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ORIGINAL RESEARCH

Elucidating the Differential Mechanisms of Jingui Shenqi Pill and Mingmu Dihuang Pill in the Treatment of Diabetic Nephropathy Based on Yin-Yang Theory

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Background: The Yin-Yang attributes of MMDH and JGSQ in treating diabetic nephropathy (DN) remain unexplored.

Methods: UPLC-MS identified formula components, network pharmacology analyzed common DN targets, and in vitro renal fibrosis models assessed efficacy. Transcriptomics revealed key pathways, and molecular docking simulated component-target interactions.

Results: UPLC-MS confirmed the compositional complexity of MMDH and JGSQ. Network pharmacology indicated their involvement in multiple DN-related pathways. In vitro, JGSQ alleviated fibrosis and enhanced adhesion via FN and E-cad, while MMDH reduced interstitial fibrosis via FN and VIM. Transcriptomics showed JGSQ regulates the TGF-β pathway, and MMDH modulates the TNF pathway. Molecular docking confirmed key components binding to TGFB1 and TNFA.

Conclusion: MMDH and JGSQ exhibit distinct chemical compositions, targets, and pathways, underscoring their Yin-Yang regulatory roles in kidney function.

Keywords: diabetic nephropathy, Mingmu Dihuang pill, Jingui Shenqi pill, network pharmacology, transcriptomics

Introduction

Diabetes is a metabolic disorder leading to chronic microvascular and macrovascular complications, with diabetic nephropathy (DN) being one of the most severe. DN is a major cause of end-stage renal disease, characterized by proteinuria resulting from podocyte injury and kidney function decline due to structural changes in the glomerular filtration barrier.[1,2] The pathogenesis of DN involves hemodynamics, metabolism, and inflammation. The renin-angiotensin system (RAS) regulates hemodynamics, and its activation generates angiotensin II, which promotes aldosterone secretion, induces fibrogenic factors, and activates macrophages, leading to inflammation.[3] Angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs) remain the primary treatments for DN. With advances in antidiabetic drug development, treatment options now also include sulfonylureas, insulin, dipeptidyl peptidase-4 (DPP-4) inhibitors, glucagon-like peptide-1 (GLP-1) agonists, and sodium-glucose co-transporter 2 (SGLT2) inhibitors.[4] Although RAS inhibitors are first-line therapies for DN, they often yield unsatisfactory results. Traditional Chinese medicine (TCM), with its multi-targeted effects, has shown significant clinical benefits as a primary or alternative therapy for DN. Research increasingly focuses on identifying TCM bioactive components and their mechanisms, including regulation of glucose and lipid metabolism, antioxidation, anti-inflammation, antifibrosis, and podocyte protection.[5,6] Among them, the activation of transforming growth factor (TGF)-β1/Smad and PI3K/Akt/

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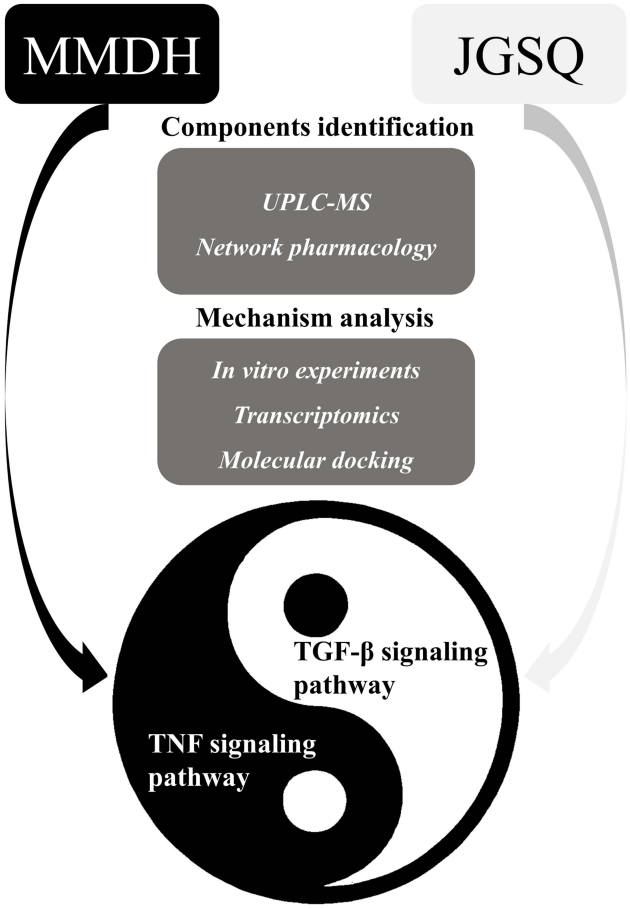
Graphical Abstract



mTOR pathways helps counter renal microinflammation. However, excessive activation leads to cell dysfunction, apoptosis, increased ECM secretion, and ultimately renal fibrosis. This imbalance reflects the Yin-Yang concept in TCM, where microinflammation and fibrosis correspond to the balance of Yin and Yang.[7,8]

Mingmu Dihuang pill (MMDH) is a classic Chinese herbal formula composed of twelve herbs: Rehmanniae radix (Dihuang), Dioscoreae rhizoma (Shanyao), Corni fructus (Shanzhuyu), Moutan cortex (Mudanpi), Alismatis rhizoma (Zexie), Poria (Fuling), Lycii fructus (Gouqizi), Chrysanthemi flos (Juhua), Angelicae sinensis radix (Danggui), Paeoniae radix alba (Baishao), Tribuli fructus (Jili), and Haliotidis concha (Shijueming).[9] MMDH was first recorded in the Ming Dynasty's "Shenshi Yaohan". It has been used to treat conditions such as liver and kidney Yin deficiency.[10] Modern pharmacological studies have shown that MMDH possesses antioxidant and retinal-protective activities.[11,12] The traditional Chinese medicine formula Jingui Shenqi pill (JGSQ) originates from the Eastern Han Dynasty's "Jin Gui Yao Lue" and is composed of Dihuang, Shanyao, Shanzhuyu, Mudanpi, Zexie, Fuling, Cinnamomi Ramulus (Guizhi), and Aconiti Lateralis Radix Praeparata (Fuzi). JGSQ has traditional effects of tonifying the kidneys and supporting yang.[13–15] In addition, JGSQ also exhibits pharmacological activities such as retinal protection, immune regulation, kidney protection, and blood glucose reduction.[16–21] Thus, it can be seen that the two formulas each focus on regulating different aspects of the Yin-Yang balance in the kidneys: JGSQ emphasizes tonifying kidney yang, while MMDH focuses on nourishing kidney Yin. In TCM theory, Yin and Yang are core concepts that permeate the properties of medicinal herbs, disease differentiation, and treatment principles. These concepts not only guide the classification and application of TCM but also provide an important theoretical basis for clinical treatment.[22] Studies have shown that JGSQ can reverse the T helper

cell 1/2 imbalance in a kidney yang deficiency animal model by upregulating pro-inflammatory factors.[23] The Yin-nourishing effect of MMDH is often linked to its antioxidant activity, which is used to screen for active components.[11] These findings suggest that the differences in the Yin-Yang properties of the two formulas play a role in shaping their biological mechanisms. Although JGSQ and MMDH have distinctive Yin-Yang properties, few studies have compared the two to analyze the modern biological significance of Yin and Yang.

This study will utilize techniques such as liquid chromatography-mass spectrometry (LC-MS), network pharmacology, in vitro validation experiments, transcriptomics, and molecular docking to conduct multi-level analyses of the Yin-Yang attributes of MMDH and JGSQ, focusing on components, targets, and pathways.

Materials and Methods

Component Identification of MMDH and JGSQ Based on UPLC-MS

MMDH and JGSQ were sourced from Beijing Tong Ren Tang (Group) Co., Ltd. A portion of the sample was dissolved in the mobile phase to obtain a final concentration of 100 mg/mL, followed by filtration using a 0.22 μm membrane for component analysis. The analysis was conducted using a Accela 600 UHPLC system coupled with an LTQ-Orbitrap mass spectrometer (Thermo Scientific, Germany) and an ACQUITY UPLC BEH C18 column (2.1 × 100 mm, 1.7 μm; Waters Corp, USA). Electrospray ionization was applied in both positive and negative modes. The capillary temperature was 320 °C, and the sheath gas flow was set to 35 arb, while the aux gas flow was 15 arb. The spare gas flow was 0, and the maximum spray current was 100. The probe heater temperature was 400 °C, and the S-Lens RF level was set to 55. The mass scan range was from 100 to 1500 m/z. The mobile phase consisted of acetonitrile (A) and 0.1% formic acid in water (B). The elution gradient was programmed as follows: 0 min, 3% A; 3 min, 5% A; 8 min, 7% A; 33 min, 20% A; 46 min, 40% A; 56 min, 90% A; 60 min, 3% A. The column temperature was maintained at 35°C, with a flow rate of 0.3 mL/min and an injection volume of 2 μL. Data processing was performed using Xcalibur 2.1 software. Preliminary screening was based on literature reports of pharmacological activity or the presence of corresponding targets in TCMSP.

Network Pharmacology Analysis of MMDH and JGSQ

Relevant targets were retrieved from the TCMSP database (https://old.tcmsp-e.com/tcmsp.php) based on the compounds identified through UPLC-MS analysis. Targets associated with DN were extracted from the GSE30529 dataset in the GEO database (https://www.ncbi.nlm.nih.gov/geo/), using a significance threshold of $P < 0.05$ for gene selection. This dataset comprises 10 kidney samples from DN patients and 12 from healthy controls. This study is exempt from ethical review based on the national legislation guidelines, specifically item 1 and 2 of Article 32 of the Measures for Ethical Review of Life Science and Medical Research Involving Human Subjects (issued on February 18, 2023, China). A Venn diagram (https://bioinfogp.cnb.csic.es/tools/venny/) was used to identify overlapping targets between DN and the formulas (MMDH and JGSQ). The shared targets were then input into STRING (https://cn.string-db.org/) to construct a protein-protein interaction (PPI) network. Cytoscape 3.7.2 was utilized to develop a network linking components, targets, and the disease, enabling the identification of active compounds. After constructing the network, all components were ranked according to their Degree values, and the top two components with Degree values greater than the median and uniqueness were selected as potential key components of JGSQ and MMDH. Additionally, Metascape (https://metascape.org/) facilitated the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway and Gene Ontology (GO) enrichment analyses.

In vitro Studies

HK2 cells are the most commonly used human proximal tubule cells for research, and the TGF-β1-induced fibrosis model is a classical model for studying the fibrotic mechanisms of DN. Fibrosis is a hallmark lesion of late-stage DN, with TGF-β1 playing a key role in this process. Therefore, this model provides a controlled and reproducible system for screening potential therapeutic compounds and focusing on fibrosis-related pathways, offering guidance for in vivo studies without the confounding effects of hyperglycemia or podocyte injury.[24] HK2 cells (Wuhan Pricella Biotechnology Co., Ltd.) were cultured in MEM supplemented with 5% FBS and 1% penicillin-streptomycin solution

in a humidified incubator at 37°C with 5% CO_2. During the logarithmic growth phase, HK2 cells were seeded in 96-well plates at a density of 2×10^5 cells per well. To induce fibrosis, cells were treated with 5 ng/mL TGF-β1 for 72 h. After establishing the fibrosis model, the following experimental groups were included: control group, model group, positive control (valsartan, 750 μM), MMDH group (2 mg/mL), and JGSQ group (2 mg/mL). The expression levels of fibrosis markers fibronectin (FN), vimentin (VIM), and E-cadherin (E-cad) were used to evaluate the fibrosis model and therapeutic effects.

Transcriptomic Analysis

Gene expression levels were measured by read counts, and DESeq2 was applied to identify differentially expressed genes (DEGs) with a significance threshold of $P < 0.05$. The gene expression difference between the control and model groups was large, with a higher fold change. Therefore, a stricter threshold of $|\log2FC| > 2$ was used to screen for DEGs with stronger biological relevance. In contrast, the differential genes between the drug-treated and model groups were primarily regulatory genes, and drug intervention generally does not fully restore gene expression to normal levels, resulting in a smaller fold change. To improve the sensitivity of screening while ensuring that the identified DEGs are biologically significant, a relatively more relaxed but widely accepted threshold of $|\log2FC| > 1$ was applied in the treatment group, aiming to identify key genes related to drug efficacy. GO and KEGG enrichment analyses for DEGs were conducted using clusterProfiler. To further explore gene sets, Gene Set Enrichment Analysis (GSEA) was used to rank genes by expression differences and assess enrichment of predefined sets. Weighted Correlation Network Analysis (WGCNA) was utilized to identify gene modules with coordinated expression and to pinpoint potential biomarkers or targets.

Quantitative Real-Time PCR Analysis

Total RNA from HK2 cells was extracted using Trizol reagent (Invitrogen) according to the manufacturer's protocol. cDNA was synthesized from the isolated RNA using the FastKing RT Kit (with gDNase, TIANGEN). Quantitative real-time PCR (qRT-PCR) was carried out using TB Green Premix Ex Taq^{TM} II (Tli RNaseH Plus, Takara). The primer sequences were listed in Table 1, with GAPDH used as the internal reference for normalization. Relative mRNA expression levels were determined using the $2^{-\Delta\Delta Ct}$ method.

Molecular Docking

Obtain the ligand structure file from PubChem (https://pubchem.ncbi.nlm.nih.gov/) and the receptor structure file from PDB (https://www.rcsb.org/). Use "Prepare Protein" to process the receptor and ensure its structure is suitable for docking. Use the "Prepare Ligand" tool to process the ligand, including optimizing its structure and adding hydrogen atoms. Define the docking area or binding site based on the receptor's characteristics. After completing all preparation work, start the docking calculation. Once docking is complete, review the docking results, including the ligand-receptor binding modes and energy scores, as well as the visualized images of the docking results.

Results

Identification of Chemical Components in JGSQ and MMDH

Under the combined identification of positive and negative ion modes, along with preliminary screening, a total of 32 chemical components were identified in JGSQ, and 48 chemical components were identified in MMDH. These

Table 1 Primer Sequences

Gene Name	Forward	Reverse
FN	GGCTTGAACCAACCTACGGATGAC	TCCTTCTGCCACTGTTCTCCTACG
VIM	GTCCGTGTCCTCGTCCTCCTAC	AGTTGGCGAAGCGGTCATTCAG
E-cad	TGGGCTGGACCGAGAGAGTTTC	TGGCTGTGGAGGTGGTGAGAG
GAPDH	TCTTCCTCTTGTGCTCTTGC	CTTTGTCAAGCTCATTTCCTGG

components mainly belong to organic acids, aldehydes, phenols, coumarins, flavonoids, terpenoids, and esters. This indicates the complexity of the components contained in JGSQ and MMDH. The mass spectrometry profiles are shown in Figure 1, and detailed component information is provided in Supplemental Table 1.

Component-Target Network Analysis of JGSQ and MMDH

Chemical components of JGSQ and MMDH were identified based on mass spectrometry, and corresponding targets were obtained from TCMSP. Subsequently, VENN analysis revealed 62 common targets between JGSQ and DN (Figure 2A), and 95 common targets between MMDH and DN (Figure 2D). Enrichment analysis of the intersecting targets of JGSQ and MMDH identified diabetes-related KEGG pathways, including Lipid and atherosclerosis, and AGE-RAGE signaling pathway in diabetic complications (Figure 2B and E). Additionally, biological functions such as response to lipopolysaccharide, adrenergic receptor activity, and membrane raft were also identified (Figure 2C and F). However, these pathways, common to both JGSQ and MMDH, elucidate potential mechanisms for treating DN but do not fully account for the Yin-Yang attributes.

Subsequently, the intersecting targets were further used to construct a PPI network to observe target interactions. Following this, a component-target network was constructed, comprising 244 nodes and 803 edges. After ranking by network degree, the top-ranking unique components of JGSQ were identified as syringaldehyde and cinnamaldehyde, while the unique components of MMDH were apigenin and kaempferol (Figure 3).

In vitro Activity Evaluation of JGSQ and MMDH

JGSQ and MMDH showed no toxicity at concentrations ranging from 0.5 to 2 mg/mL. Therefore, the maximum non-toxic concentration of 2 mg/mL was selected for subsequent evaluation. Compared to the control group, the expression of FN and VIM in the model group was significantly increased, indicating renal fibrosis and deterioration of renal function. The decrease in E-cad suggests that renal fibrosis is further exacerbated. JGSQ can reverse the expression of FN and

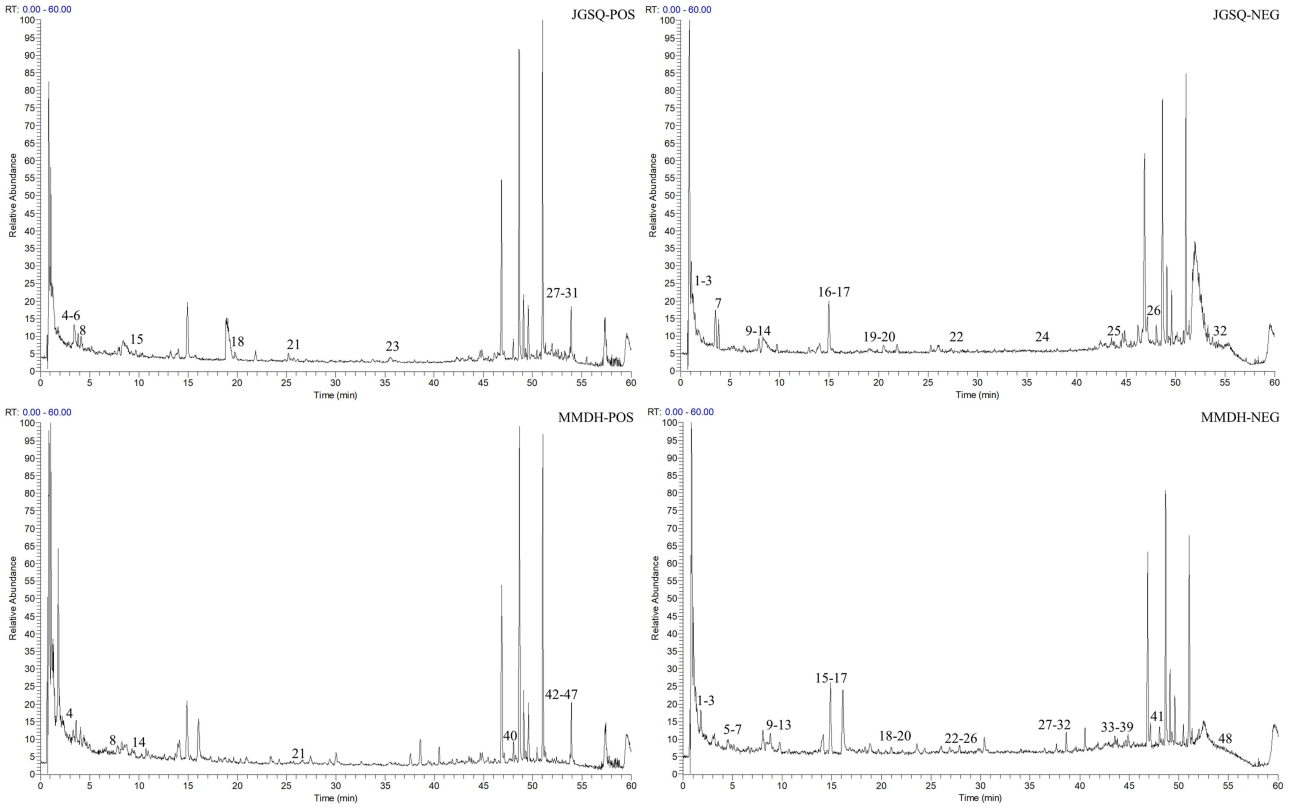


Figure 1 Positive (POS) and negative (NEG) ion MS of JGSQ and MMDH.

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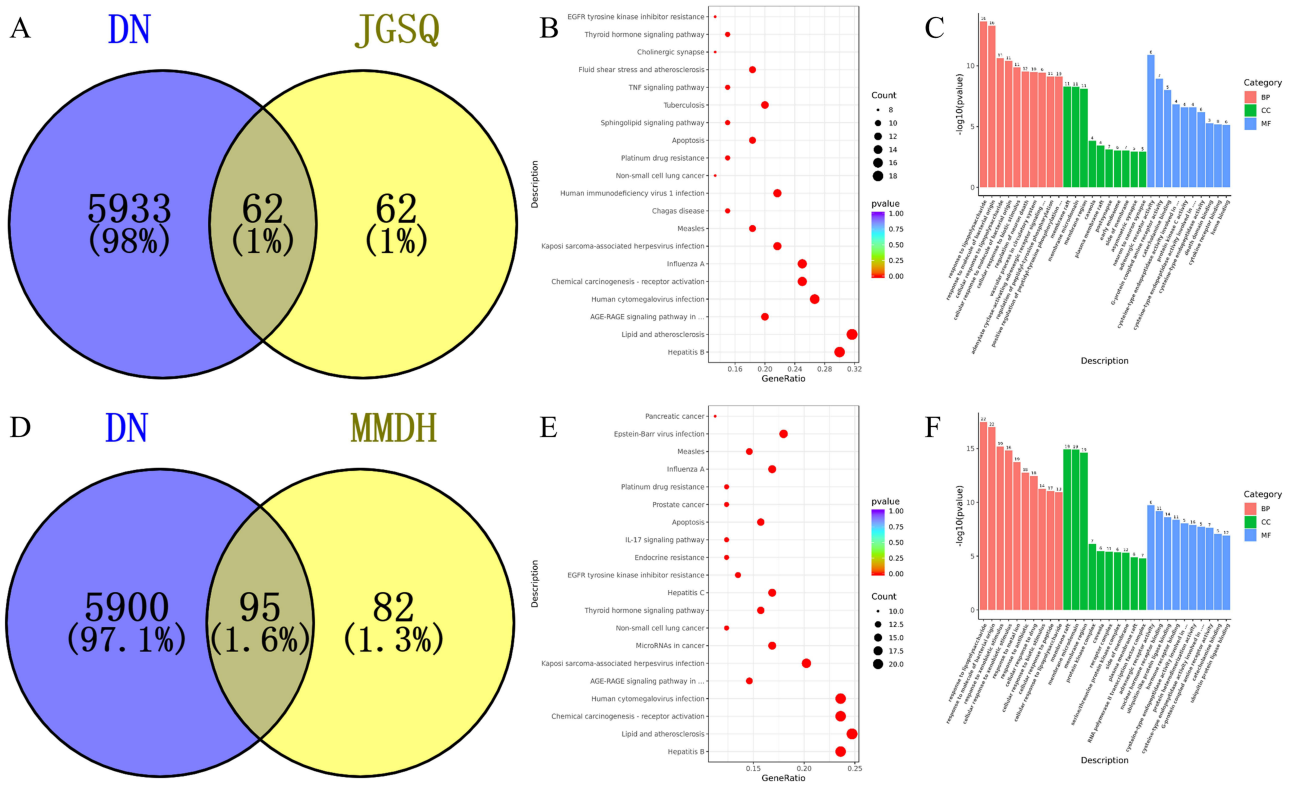

Figure 2 VENN diagram of intersecting targets between JGSQ (**A–C**)/MMDH (**D–F**) and DN, along with KEGG and GO pathway enrichment.

E-cad, indicating that JGSQ can alleviate fibrosis while enhancing epithelial cell adhesion. MMDH can reverse the expression of FN and VIM, indicating that MMDH can alleviate interstitial fibrosis (Figure 4). This reflects the functional differences of JGSQ and MMDH in regulating the balance of kidney Yin and Yang.

Transcriptional Regulatory Analysis of JGSQ and MMDH

PCA shows a clear separation trend between the control group and the model group, while both the JGSQ and MMDH groups exhibit effects that distinguish them from the model group. This is further supported by the clustering heatmap (Figure 5A). Based on the predetermined DEGs screening criteria, 470 genes were upregulated and 470 genes were downregulated between the control group and the model group. Between the model group and the JGSQ group, 430 genes were upregulated and 426 genes were downregulated. Between the model group and the MMDH group, 788 genes were upregulated and 725 genes were downregulated (Figure 5B). The KEGG enrichment results show that the differential pathways between the control group and the model group include neuroactive ligand-receptor interaction, between the model group and the JGSQ group include steroid biosynthesis, and between the model group and the MMDH group include protein processing in the endoplasmic reticulum (Figure 5C). The GO enrichment results indicate that the key biological functions between the control group and the model group include receptor regulator activity, extracellular matrix, and blood vessel morphogenesis; between the model group and the JGSQ group include steroid metabolic process, extracellular matrix, and serine-type peptidase activity; and between the model group and the MMDH group include hormone metabolic process, endoplasmic reticulum lumen, and receptor regulator activity (Figure 5D).

Analysis of Differential Mechanisms Between JGSQ and MMDH

Based on DEGs, VENN analysis identified 226 shared regulatory genes between JGSQ and MMDH, 193 unique regulatory genes for JGSQ, and 371 unique regulatory genes for MMDH (Figure 6A). JGSQ and MMDH shared six identical herbal ingredients in their formulations, which corresponded to the 226 commonly regulated DEGs between them. This suggested that the addition of new herbs did not diminish the fundamental pharmacological effects of the

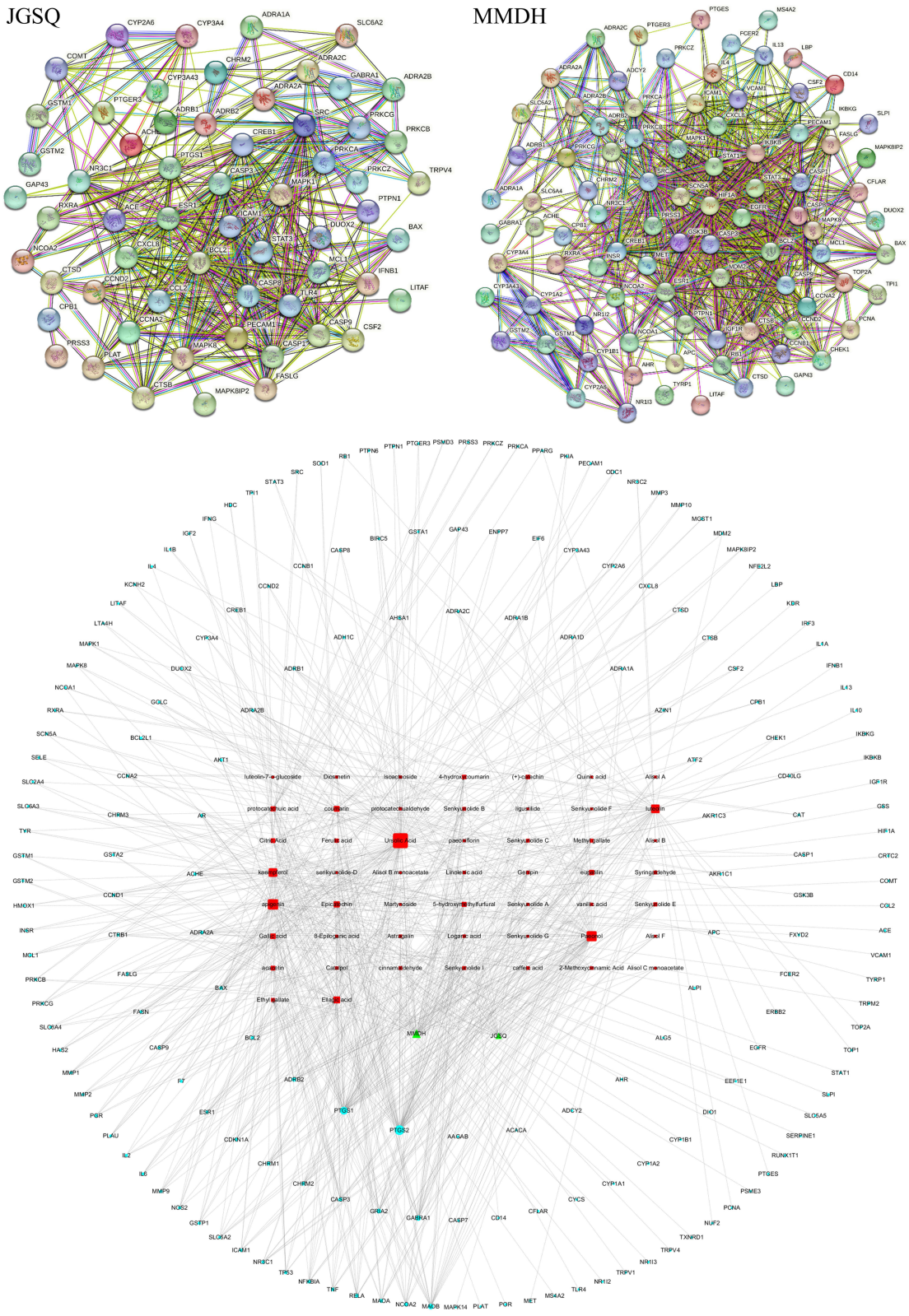

Figure 3 PPI and component-target network diagrams of the intersecting targets.

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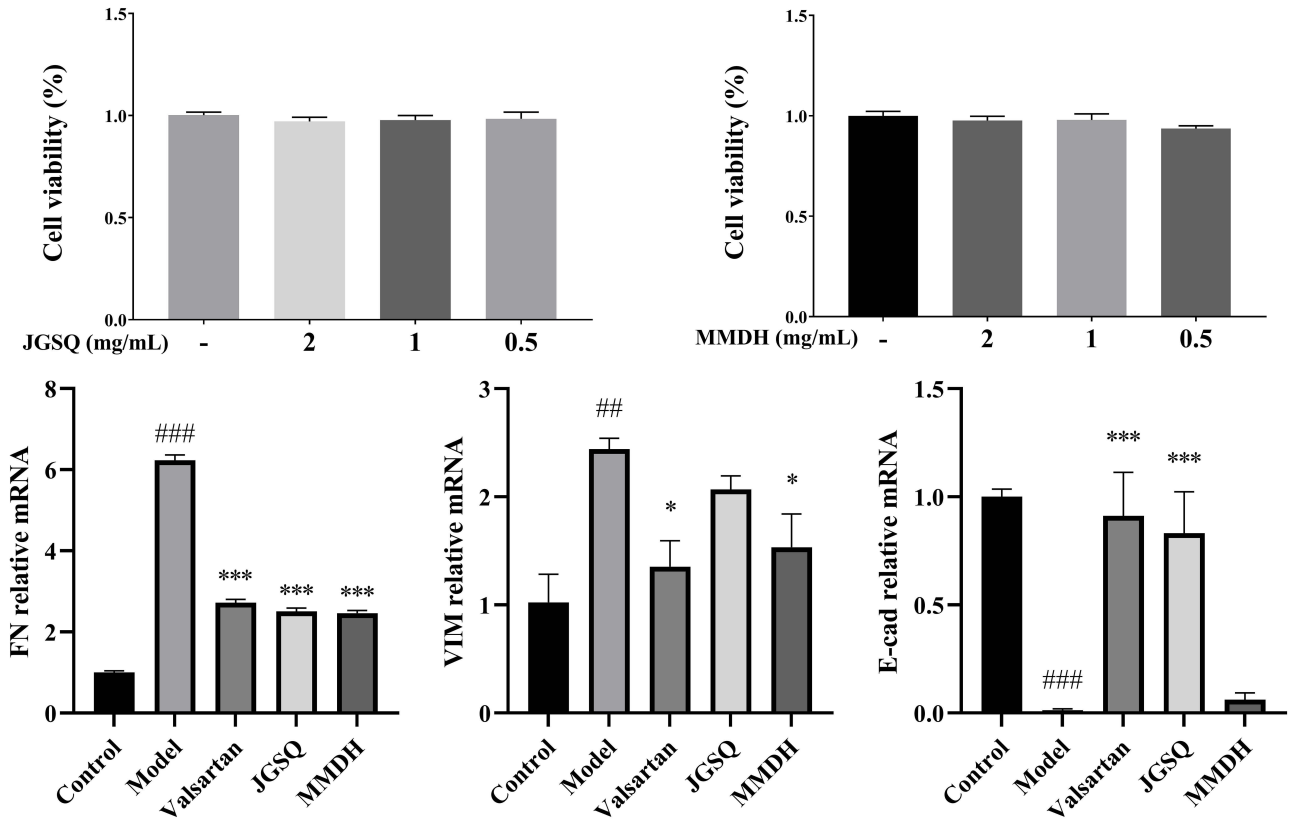

Figure 4 In vitro pharmacological evaluation of JGSQ and MMDH. The data presented here are the combined results of five separate experiments. Compared with control group: $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$. Compared with model group: $^{*}P < 0.05$, $^{***}P < 0.001$.

original formula. Furthermore, JGSQ and MMDH each incorporated two additional herbs on top of the shared base formula, thereby conferring unique regulatory genes and signaling pathways to each. This phenomenon further elucidated the combinatorial characteristics of TCM formulas, demonstrating that while preserving the core therapeutic effects, the adjustment of herbal composition enabled specific regulation of distinct biological pathways. Consequently, the Yin-Yang properties of these formulas became concretely manifested at the molecular mechanism level. KEGG enrichment results revealed that shared pathways include Steroid biosynthesis, JGSQ-specific pathways include the TGF-beta signaling pathway, and MMDH-specific pathways include the TNF signaling pathway (Figure 6B). Subsequently, PPI networks were constructed using genes from the JGSQ and MMDH-specific regulatory pathways to observe target interactions (Figure 6C).

Using the complete set of genes obtained from transcriptomics, WGCNA was constructed to identify key network modules associated with JGSQ and MMDH. The results showed that the MEdarkgreen and MEgreenyellow modules were significantly associated with JGSQ, while the MEpink and MEyellow modules were significantly associated with MMDH (Figure 7A). Further KEGG enrichment analysis of each significantly related color module revealed that MEdarkgreen was enriched in the notch signaling pathway, MEgreenyellow in nucleocytoplasmic transport, MEpink in aminoacyl-tRNA biosynthesis, and MEyellow in the notch signaling pathway (Figure 7B). Notch signaling pathway, nucleocytoplasmic transport, and aminoacyl-tRNA biosynthesis are closely associated with the pathogenesis of diabetes. However, whether these pathways can be directly linked to the Yin-Yang properties of JGSQ and MMDH remains to be further investigated. This also suggests that WGCNA analysis may have limitations in precisely identifying the complex mechanisms of TCM formulas within the context of the entire gene network.[25–27] Subsequently, GSEA was used to evaluate the gene sets associated with JGSQ and MMDH. The results showed that JGSQ is primarily involved in regulating the TGF-beta signaling pathway, while MMDH is mainly involved in regulating TNFA signaling via NFκB (Figure 7C).

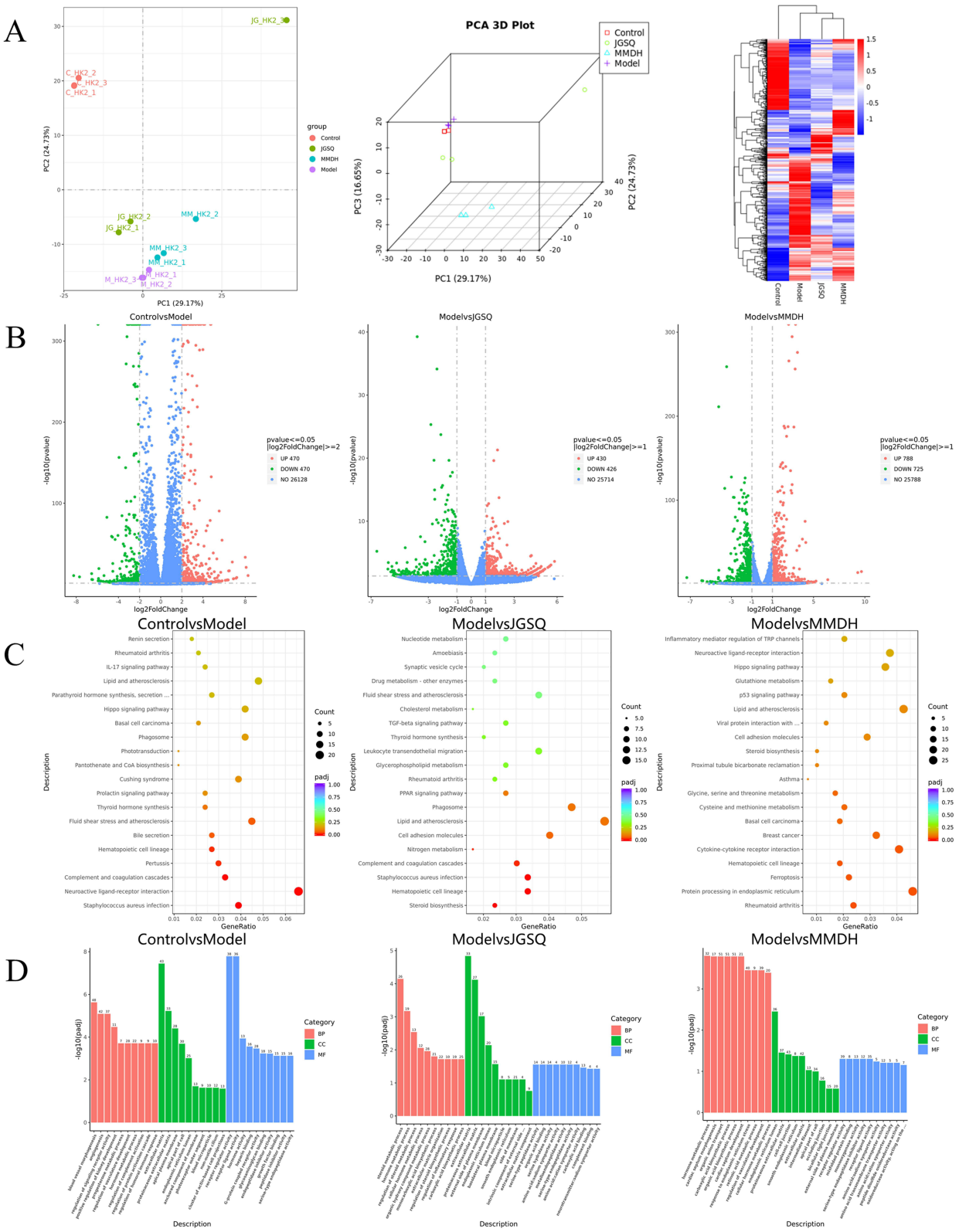

Figure 5 Transcriptional regulatory analysis. PCA distribution, clustering heatmap (**A**), DEGs volcano plot (**B**), KEGG enrichment (**C**), and GO enrichment (**D**) for the control group, model group, JGSQ group, and MMDH group.

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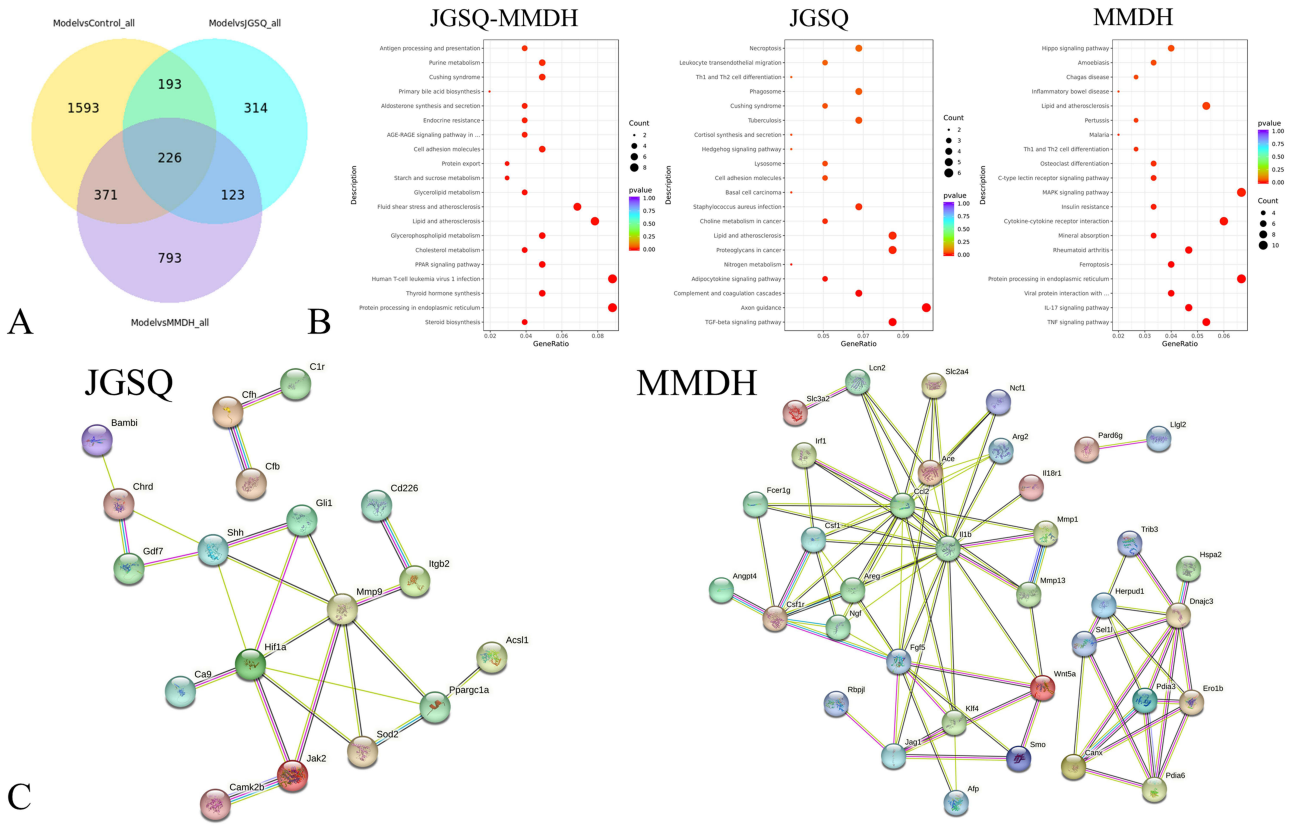

Figure 6 Analysis of unique regulatory genes and pathways of JGSQ and MMDH based on DEGs. VENN diagram (**A**), KEGG pathway enrichment (**B**), and PPI network (**C**).

Molecular docking was used to simulate the binding modes of key components from JGSQ and MMDH, including syringaldehyde, cinnamaldehyde, apigenin, and kaempferol, with the targets TGFB1 and TNFA (Figure 8). Syringaldehyde binds to HIS221 and ASP92 of TGFB1, while cinnamaldehyde binds to HIS221 and MET103 of TGFB1. Apigenin binds to GLU116 and TYR115 of TNFA, and kaempferol binds to GLU116 and LYS98 of TNFA.

Discussion

In this study, we conducted a systematic analysis of the chemical components, network pharmacology, in vitro experiments, transcriptomics, and molecular docking of the TCM formulas MMDH and JGSQ. The results revealed differences in their chemical components and potential pharmacological mechanisms. Through UPLC-MS analysis, we found that MMDH and JGSQ contain various chemical components, including organic acids, aldehydes, phenols, coumarins, flavonoids, terpenoids, and esters. The diversity of these chemical components suggests the complex pharmacological effects of both formulas in regulating the Yin-Yang balance in the kidneys. Network pharmacology analysis further indicated that these components may play roles in the treatment of DN by regulating multiple biological pathways. In vitro experiments showed that JGSQ primarily alleviates fibrosis and enhances epithelial cell adhesion by regulating the expression of FN and E-cad, while MMDH alleviates interstitial fibrosis by regulating the expression of FN and VIM. These results highlight the different mechanisms by which JGSQ and MMDH regulate the Yin-Yang balance. Transcriptomic analysis revealed significant differences in gene expression between JGSQ and MMDH, with JGSQ primarily regulating the TGF-β signaling pathway and MMDH primarily regulating the TNF signaling pathway, which was further supported by GSEA analysis. Finally, molecular docking analysis further elucidated the unique mechanisms by which the key components of JGSQ and MMDH regulate the Yin-Yang balance.

High blood glucose levels in DN patients lead to significant abnormalities in the glomeruli, with the most severe damage caused by the excessive secretion of TGF-β1 by mesangial cells. Excessive TGF-β1 promotes the accumulation of extracellular matrix proteins such as FN. TGF-β1 also induces podocyte apoptosis, further increasing the permeability of

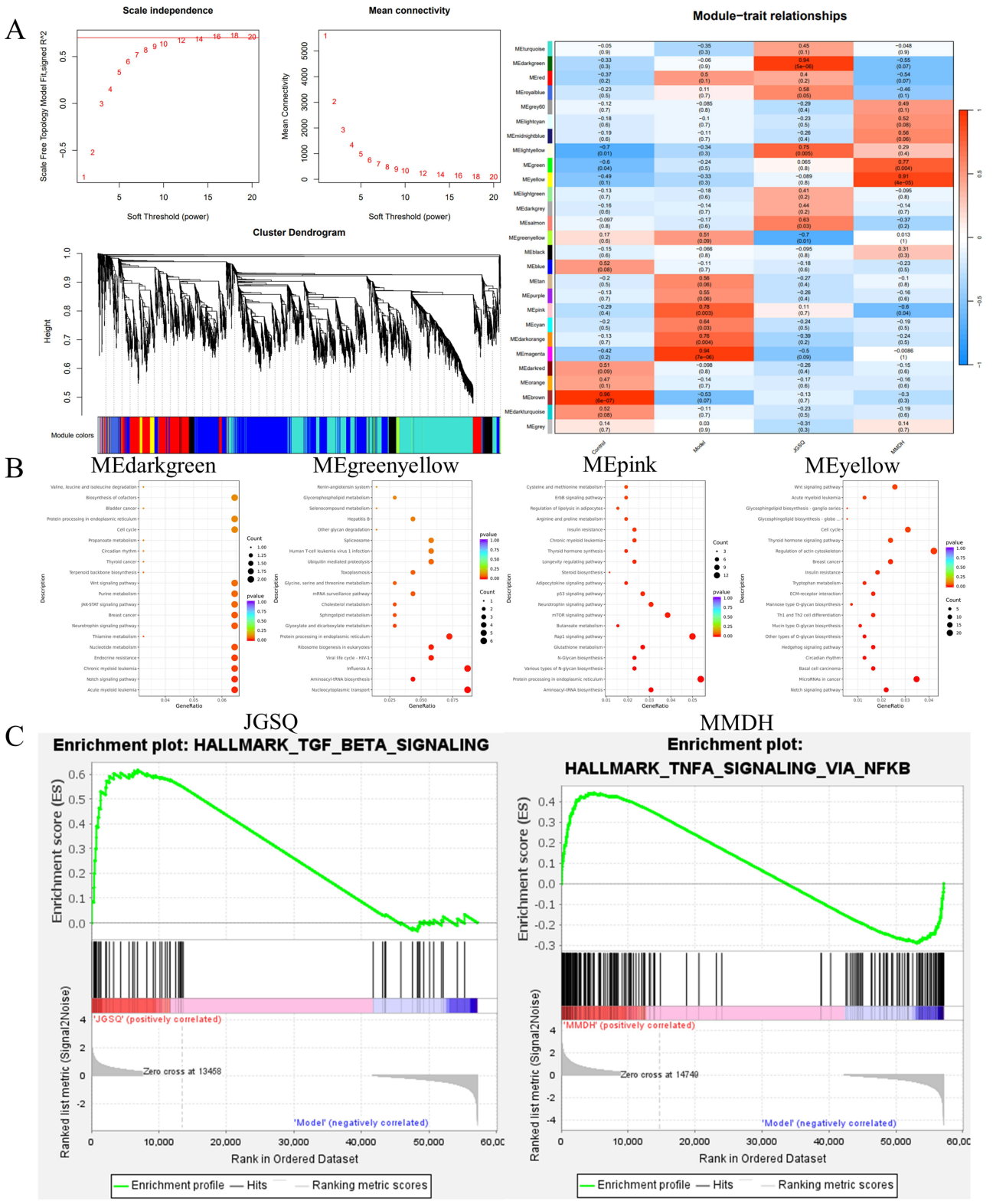

Figure 7 Key pathway analysis of JGSQ and MMDH based on WGCNA and GSEA. WGCNA construction (**A**), KEGG enrichment of network modules (**B**), GSEA enrichment (**C**).

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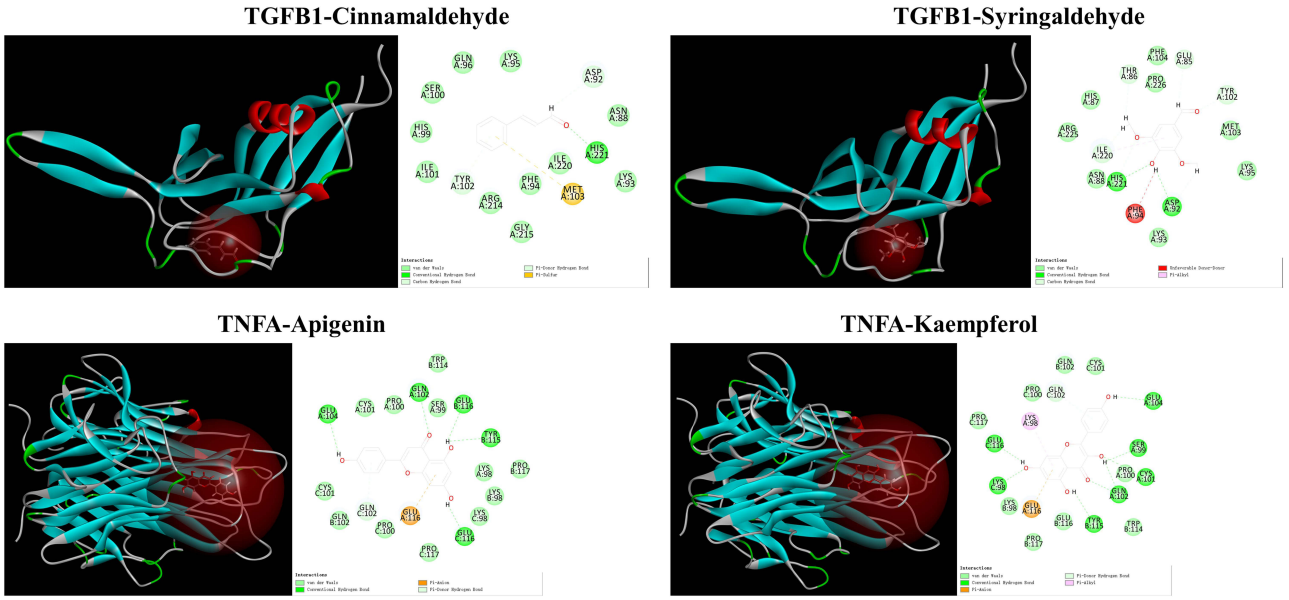

Figure 8 Binding patterns of key components and targets of JGSQ and MMDH.

the glomerular basement membrane, exacerbating diabetic proteinuria and renal fibrosis. Therefore, inhibiting TGF-β1 is considered a promising therapeutic target for the treatment of DN.[28–31] TNF-α is a pleiotropic cytokine whose receptors 1 and 2 (TNFR1, TNFR2) are members of the TNFR superfamily and serve as key mediators of chronic inflammation. By stimulating signaling pathways, including the downstream cascades of MAPK and NF-κB, TNF-α triggers the production of relevant cytokines and the expression of adhesion molecules in the kidney, ultimately leading to the development of glomerulosclerosis and tubulointerstitial fibrosis.[32–37] On this basis, TNF-α has been recognized as a key therapeutic target for treating DN. Through analyses at various levels, we have found that JGSQ may exert its antifibrotic activity via the TGF-β signaling pathway, while MMDH may display its anti-inflammatory effects through the TNF signaling pathway. This also suggests the potential underlying mechanisms of JGSQ in tonifying kidney Yang and MMDH in nourishing kidney Yin (Figure 9). At the same time, JGSQ and MMDH also involve common regulatory signaling pathways in their mechanisms of action, such as steroid biosynthesis, protein processing in the endoplasmic reticulum, PPAR signaling pathway, and cholesterol metabolism. The involvement of these shared pathways not only highlights the "multi-component, multi-target, and multi-pathway" characteristics of TCM but also further reveals its complexity and systemic regulatory effects. Moreover, this shared regulatory mechanism suggests that the properties of TCM are not strictly categorized as either Yin or Yang but rather exist in a dynamic balance and mutual transformation between the two. In other words, the therapeutic effects of TCM are not solely dependent on an extreme action of one aspect but are instead achieved through the mutual restriction and regulation of Yin and Yang, ultimately stabilizing and improving physiological functions. Therefore, this transitional process between Yin and Yang may be the key mechanism through which TCM achieves synergistic regulation and maintains homeostasis in the body. This study has partially revealed the Yin-Yang properties of JGSQ and MMDH, as well as their potential mechanisms in regulating DN. However, certain limitations remain. Future research will be conducted using DN animal models to explore the in vivo mechanisms by which MMDH and JGSQ regulate Yin-Yang balance from multiple perspectives, including physiological and biochemical analysis, pathology, and molecular mechanisms. Furthermore, the bioactive components responsible for their therapeutic effects will be further elucidated. Systematic experimental validation will help to solidify the scientific foundation of the Yin-Yang harmony theory in DN treatment, while also providing stronger experimental support for the modernization of TCM and the advancement of precision medicine.

Conclusion

In conclusion, a comprehensive analysis showed that MMDH and JGSQ exhibit significant differences in their chemical components, targets, and regulatory pathways. These differences highlight the distinct roles each formula plays in

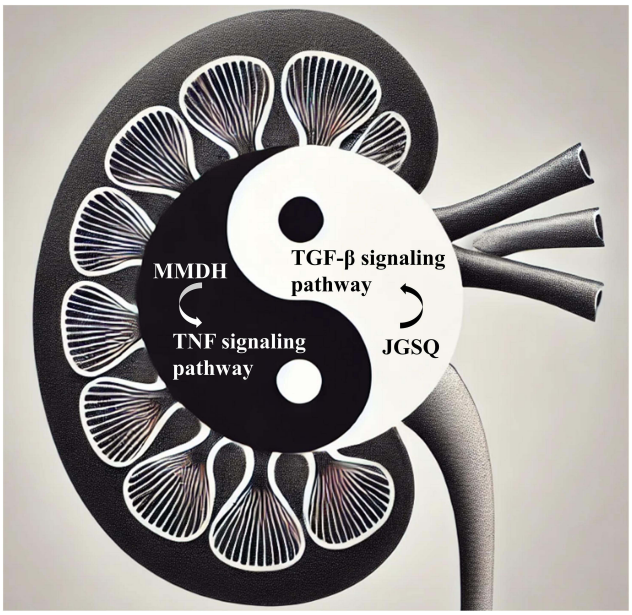

Figure 9 Potential mechanisms of JGSQ and MMDH regulating kidney Yin and Yang.

regulating the Yin-Yang balance in the kidneys, and provide important insights for further pharmacological research. Future studies can further explore the in vivo interaction mechanisms of these components and pathways.

Abbreviations

DN, diabetic nephropathy; RAS, renin-angiotensin system; ACEIs, angiotensin-converting enzyme inhibitors; ARBs, angiotensin receptor blockers; DPP-4, dipeptidyl peptidase-4; GLP-1, glucagon-like peptide-1; SGLT2, sodium-glucose co-transporter 2; TCM, traditional Chinese medicine; TCM, traditional Chinese medicine; MMDH, Mingmu Dihuang pill; JGSQ, Jingui Shenqi pill; LC-MS, liquid chromatography-mass spectrometry; KEGG, Kyoto Encyclopedia of Genes and Genomes; GO, Gene Ontology; VIM, vimentin; FN, fibronectin; E-cad, E-cadherin; DEGs, differentially expressed genes; GSEA, Gene Set Enrichment Analysis; WGCNA, Weighted Correlation Network Analysis; qRT-PCR, Quantitative real-time PCR.

Data Sharing Statement

Data will be made available on request.

Acknowledgments

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Disclosure

All authors declare no conflict of interests.

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