectively. The herbal mixture was then extracted twice with water at an 8:1 volume-to-weight ratio under reflux for 2 hours each session. The combined extracts were subsequently filtered, concentrated to a relative density of 1 g/mL. Based on dose conversion factors between humans and rats, the administered dose of SMYA was set at 20 g/kg/day for this experiment.

Animals

The Ethical Committee of Zhengzhou Central Hospital Affiliated to Zhengzhou University granted approval for this study (Ethics number: 202318). All procedures involving animals were conducted in accordance with the guidelines for the care and use of laboratory animals set forth by the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the ARRIVE guidelines. We acquired male db/db mice, C57BL/6J mice, and Sprague-Dawley (SD) rats from Cyagen Biotechnology Co., Ltd. in China. These animals were kept under specific pathogen-free conditions, with their environment controlled at 22 ± 1°C and 50 ± 5% relative humidity, complemented by a consistent 12-hour light/dark cycle. Unlimited access to food and water was provided throughout the study for all rodents.

Animal Model

WT mice and db/db mice aged 8 weeks were anesthetized, and two 6-mm circular full-thickness wounds were created on their dorsum. The wounds were located in the central area of the dorsum, approximately 1 cm from the midline, avoiding the spine and major muscle groups (Figure S1). WT mice (n = 5) were designated as the control group (NC group), while db/db mice (n = 5) were assigned to the diabetic mellitus group (DM group). The db/db mice (n = 5) treated with metformin (250 mg/kg/day, MedChemExpress, USA) were included in the MET group, and db/db mice (n = 5) receiving intragastric administration of SMYA were assigned to the SMYA group. The wound areas were photographed on days 0, 3, 7, 10, and 14 using a digital camera. Images were analyzed using ImageJ software (National Institutes of Health, USA). The wound area was manually outlined, and the pixel count within the outlined area was calculated. The percentage of wound area was determined using the following formula: wound area (%) = remaining wound area at each time point / initial wound area × 100. Random blood glucose levels were monitored at baseline (Day 0), Day 3, Day 7, Day 10, and Day 14 to assess changes in blood glucose fluctuations during the treatment period.

Preparation of SMYA-Containing Serum

The SD rats (n = 10) were administered SMYA via intragastric injection for 1 week. After 1 hour of the last administration, the SD rats were anesthetized with uratan and blood was collected from the abdominal aorta. The blood was centrifuged, inactivated and filtered for subsequent experiments.

Histological Analysis

Mice skin tissues were collected on day 14, fixed in 4% paraformaldehyde, and embedded in paraffin. Paraffin-embedded tissue was sectioned into 5 μm-thick slices for hematoxylin and eosin (H&E) staining and Masson's trichrome staining. For collagen content quantification, Masson's trichrome-stained sections were analyzed using ImageJ software to measure the area of collagen deposition, which was expressed as a percentage of the total tissue area. Tissue thickness was measured at multiple points in the wound area using ImageJ software, and the average tissue thickness was calculated for each group.

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

Total RNA from wound tissues was extracted by Trizol (Invitrogen, USA) method. The extracted RNA was reverse transcribed into cDNA by a reverse transcription kit (Thermo Scientific, USA). Finally, qRT-PCR reaction was carried out on 7500 Fast Dex Real-Time PCR System (Applied Biosystems, USA) through the SYBR Green I Master Mix Kit (Vazyme, China). The relative standard curve method ($2^{-\Delta\Delta CT}$) was used to determine the relative RNA expression, using β-actin as the reference. The qRT-PCR primers used in this study are shown in Table 1.

Table 1 Primer Sequences

Gene		Sequence
Il1b	Forward	CTCGTGCTGTCGGACCCAT
	Reverse	GCTTGTGCTCTGCTTGTGA
Il6	Forward	TTCCATCCAGTTGCCTTCTT
	Reverse	TCCACGATTTCCCAGAGAAC
Tnf	Forward	CACCACGCTCTTCTGTCTACTG
	Reverse	GGGCTACAGGCTTGTCACTC
Rage	Forward	CCCTGAGACGGGACTCTTTA
	Reverse	GTTGGATAGGGGCTGTGTTC
β-actin	Forward	TCACCATGGATGATGATATCGC
	Reverse	ATAGGAATCCTTCTGACCCATGC

Cell Culture

Human umbilical vein endothelial cells (HUVECs) were purchased from Procell (China) and cultured at 37° with 5% CO_2 in endothelial cell medium (ScienCell, USA). To simulate the high glucose microenvironment in vitro, HUVECs were treated with D (+)-glucose (Yuanye, China) at 35 mmol/L concentration (HG group). The control group (NC group) was treated with 5.5 mmol/L glucose. Based on the high-glucose treatment, cells were further treated with metformin (100 μM) or rat serum containing SMYA, which were set as the MET group and SMYA group, respectively.

Wound Healing Assay

HUVECs were plated on 6-well plates and cultured until they reached 80% confluence. A uniform scratch was made using the tip of a 1 mL pipette across the center of each well. The cells were then rinsed with PBS to remove debris from the scratched area. The healing process of the scratch was monitored under a microscope at 0- and 24-hours post-treatment. The migration area within the scratch was quantified using ImageJ software (National Institutes of Health, USA). The calculation was performed by comparing the post-migration scratch area to the initial scratch area, using the formula: (post-migration scratch area / initial scratch area) × 100%.

Trans-Well Migration Assay

We assessed the migration of HUVECs in 24-well plates using trans-well chambers that feature 8-μm pores (Corning, USA). Following treatment under various conditions, a cell suspension of 100 μL (1×10^5 cells/mL) was transferred to the upper chamber of each migration setup. Subsequently, 600 μL of DMEM containing 10% FBS was introduced into the lower chamber of each well. After a 24-hour incubation, cells that migrated through the membrane of the upper chamber were fixed with 4% paraformaldehyde (Solarbio, China) and subsequently stained using a 1% solution of crystal violet dye (Solarbio, China). Micrographs of each chamber were captured, and cells were counted manually across three replicates per group.

Tube Formation Assay

Overnight at 4°C, Matrigel (Corning, USA) was thawed and subsequently dispensed into a 48-well plate, where it was incubated at 37°C for one hour to solidify into a gel. HUVECs were then plated at a density of 1×10^5 cells per well, with three wells per experimental group. Following a 6-hour incubation at 37°C, tube formation was assessed using an inverted light microscope. The lengths of the formed tubes in each well were measured using ImageJ software to quantify tube formation.

Screening of Active Ingredients and Targets

The Traditional Chinese Medicine Systems Pharmacology (TCMSP) Database (https://tcmsp-e.com/) was used to identify the ingredients of SMYA, which include Jinyinhua, Xuanshen, Danggui, and Gancao. Oral bioavailability

(OB) quantifies the fraction of an orally administered drug that enters systemic circulation, a critical pharmacokinetic parameter for drug selection. Drug likeness (DL) reflects a compound's qualitative "drug-like" characteristics, aiding in the exclusion of chemically unsuitable compounds. The ingredients selected met both OB of ≥30% and DL of ≥0.18 were considered to be active ingredients. Active ingredients' target genes were sourced from the DrugBank database. To consolidate the targets from the four herbs, duplicates were removed to compile a comprehensive list of SMYA target genes. These targets were then normalized for gene nomenclature using the UniProt database (https://www.uniprot.org/).

Acquisition of Diabetic Wound-Related Targets

Disease targets were obtained using GeneCards (https://www.genecards.org/), OMIM (http://www.omim.org) and DisGeNET (http://www.disgenet.org/home/) online databases. Relevant target genes were obtained by searching with the keyword "diabetic wound". The contents of the different databases were combined, and duplicates were removed.

Protein–Protein Interaction Network Construction and Hub Gene Screening

Potential targets of SMYA for the treatment of diabetic wounds obtained by Venn diagram. These overlapping genes were then uploaded to the STRING online database (http://string-db.org/) for constructing the protein–protein interaction (PPI) network. Network topology was analyzed using Cytoscape V3.8.2 (http://cytoscape.org). Additionally, a "drug-ingredient-target" network was developed, through which key ingredients and central hub genes were pinpointed based on their degree values.

Enrichment Analysis

To explore the main biological functions and signaling pathways of SMYA for the treatment of diabetic wounds. The overlapping genes were submitted to the DAVID online database (https://david.ncifcrf.gov/) for Gene Ontology (GO) functional enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis. The top 20 biological processes and pathways ranked by P-value were visualized in bubble charts.

Molecular Docking

The 2D structures of the pharmaceutical bioactive ingredients were obtained from the PubChem (https://pubchem.ncbi.nlm.nih.gov) database and converted to the mol2 format using the OpenBabel software. The Protein crystal structures were downloaded from the Protein Data Bank (PDB) database (https://www.rcsb.org) and removed water molecules and ligand using the PyMOL software.[10] Polar hydrogen was added to target protein receptor molecules using Autodock Tools software. Finally, molecular docking is performed by Autodock Vina and Python scripts.

Molecular Dynamics Simulation

The protein-ligand complex was subjected to a 200 ns molecular dynamics simulation using Gromacs 2023. The protein was parameterized using the CHARMM 36 force field, and the ligand topology was constructed with the GAFF2 force field parameters.[11] Periodic boundary conditions were applied, and the protein-ligand complex was placed in a cubic box. Water molecules were added using the TIP3P water model to fill the box. Electrostatic interactions were treated using the Particle Mesh Ewald (PME) method, and the Verlet algorithm was used for calculating non-bonded interactions. A 200,000-step equilibration was performed under the NVT (constant volume and temperature) ensemble followed by the NPT (constant pressure and temperature) ensemble, with a coupling constant of 0.1 ps and a simulation duration of 200 ps. Van der Waals and Coulomb interactions were calculated with a cutoff value of 1.0 nm. Finally, a 200 ns production molecular dynamics simulation was carried out at a constant temperature of 300 K and pressure of 1 bar using Gromacs 2023.

Statistical Analysis

Statistical analysis was performed using GraphPad Prism 8 software (San Diego, USA). All experiments were conducted at least three times and the data were expressed as mean ± standard deviation. One-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test was used to compare the data involving multiple group comparisons. $P < 0.05$ was considered statistically significant difference.

Results

SMYA Promotes Wound Healing in Db/Db Mice

We first explored the efficacy of SMYA in promoting skin wound healing among diabetic mice. Compared to the NC group, the wound healing in the DM group mice was significantly delayed, while the wound healing in the MET and SMYA groups was notably accelerated (Figures 2A, B and S1). In addition, the random blood glucose levels in the DM group mice were significantly higher than those in the NC group. Metformin treatment significantly reduced the blood glucose levels in the diabetic mice, but SMYA did not lower the blood glucose levels in the diabetic mice (Figure 2C). The results of H&E and Masson staining revealed that the wounds of DM group mice had thinner epidermis, irregular tissue morphology, and reduced collagen deposition. These conditions were significantly improved in both the MET and SMYA groups (Figure 2D). These findings support the potential of SMYA in promoting wound healing in diabetic db/db mice.

Network Pharmacological Analysis of SMYA in the Treatment of Diabetic Wounds

In the TCMSP database, 3 components of Danggui, 88 components of Gancao, 17 components of Jinyinhua, and 5 components of Xuanshen were obtained (Table S1). A total of 254 drug targets of SMYA were obtained after removing duplicates (Table S2). Relevant disease targets were extracted from GeneCards, selecting the top 25% by relevance score, which were then integrated with data from the DisGeNET and OMIM databases. Elimination of repeated targets yielded a comprehensive list of 1611 unique genes linked to diabetic wound healing (Table S3).

We then identified 140 "drug-disease" overlapping genes through Venn diagrams (Figures 3A and S2). Furthermore, the TOP 10 genes were screened by degree value ranking as the hub genes for the treatment of diabetic wounds by SMYA (Figure 3B). GO functional enrichment analysis of 140 overlapping genes showed that these genes are involved in angiogenesis and inflammatory response which are closely associated with diabetic wounds (Figure 3C). The results of KEGG pathway enrichment analysis indicated that SMYA may exert therapeutic effects on diabetic wounds through the AGE-RAGE signaling pathway in diabetic complications (Figure 3D).

Network Analysis, Molecular Docking, and Dynamics Reveal Stability and Key Interactions of Active Ingredients

We constructed a "drug-ingredient-target" network to illustrate the relationship between active ingredients and their corresponding targets in drugs (Figure 4). PPI network analysis revealed that quercetin (Figure 4, B2, degree = 205) and kaempferol (Figure 4, B1, degree = 82), shared ingredients of Gancao and Jinyinhua, play a key role in the therapeutic process of the disease. Therefore, we verified the binding energies of quercetin and kaempferol to the angiogenesis- and inflammation-related hub targets by molecular docking (Figure 5A–F). Results from molecular docking revealed that the binding energies for both quercetin and kaempferol with the hub targets were below −5.0 kcal/mol (Table 2).

Molecular dynamics simulations were subsequently performed and the equilibrium state of the simulated system was assessed using root mean square deviation (RMSD). As shown in Figure 6A, the IL-6-quercetin, TNF-quercetin, and TNF-kaempferol complexes reached equilibrium after 10 ns, with fluctuations stabilizing at approximately 4 Å, 2 Å, and 1.9 Å, respectively. The IL1B-quercetin complex equilibrated after 170 ns and exhibited fluctuations around 17.8 Å. In contrast, the IL-6-kaempferol complex achieved equilibrium after 60 ns, with fluctuations stabilizing at approximately 3.8 Å. These results indicate that quercetin and kaempferol exhibit high stability when bound to IL-6, TNF, and IL1B target proteins. Further analysis revealed that the radius of gyration (Rg) and solvent-accessible surface area (SASA) of these complexes showed minor fluctuations during the simulations, suggesting conformational changes in the complexes over time (Figure 6B and C). Most of the complexes maintained 3–4 hydrogen bonds during the dynamic process, which indicates strong hydrogen bonding interactions (Figure 6D). In addition, the root mean square fluctuation (RMSF) values of these complexes were relatively low (mostly below 6 Å), indicating low flexibility and high stability of the amino acid residues (Figure 6E).

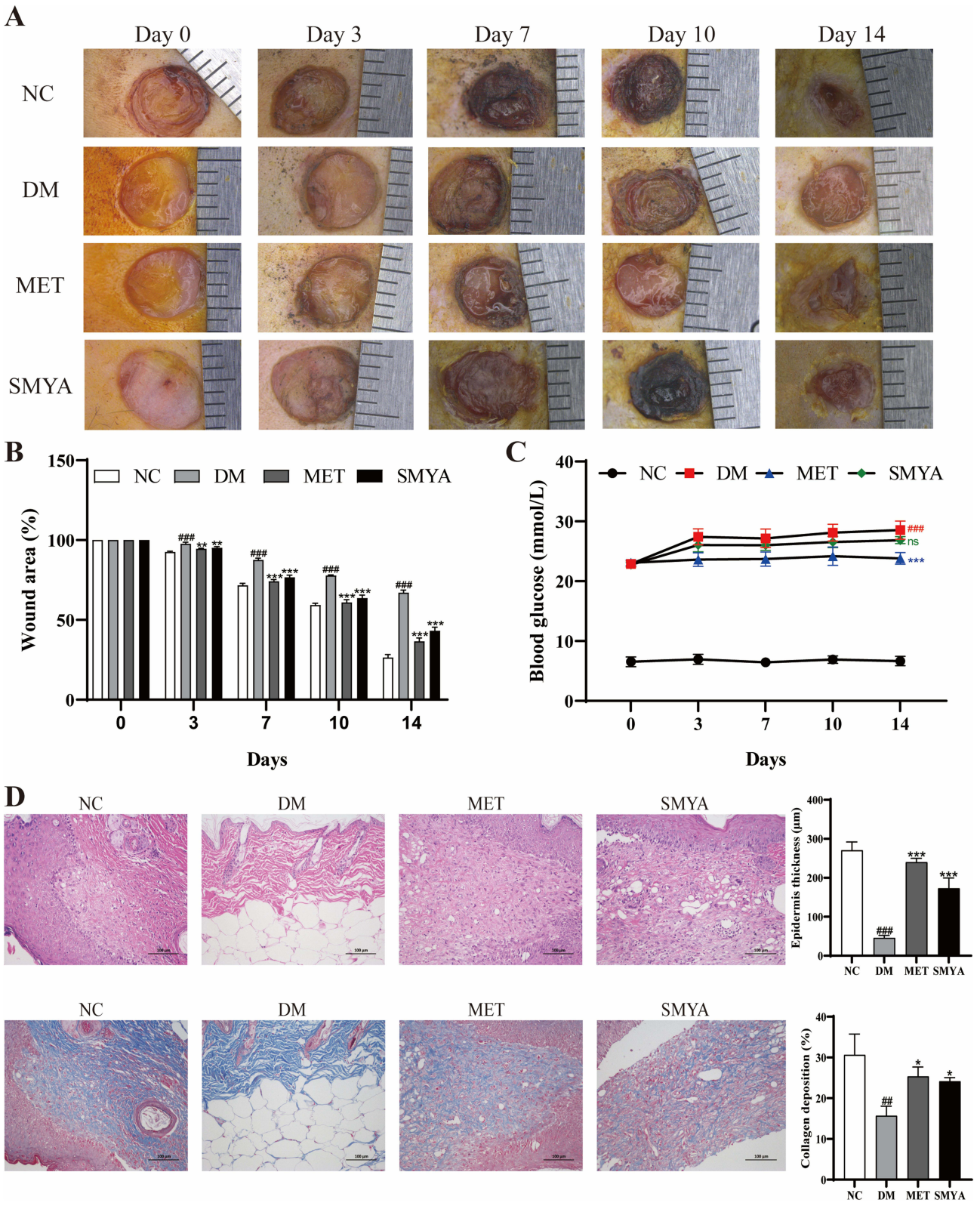

Figure 2 SMYA promotes diabetic wound healing. (**A**) Images of skin wounds in mice. (**B**) The percentage of wound area. (**C**) Randomized blood glucose levels in mice. (**D**) Images of wound histopathology and quantitative analysis of epidermal thickness and collagen deposition. $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$, compared with NC group. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, ns (no significance), compared with DM group.

Abbreviations: NC, control group; DM, diabetic group; MET, diabetic + metformin group; SMYA, diabetic + SMYA group.

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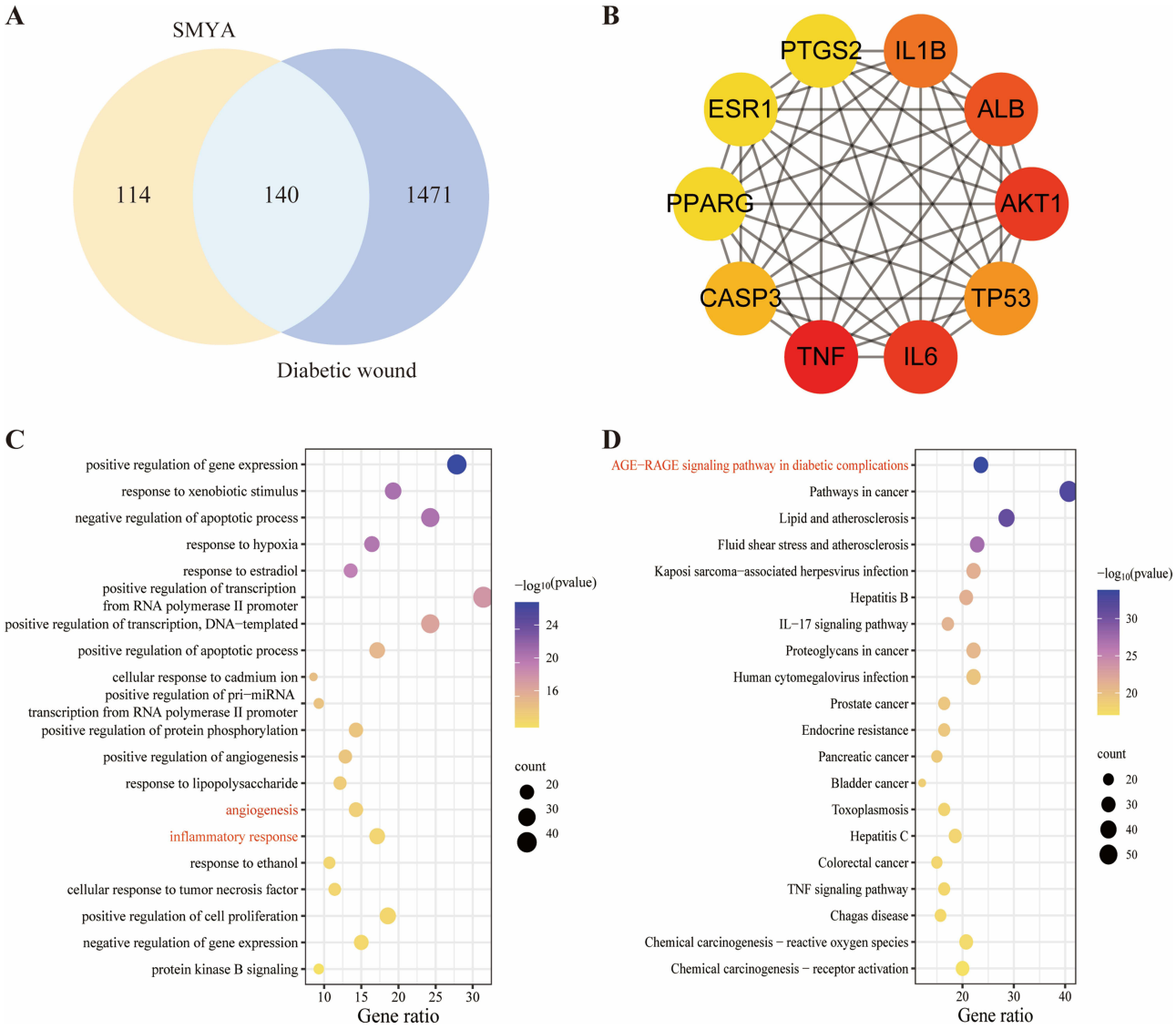

Figure 3 Network pharmacological analysis of SMYA in the treatment of diabetic wounds. (**A**) Identification of overlapping genes between SMYA and diabetic wounds. (**B**) The hub gene of SMYA in the treatment of diabetic wounds. (**C**) GO functional enrichment analysis of overlapping genes. (**D**) KEGG pathway enrichment analysis of overlapping genes.

SMYA Ameliorates HUVECs Injury Induced by High Glucose Concentration

To confirm the findings from our network pharmacology analysis, we incubated HUVECs with 35 mmol/l glucose to establish an in vitro high-glucose microenvironment. At high glucose concentrations, the wound healing ability of HUVECs was diminished and the number of HUVECs crossing the polycarbonate membrane was reduced (Figure 7A–D). In contrast, both metformin and serum containing SMYA significantly enhanced the migration ability of HUVECs (Figure 7A–D). On the other hand, the tube formation assay revealed that HUVECs in the HG group exhibited reduced junctions and total branch length (Figure 7E–G). Metformin and serum containing SMYA alleviated the negative effects of high glucose concentrations on angiogenesis (Figure 7E–G).

SMYA Promotes Diabetic Wound Healing by Modulating Inflammation and Suppressing RAGE Expression

To further elucidate the mechanism of effect of SMYA, we collected tissue from the wound edges and performed qRT-PCR analysis. After treatment with SMYA, the elevated inflammatory factors Tnf, Il1b, and Il6 were significantly

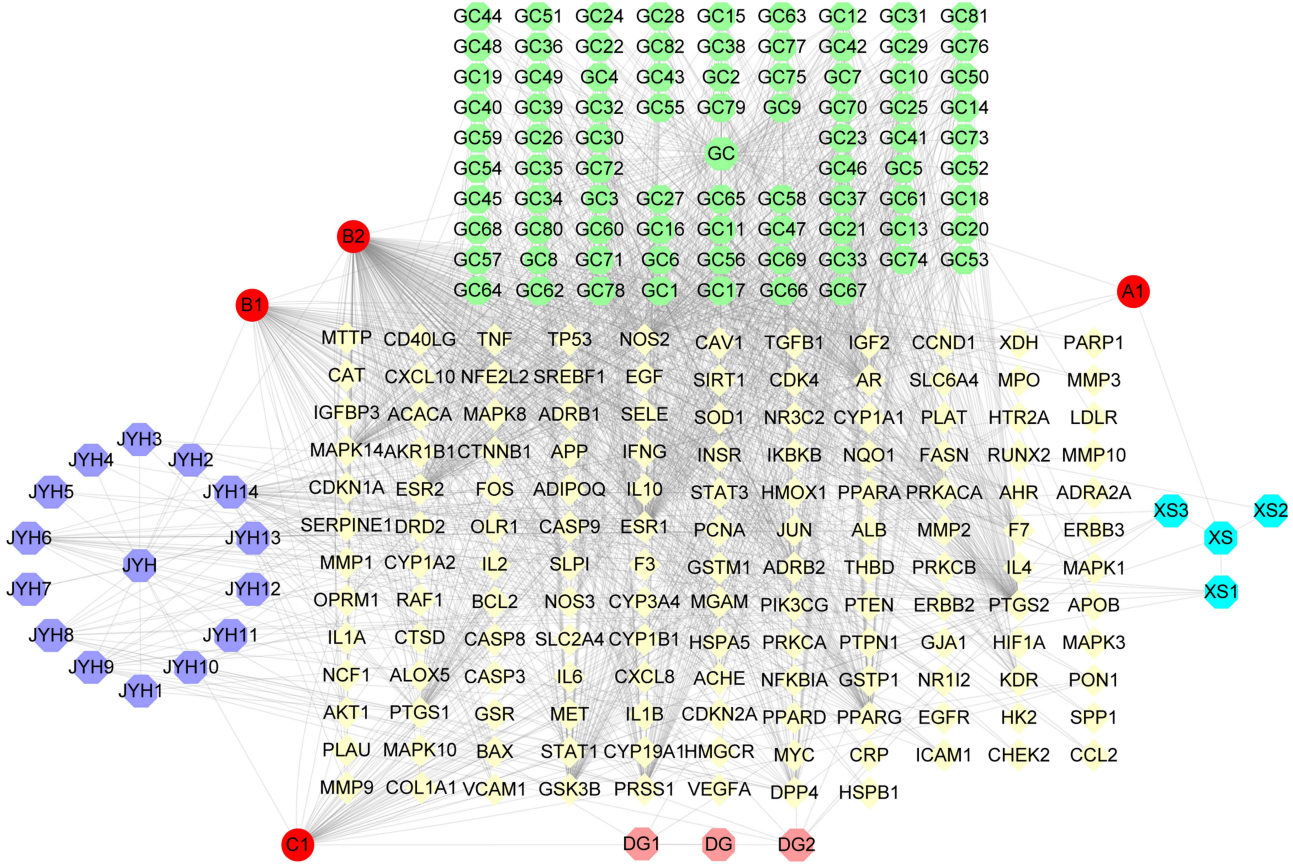

Figure 4 "Drug-ingredient-target" network.
Abbreviations: DG, Danggui; GC, Gancao; JYH, Jinyinhua; XS, Xuanshen. A1, common ingredients of Gancao and Xuanshen; B1 and B2, common ingredients of Gancao and Jinyinhua; C1, common ingredients of Danggui, Jinyinhua and Xuanshen.

downregulated in the DM group (Figure 8A–C). Additionally, network pharmacology studies suggested the AGE-RAGE signaling pathway as a critical mediator in treating diabetic wounds with SMYA. Consequently, we examined the expression markers of this pathway within the wound tissues. Results indicated that while Rage expression was significantly elevated in the DM group, it decreased following SMYA treatment (Figure 8D). These findings suggest that SMYA promotes diabetic wound healing primarily by inhibiting the expression of Rage and inflammatory factors.

Discussion

SMYA, a traditional Chinese prescription known for its effectiveness in detoxification, enhancing blood flow, reducing retention, and nourishing blood vessels. It has been widely used in the treatment of peripheral vascular conditions, such as thromboangiitis obliterans.[12] Recent studies have also highlighted its efficacy in promoting the healing of acute radiation-induced skin injuries.[13] Given its potential therapeutic effects on diabetic complications, this study aimed to explore the role of SMYA in diabetic wound healing. Our results demonstrated that SMYA significantly promotes skin wound closure in db/db diabetic mice. To elucidate the underlying mechanisms, we employed a comprehensive approach combining network pharmacology, molecular docking, molecular dynamics simulations, and experimental validation.

Critical molecular targets, including TNF, AKT1, IL6, and IL1B, have been identified as central mediators in the therapeutic effects of SMYA on diabetic wounds. Wound healing is a complex and finely regulated process that is often impaired in diabetic conditions due to persistent inflammation and reduced angiogenesis.[14] In particular, the hyperglycemic state in diabetic wounds elevates reactive oxygen species levels and induces macrophages to release pro-inflammatory cytokines, such as TNF-α, IL-1β, and IL-6, thereby promoting M1 macrophage polarization and sustaining chronic inflammation.[15] Our GO functional enrichment analysis further revealed that overlapping genes were involved in

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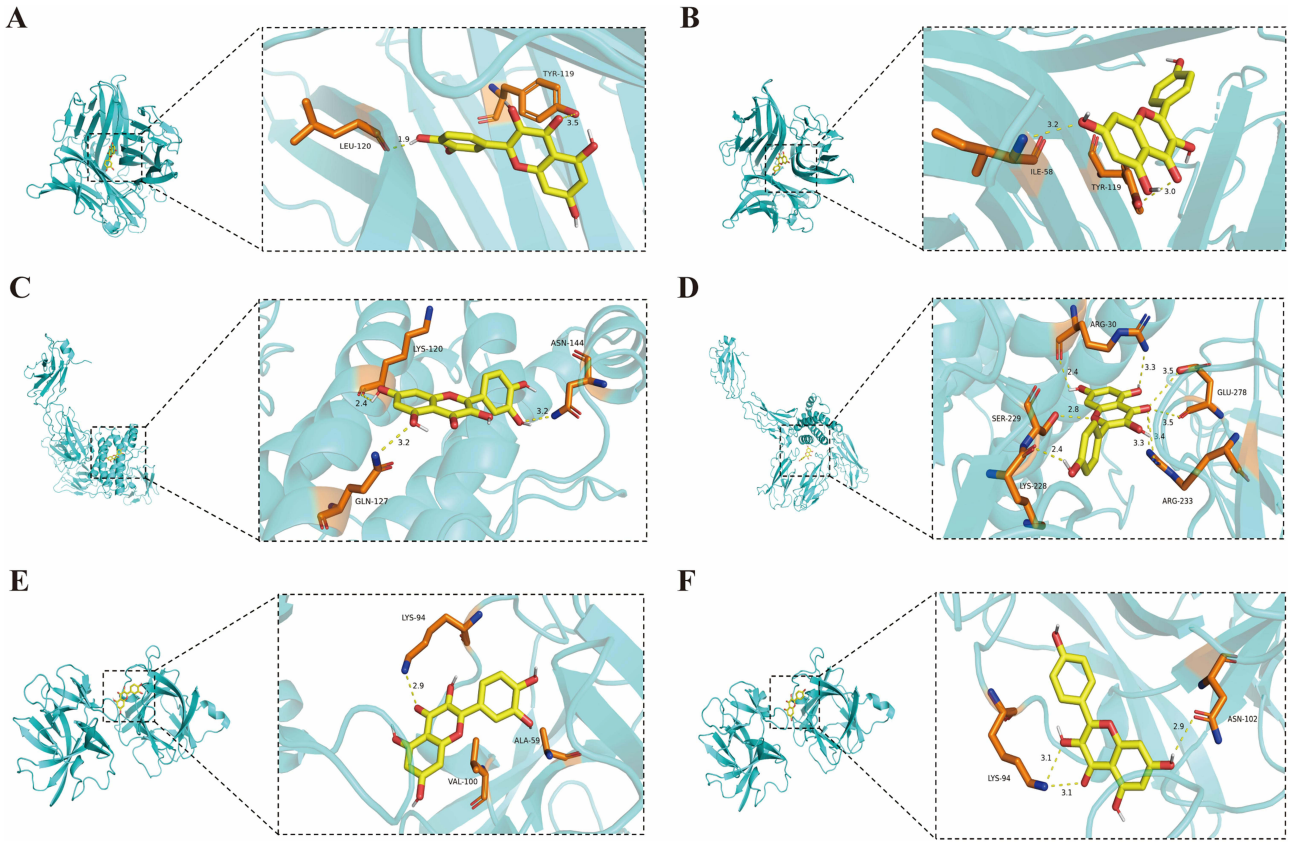

Figure 5 Molecular docking binding modes of quercetin and kaempferol to target proteins. The docking of (**A**) quercetin with TNF, (**B**) kaempferol with TNF, (**C**) quercetin with IL6, (**D**) kaempferol with IL6, (**E**) quercetin with IL1B, and (**F**) kaempferol with IL1B.

pathways related to inflammation and angiogenesis. In vitro experiments demonstrated that SMYA-containing serum mitigated the adverse effects of high glucose concentrations on the migration and angiogenesis capabilities of HUVECs. Additionally, SMYA treatment significantly reduced the levels of inflammatory factors, including Tnf, Il1b, and Il6, in the wound tissues of db/db mice.

Due to the multi-ingredient and multi-target characteristics of traditional Chinese medicine, we have constructed a "drug-ingredient-target" network. Quercetin and kaempferol, shared components in Gancao and Jinyinhua, had the strongest interactions with targets in the network. Quercetin and kaempferol are flavonoids widely distributed in plants with strong anti-inflammatory properties.[16,17] Existing studies have demonstrated that quercetin plays a pivotal role in accelerating diabetic wound healing by modulating macrophage polarization from the M1 to the M2 phenotype, thereby suppressing inflammatory responses.[18] Furthermore, quercetin has been shown to improve the antioxidative state of wounds in diabetic rats and stimulate the proliferative phase of wound healing.[19] Advanced quercetin-loaded nanocarrier formulations have been developed to improve its solubility, permeability, and bioavailability, thereby enhancing its therapeutic potential for diabetic wound treatment.[20] Similarly, the topical application of kaempferol-based ointments has been reported to elevate hydroxyproline levels and promote re-epithelialization in diabetic wounds.[21] These findings

Table 2 Binding Energy of Quercetin and Kaempferol With Hub Genes

	Quercetin	Kaempferol
TNF	−9.7 kcal/mol	−9.6 kcal/mol
IL6	−7.5 kcal/mol	−7.1 kcal/mol
IL1B	−8.8 kcal/mol	−8.5 kcal/mol

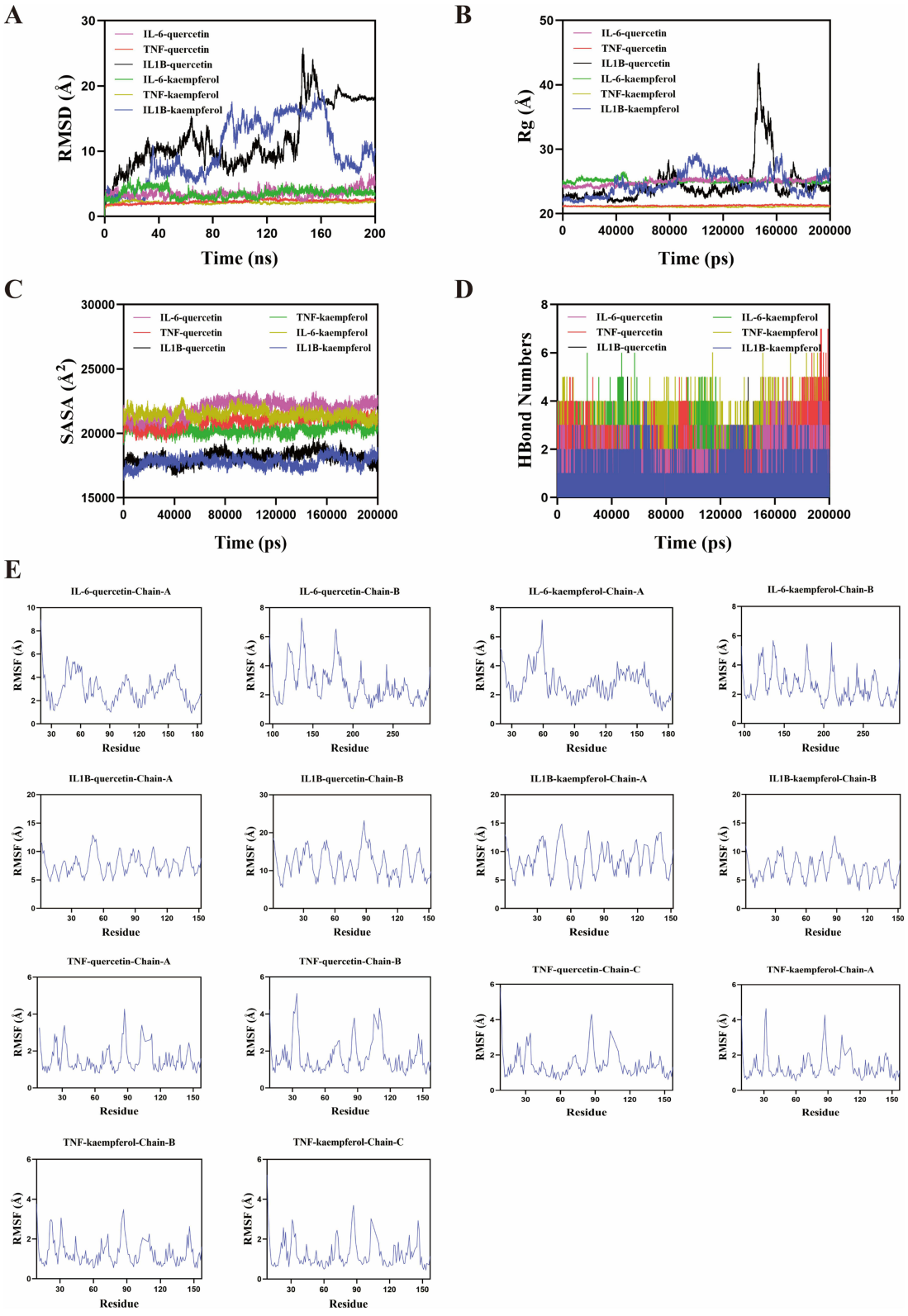

Figure 6 Molecular dynamics simulations of quercetin and kaempferol with target proteins. (**A**) Root mean square deviation. (**B**) Radius of gyration. (**C**) Solvent-accessible surface area. (**D**) The number of hydrogen bonds. (**E**) Root mean square fluctuation.

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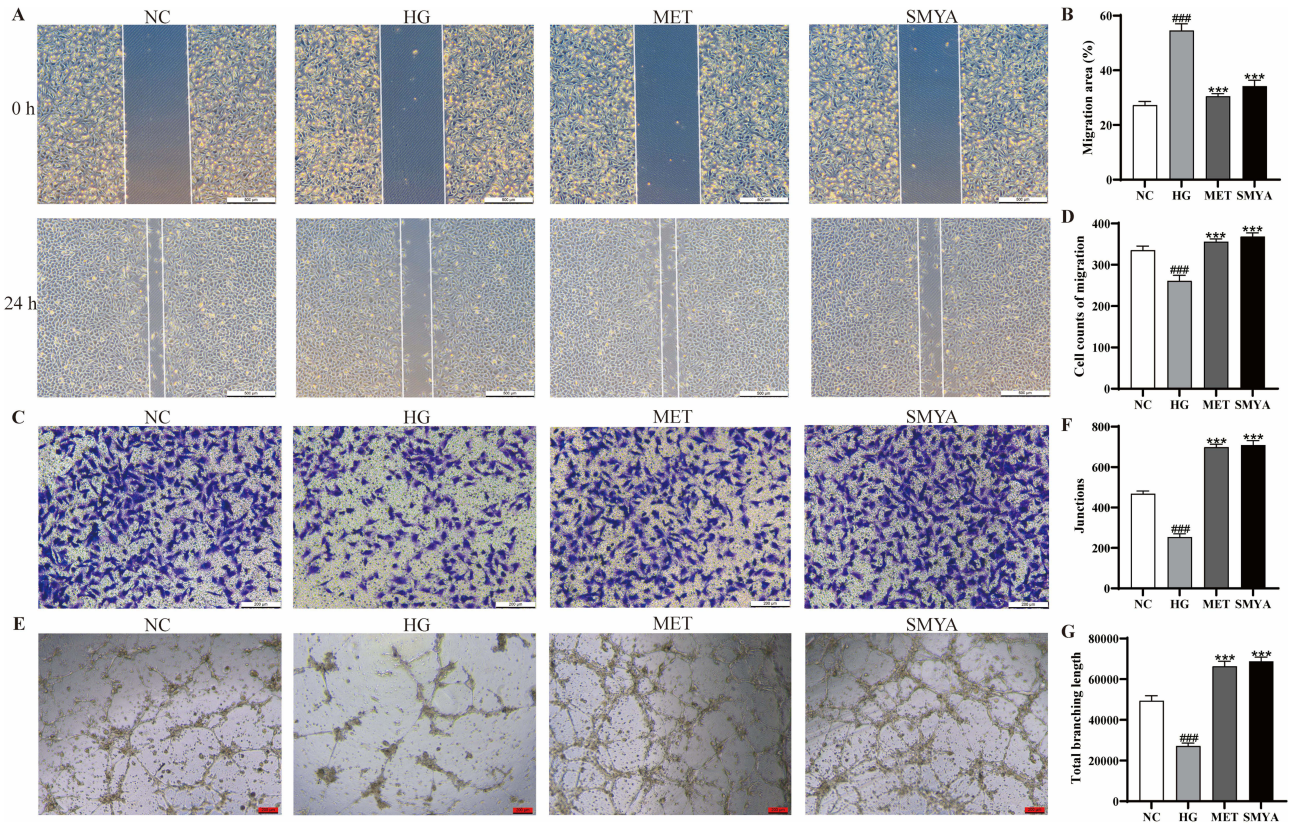

Figure 7 Effect of SMYA on HUVECs injury induced by high glucose concentration. (**A**) HUVECs migration was determined by wound healing assay. (**B**) Migration area of HUVECs in the wound healing assay. (**C**) The migration ability was assessed by the number of HUVECs crossing the polycarbonate membrane. (**D**) Cell counts across the polycarbonate membrane. (**E**) Tube formation of HUVECs was assessed on solidified Matrigel. (**F**) Junctions of angiogenic network. (**G**) Total branching length of angiogenic network. $^{\#\#\#}P < 0.001$, compared with NC group. $^{***}P < 0.001$, compared with DM group.
Abbreviations: NC, control group; HG, high glucose concentration group; MET, high glucose concentration + metformin group; SMYA, high glucose concentration + SMYA group.

suggest that quercetin and kaempferol, as bioactive compounds, exhibit significant therapeutic effects on diabetic wound healing through distinct yet complementary mechanisms.

Our molecular docking results confirmed the strong binding affinity of quercetin and kaempferol to key target proteins, including TNF, IL6, and IL1B, with binding energies below −5.0 kcal/mol. These results suggest stable binding, indicating that both compounds could modulate key inflammatory pathways involved in wound healing. RMSD serves as a reliable metric for evaluating the conformational stability of protein-ligand complexes and the deviation of atomic positions from their initial states. Lower RMSD values indicate greater structural stability. In this study, RMSD analysis revealed that quercetin and kaempferol exhibit high stability when bound to their target proteins. Hydrogen bonds play a critical role in protein-ligand interactions. The complexes consistently maintained 3–4 hydrogen bonds during the simulation, demonstrating strong hydrogen bonding interactions. Additionally, RMSF was used to assess the flexibility of amino acid residues in the complexes. The observed low RMSF values across all systems indicate reduced flexibility and enhanced stability of the complexes. In summary, the protein-ligand complexes exhibited stable binding and strong hydrogen bonding interactions, underscoring the robust interaction of quercetin and kaempferol with the IL6, TNF, and IL1B target proteins.

KEGG enrichment analysis suggested that the AGE-RAGE signaling pathway in diabetic complications may play a pivotal role in the treatment of diabetic wounds by SMYA. Chronic hyperglycemia results in elevated levels of advanced glycosylation end products (AGE), which bind to the receptor for AGEs (RAGE), causing tissue damage and eliciting an inflammatory response.[22] Studies have shown that excessive AGEs in wound tissues amplify the pro-inflammatory responses of M1 macrophages while suppressing the polarization and anti-inflammatory functions of M2 macrophages. Early inhibition of the AGE-RAGE pathway has been shown to mitigate these effects on macrophages during the initial stages of inflammation.[23] Blocking RAGE has been demonstrated to inhibit inflammatory cell

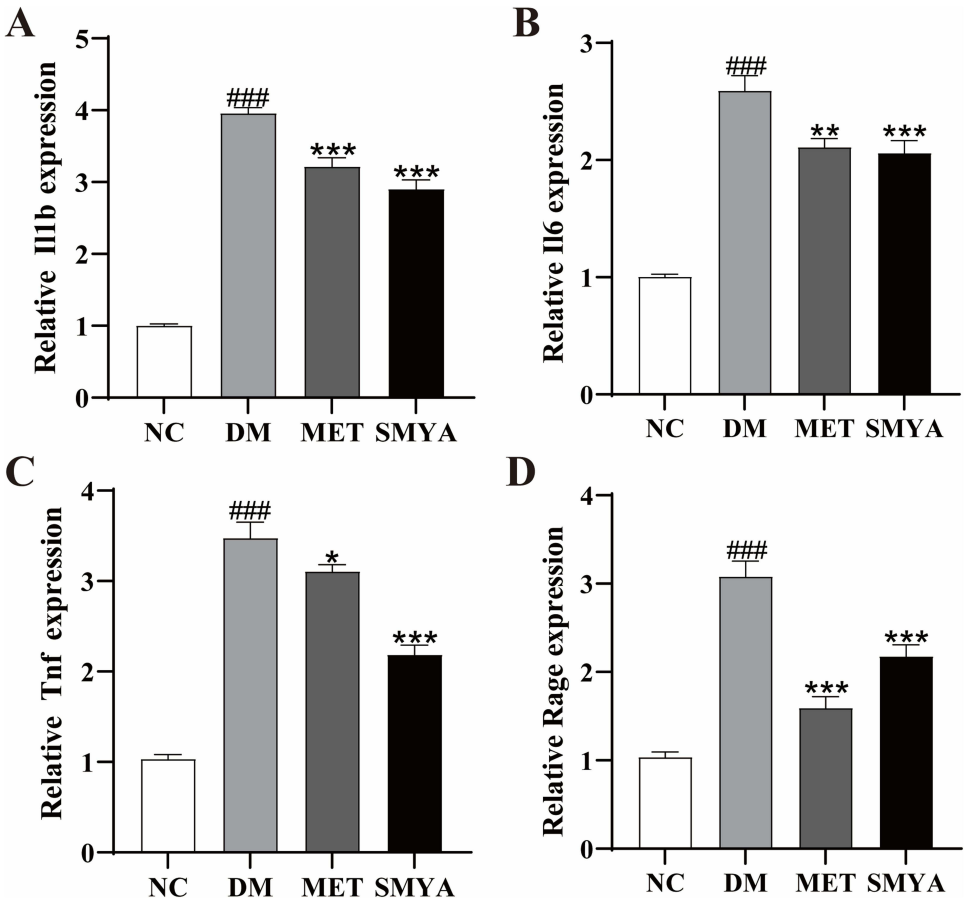

Figure 8 The expression of inflammatory factors and Rage in wound tissue. (**A**) The relative mRNA expression of Tnf. (**B**) The relative mRNA expression of Il1b. (**C**) The relative mRNA expression of Il6. (**D**) The relative mRNA expression of Rage. $^{\#\#\#}P < 0.001$, compared with NC group. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, compared with DM group.

Abbreviations: NC, control group; DM, diabetic group; MET, diabetic + metformin group; SMYA, diabetic + SMYA group.

infiltration and activation in diabetic wounds, thereby effectively restoring wound healing in diabetic mice.[24] In this study, treatment with SMYA downregulated Rage expression in wound tissues, suggesting that SMYA promotes diabetic wound healing by improving the local inflammatory microenvironment through inhibition of the AGE-RAGE signaling pathway.

Compared to existing studies on wound healing, the current study offers several key advantages. Firstly, while many studies have focused on individual compounds, our research provides a comprehensive understanding of the potential therapeutic effects of SMYA by integrating network pharmacology, molecular docking, molecular dynamics simulation, and experimental validation. This multi-faceted approach allows us to identify not only key target proteins but also the specific mechanisms through which SMYA may promote diabetic wound healing. Secondly, although prior studies have examined the effects of SMYA in other disease models, its therapeutic potential in diabetic wound healing remains largely unexplored. This study is among the first to address this gap by investigating its specific role in promoting diabetic wound healing. By focusing on the AGE-RAGE signaling pathway and inflammation, our research provides novel insights into how SMYA might address key challenges in diabetic wound healing, including persistent inflammation and delayed tissue repair.

Although this study provides valuable insights into the potential of SMYA for promoting diabetic wound healing, several limitations need to be addressed. Firstly, while network pharmacology offers a powerful method for identifying potential targets, the accuracy and completeness of the databases used in such analyses may introduce biases, potentially affecting the reliability of the results.[25] To address these limitations, future studies should incorporate additional bioinformatics tools to cross-validate the findings and enhance their robustness. Secondly, this study exclusively used male db/db mice to evaluate the effects of SMYA, which may limit the generalizability of the findings. Future research

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should include both male and female mice to better understand the broader applicability of SMYA and to account for any potential sex-related variations in therapeutic outcomes. Furthermore, despite the promising preclinical results, the clinical translation of SMYA faces significant challenges. The feasibility of using SMYA in human patients requires careful consideration of dosing regimens, potential drug interactions, and side effects. Clinical trials are essential to determine the optimal dosage, safety, and efficacy of SMYA in humans, and dosage adjustments may be necessary for different patient populations. Additionally, rigorous safety assessments must be conducted to evaluate any potential adverse effects, such as toxicity or allergic reactions.

Conclusion

This study demonstrated that SMYA effectively promotes diabetic wound healing through multiple mechanisms, including angiogenesis enhancement, inflammatory factor suppression, and modulation of the AGE-RAGE signaling pathway. Quercetin and kaempferol, key components of SMYA, were identified as critical mediators in this process due to their stable interactions with inflammation-related targets. These findings highlight the potential of SMYA as a multi-target therapeutic agent for diabetic wound management. Further clinical studies are warranted to validate its efficacy and safety in human populations.

Abbreviations

AGE, advanced glycosylation end products; DL, drug likeness; GO, Gene Ontology; HUVECs, human umbilical vein endothelial cells; KEGG, Kyoto Encyclopedia of Genes and Genomes; LPS, lipopolysaccharide; OB, oral bioavailability; PPI, protein–protein interaction; qRT-PCR, quantitative real-time polymerase chain reaction; RAGE, receptor for advanced glycosylation end products; Rg, radius of gyration; RMSD, root mean square deviation; RMSF, root mean square fluctuation; SASA, solvent-accessible surface area; SMYA, Si-Miao-Yong-An; SD, Sprague-Dawley; TCMSP, Traditional Chinese Medicine Systems Pharmacology.

Data Sharing Statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author/s.

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Disclosure

The author(s) report no conflicts of interest in this work.

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