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Exploring the pharmacological mechanisms of *Tripterygium wilfordii* against diabetic kidney disease using network pharmacology and molecular docking

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ABSTRACT

Background: Tripterygium wilfordii (TW), when formulated in traditional Chinese medicine (TCM), can effectively treat diabetic kidney disease (DKD). However, the pharmacological mechanism associated with its success has not yet been elucidated. The current work adopted network pharmacology and molecular docking for exploring TW-related mechanisms in treating DKD. **Methods**: In the present work, the Traditional Chinese Medicine Systems Pharmacology (TCMSP) database was employed to obtain the effective components and candidate targets of TW. Additionally, this work utilized the UniProt protein database for screening and standardizing human-derived targets for effective components. The Cytoscape software was utilized to construct an effective component-target network for TW. Targets for DKD were acquired in the GEO, DisGeNET, GeneCards, and OMIM databases. Additionally, a Venn diagram was also plotted to select the possible targets of TW for treating DKD. Gene ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis were conducted to explore the TW-related mechanism underlying DKD treatment. This work also built a protein-protein interaction (PPI) network based on the Cytoscape and String platform. Then, molecular docking was conducted in order to assess the affinity of key proteins for related compounds. **Results**: In total, 29 active components and 134 targets of TW were acquired, including 63 shared targets, which were identified as candidate therapeutic targets. Some key targets and important pathways were included in the effect of TW in treating DKD. Genes with higher degrees, including TNF and AKT1, were identified as hub genes of TW against DKD. Molecular docking showed that TNF and AKT1 bind well to the main components in TW (kaempferol, beta-sitosterol, triptolide, nobiletin, and stigmasterol).

Abbreviations: TW, Tripterygium Wilfordii; TCM, Traditional Chinese Medicine; DKD, Diabetic Kidney Disease; TCMSP, Traditional Chinese Medicine Systems Pharmacology; GO, Gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; PPI, Protein-Protein Interaction; ESRD, end-stage renal disease; ARB, angiotensin receptor blocker; ACEI, angiotensin-converting enzyme inhibitor; GLP-1R, glucagon-like peptide 1 receptor; SGLT-2, sodium-dependent glucose transporters 2 inhibitors; NP, Network Pharmacology; MD, Molecular Docking; DL, drug-likeness; OB, oral bioavailability; DEGs, differentially expressed genes; CC, cellular component; BP, biological pathway; MF, molecular function; PDB, Protein Data Bank; DAVID, Database for Annotation, Visualization and Integrated Discovery; DM, diabetes mellitus.

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Conclusions: TW primarily treats DKD by acting on two targets (AKT1 and TNF) via the five active ingredients kaempferol, beta-sitosterol, triptolide, nobiletin, and stigmasterol.

1. Introduction

It is estimated that 10.5% of the population has developed diabetes mellitus (DM) in 2021 [1], with this figure expected to increase to 10.9% by 2045 [2]. Diabetic kidney disease (DKD), a frequently observed microvascular pathology of DM, usually induces end-stage renal disease (ESRD) [3]. Recently, however, owing to lifestyle alterations and the introduction of therapy, DKD treatment has been greatly improved. Current research into treatments focuses on the angiotensin receptor blocker (ARB), angiotensin-converting enzyme inhibitor (ACEI), glucagon-like peptide 1 receptor (GLP-1R), sodium-dependent glucose transporters 2 inhibitors (SGLT-2), and others [4,5]. These treatments can offer favorable outcomes for DKD patients, but numerous DKD cases still lead to the development of ESRD. Irreversible kidney failure cannot be avoided when DKD leads to ESRD, and kidney transplantation is the only way to treat this condition [6,7]. Due to the restrictions of traditional Western medicine, a number of DKD cases receive other treatments, including traditional Chinese medicine (TCM) [8]. TCM can offer multi-target, multi-mechanism, and safety advantages when treating DKD, which have been highlighted in the research.

In China, TCM has been extensively adopted for treating DKD and, typically, *Tripterygium wilfordii* (TW) has been utilized. TW, also known as Leigongteng, possesses diverse bioactive molecules and their associated pharmacological pathways, has different molecular/cellular targets, has few adverse reactions, and has been utilized in TCM for more than 2,000 years [9]. Due to its multiple pharmacological effects, including anti-inflammation, antifibrosis, anti-autoimmune disorders, and antiatherosclerosis [10–12], TW has

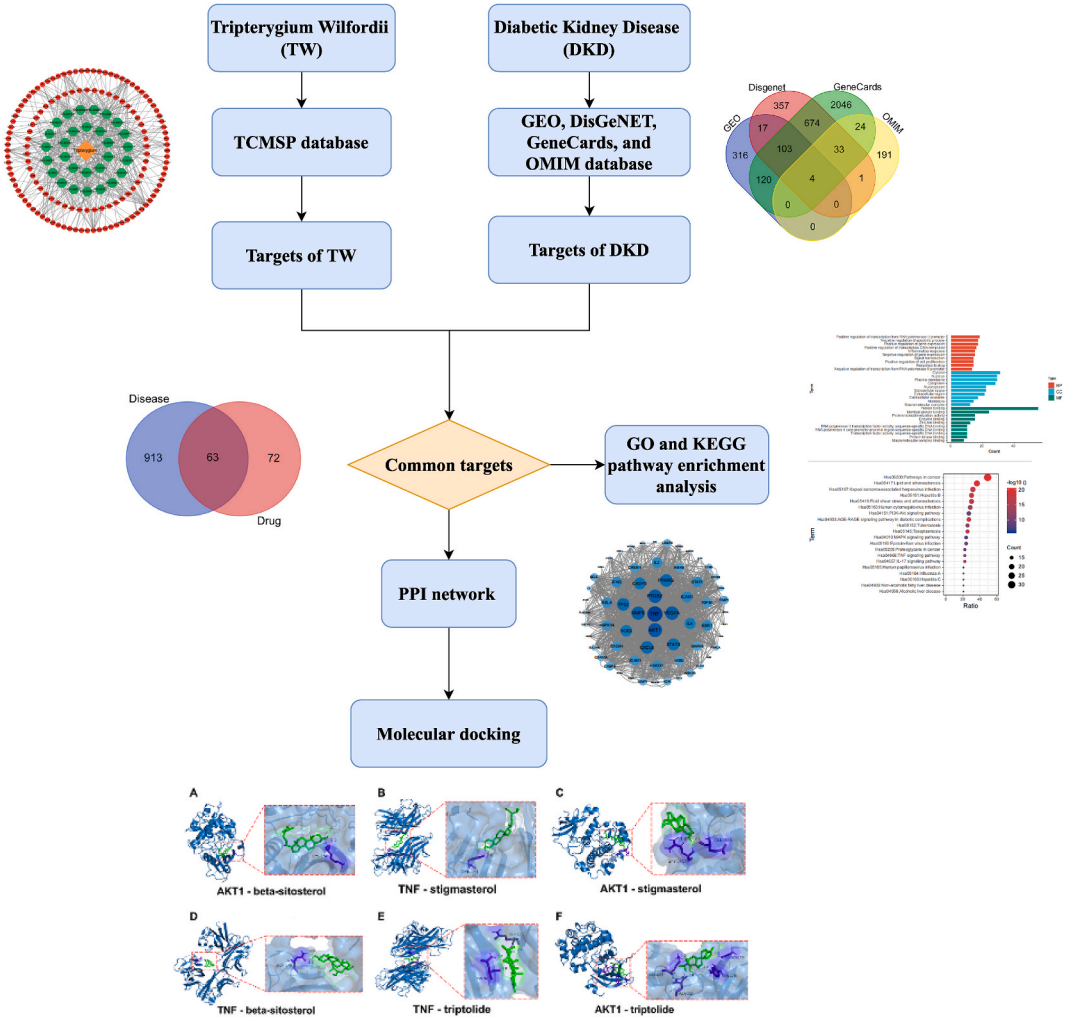

Fig. 1. A flow chart exploring TW against DKD based on network pharmacology.

been adopted for treating diverse kinds of glomerulonephritis, including DKD [13]. More studies are, however, necessary to investigate related pharmacological mechanisms.

Network pharmacology (NP) integrates massive biological data using computer technology and bioinformatics analysis [14]. NP can be used for elucidating the mechanisms underlying bioactive components as well as for studying the therapeutic efficacy of TCM, which is used for exploring the TW-related mechanisms in disease treatment, including IgA nephropathy [15] and cardiovascular disease [16]. Molecular docking (MD) can enhance the molecular pharmacological effect and illustrate the mechanism of drug action at the molecular level [17]. The combination of NP and MD offers a way to conduct unsystematic and one-sided research on therapeutic drug mechanisms. The present work adopted NP technology for exploring the major bioactive components in TW and predicting the corresponding molecular targets as well as its associated mechanisms for treating DKD, providing a reference for clinical uses and basic study. Fig. 1 illustrates our workflow.

2. Materials and methods

2.1. Selection of bioactive components and targets for TW

The Traditional Chinese Medicine Systems Pharmacology (TCMSP, http://tcmspw.com/tcmsp.php) database was searched using the term "Leigongteng" in order to obtain bioactive components and targets for TW [18]. The ingredients were selected according to drug-likeness (DL) $\geq$ 0.18 and oral bioavailability (OB) $\geq$ 30% as the bioactive components [19]. The UniProt (https://www.uniprot.org) database was then employed for target standardization, with the species and status set as "human" and "reviewed", separately [20]. Additionally, the Cytoscape 3.9.1 software was adopted for constructing the compound-targets network for TW [21].

2.2. Screening of possible targets of TW for treating DKD

To identify related disease targets, the DKD-associated proteins were obtained in 4 databases using the keyword "diabetic nephropathy", including Gene Expression Omnibus (GEO, https://www.ncbi.nlm.nih.gov/geo/) [22], DisGeNET (https://www.disgenet.org/home/) [23], GeneCards (https://www.genecards.org/) [24], and the Online Mendelian Inheritance in Man (OMIM, https://omim.org/) [25] databases. From the GEO database, GSE30122, GSE47185, GSE104948, and GSE104954 were chosen for exploring differentially expressed genes (DEGs) in DKD compared with normal samples using the online approach GE02R (https://www.ncbi.nlm.nih.gov/geo/geo2r/) using the thresholds of $|\log2FC| > 1$ and $p < 0.05$ (Table 1). This study also plotted a Venn diagram (http://bioinfogp.cnb.csic.es/tools/venny/index. html) for visualizing the intersecting genes which from GEO, DisGeNET, GeneCards, and OMIM [26]. When the genes existed in more than one database, this was deemed to indicate that they were reliable DKD-related disease targets. Targets shared by TW and DKD were obtained via Venny for further analysis.

2.3. Functional annotations of TW-DKD common genes

For exploring the TW-related mechanism in treating DKD, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment were used on the chosen targets based on the Database for Annotation, Visualization and Integrated Discovery (DAVID, https://david.ncifcrf.gov) [27]. GO functional annotation contributed to the provision of the biological functions of gene products and divided possible targets into diverse functional modules, namely, cellular component (CC), biological pathway (BP), and molecular function (MF) [28]. In addition, KEGG analysis helps to understand gene functions at the molecular level or higher [29]. Results with $P < 0.05$ were destined for further analysis.

2.4. Protein-protein interaction (PPI) network construction and core targets identification

Using the STRING database (https://cn.string-db.org/) [30] and by collecting nearly every known and predicted expressed protein interaction, this work attempted to construct a PPI network for target genes using the Cytoscape 3.9.1 software [21]. All factors were set as defaults, except for the species type, which was set to "*Homo sapiens*". Core targets were analyzed by calculating topology parameters, containing betweenness centrality (BC), closeness centrality (CC), and degree centrality (DC). Betweenness represents the ratio of the shortest path number crossing one point to the overall shortest path number within the network; closeness stands for the distance between two nodes, and degree centrality indicates the connection number between nodes in the network and others. Betweenness, closeness, and degree of centrality represent the major topological factors for measuring node importance within the network and determining the importance of target proteins for key targets [31].

Table 1
A summary of microarray datasets from gene expression omnibus (GEO) datebase.

Series	Platform	Affymetrix GeneChip	Samples
GSE30122	GPL571	Affymetrix Human Genome U133A 2.0 Array	69
GSE47185	GPL14663	Affymetrix GeneChip Human Genome HG-U133A Custom CDF	22
GSE104948	GPL22945	Affymetrix Human Genome U133 Plus 2.0 Array	25
GSE104954	GPL22945	Affymetrix Human Genome U133 Plus 2.0 Array	13

2.5. Molecular docking (MD)

Molecular docking is an approach that analyzes binding affinity and binding sites between macromolecular targets and molecular conformation, which is an important technology for discovering drugs based on structural aspects [32]. A small lowest energy indicates higher stability and binding affinity between receptor and ligand. Autodocktools 1.5.7 [33]was utilized to assess associations of TW (the ligand) with key DKD targets (the receptors), and PyMoL [34] was used for visualization: as a result, binding energy and binding affinity could be determined. Based on the TCMSP database, this work obtained TW structure (mol2 file), while the Protein Data Bank (PDB) database (https://www.rcsb.org/pages/contactus) was applied to acquire the target structure (PDB file). Fifty runs were obtained from searching using the Lamarckian genetic algorithm.

3. Result

3.1. Bioactive components and corresponding targets of TW

This work selected 29 bioactive ingredients and 134 targets of TW using TCMSP. Using Cytoscape 3.9.1 software, a bioactive component-target network was obtained, including 446 edges and 164 nodes (Fig. 2). In accordance with topological parameters, the key compounds of "degree > 25" were kaempferol, beta-sitosterol, triptolide, nobiletin, and stigmasterol, all of which might have important effects on TW.

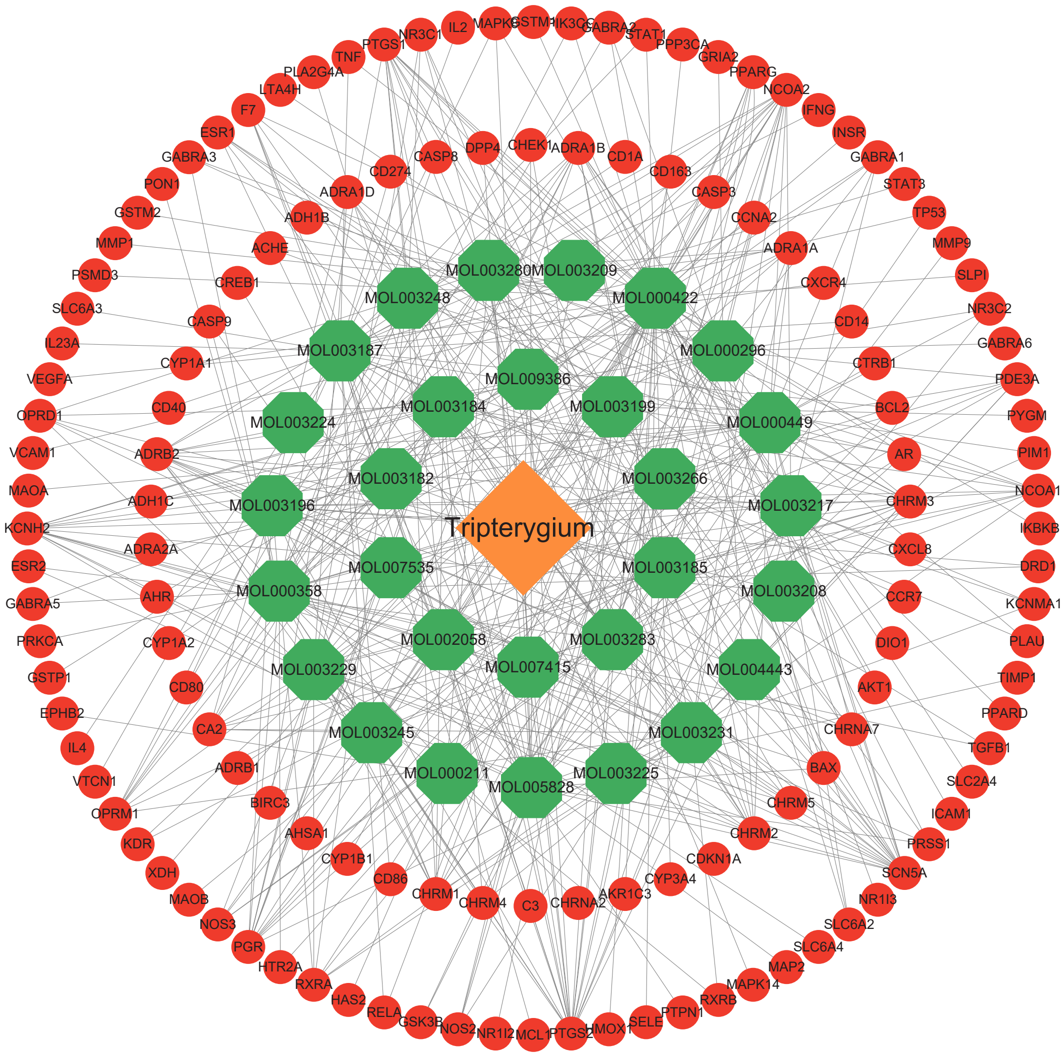

Fig. 2. Active ingredient-target network of TW. The orange diamond represents the TW; green octagons represent active ingredients; red ellipses represent potential targets.

3.2. DKD-related genes and Drug–Disease intersection targets

The present work discovered 838 DEGs in DKD compared with corresponding healthy nephrology samples using the thresholds of $P < 0.05$ and $|\log2FC| > 1$ using the GE02R online tool. Altogether, 3886 DKD-related targets were acquired in the GEO, DisGeNET, GeneCards, and OMIM databases (n = 838 in GEO, n = 1189 in DisGeNET, n = 3004 in GeneCards, and n = 253 in OMIM) (Fig. 3). We considered the genes that existed in more than one database as being reliable genes. Later, the Venn diagram was plotted based on the targets of DKD and TW (to map and intersect), and finally, 63 intersected targets were acquired, which might be the possible targets of TW for DKD treatment (Fig. 4).

3.3. GO and KEGG functional annotation

On 63 potential targets, both GO and KEGG pathway enrichment were performed. The 10 most significant GO in the BP, CC, and MF categories were chosen (Fig. 5). In terms of BP terms, co-target genes demonstrated significant relationships to gene expression, transcription, and the apoptotic process. Furthermore, these genes are linked to inflammation response, drug response, and signal transduction. CC analysis revealed that the nucleus, cytosol, and membrane accounted for most of the total. The target proteins in the MF category were primarily involved in protein binding, zinc ion binding, and enzyme binding. A total of 137 potential co-target gene signaling pathways were enriched (Fig. 6), including lipid, atherosclerosis, AGE-RAGE pathway in diabetic complications, and PI3K-Akt pathway. These pathways are linked to inflammation and oxidative stress and play a role in developing DKD.

3.4. PPI network construction and analysis

To deeply explore the results and identify key targets of TW interacting with DKD, this work mapped the PPI network, including 1412 edges and 63 nodes with shared possible targets between TW and DKD (Fig. 7). Greater nodes represent a higher node degree level and a higher possibility of an effect via this target. A denser connection indicates increased importance. Ten hub targets (TNF, AKT1, PTGS2, VEGFA, MMP9, PPARG, CXCL8, TP53, STAT3, and CASP3) were screened after being investigated by BC, CC, and DC filtering (Table 2). Based on degree, the top 2 genes were chosen in subsequent analysis: TNF and AKT1.

3.5. Molecular docking

For validating our network analysis results, MD was performed between two targets (TNF and AKT1) and the five compounds (kaempferol, beta-sitosterol, triptolide, nobiletin, and stigmasterol). As for candidate targets, including TNF (PDB: 2AZ5) and AKT1 (PDB: 3CQW), their crystal structures were collected, and protein structure data were obtained from the PDB database. As a result, the binding energy of < -5.0 kcal/mol was obtained, suggesting favorable binding affinity and stability (Table 3) [35]. Among them, beta-sitosterol, stigmasterol, and triptolide showed potent binding to the key targets (docking score < -7.0 kcal/mol) [36], and structure matching analysis of them can be observed in Fig. 8. Beta-sitosterol generated a hydrogen bond (H-bond) with LYS-158 amino acid (aa) residues within AKT1; stigmasterol produced an H-bond with TYR-151 aa residues within TNF; stigmasterol generated H-bonds with GLU-365 and ARG-367 aa residues within AKT1; beta-sitosterol formed H-bonds with GLN-149 aa residues in TNF; triptolide formed H-bonds with GLN-125 and ASN-92 aa residues within TNF; and triptolide formed H-bonds with LYS-284, TYR-175,

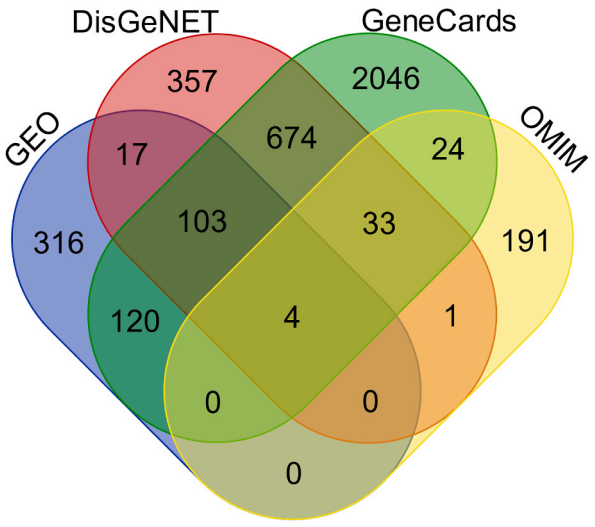

Fig. 3. Venn diagram of DKD disease targets. 838 targets were retrieved from GEO, 1189 from DisGeNET, 3004 from GeneCards, and 253 from OMIM.

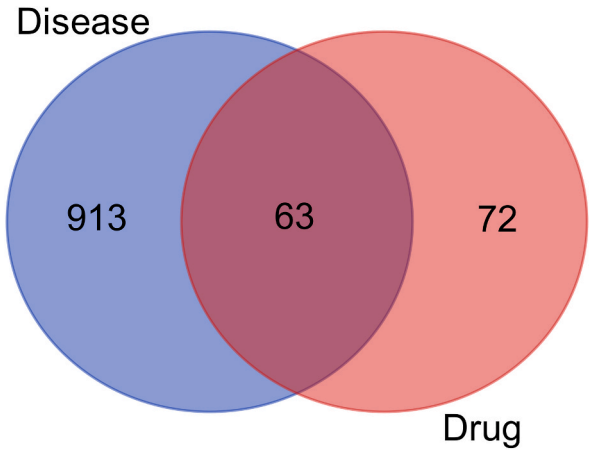

Fig. 4. Venn diagram of DKD and TW targets.

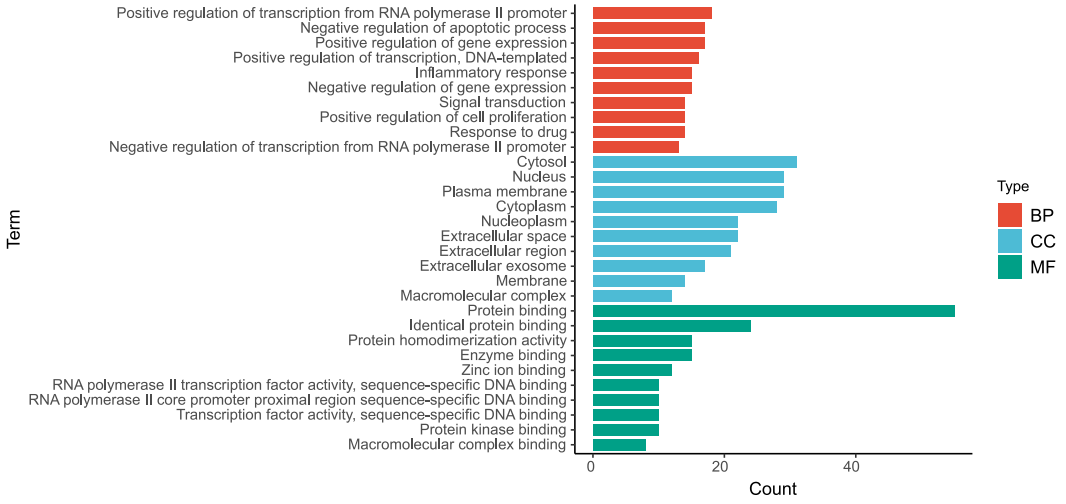

Fig. 5. GO functional enrichment analyses of DKD-TW common targets. Red columns represent biological process (BP); blue columns represent cellular component (CC); green columns represent molecular function (MF). The top 10 terms of BP, CC, and MF are displayed in the figure.

GLY-173 and ASN-231 aa residues within AKT1.

4. Discussion

Diabetic kidney disease (DKD) represents a frequently seen complication of DM, which threatens the life and health of individuals with DM. Many DM cases do not, however, obtain favorable therapeutic effects using conventional treatments. In recent decades, and with multiple advantages, TCM has emerged as the efficient candidate with which to treat DKD [37]. Tripterygium wilfordii (TW) is therefore suggested as the best possible adjuvant therapy for DKD [38,39]. As a cutting-edge approach, network pharmacology provides a preliminary understanding of the principles of network theory and systems biology [40]. Based on the advancements in network pharmacology, many drugs, especially traditional Chinese medicine, can treat a variety of diseases [41–43]. Consequently, this work analyzed TW for its role as adjuvant therapy for DKD via network pharmacology analysis. In addition, MD was conducted to analyze the association of DKD with TW. Our results highlighted new perspectives on the TW-related molecular mechanism for DKD treatment, which provides a foundation for its clinical application and basic research.

In our study, 29 bioactive components were chosen in TW, and thereafter, 63 intersected targets of TW with DKD were obtained. We analyzed the hub target genes using GO and KEGG analyses to explore TW-related mechanisms for treating DKD. PPI network analysis selected 10 key targets in line with BC, CC, and DC. According to MD verification, the bioactive compounds of TW exhibited a high binding affinity for the key candidate targets TNF and AKT1, which has the potential to provide a direction for further research on TW in treating DKD.

For GO enrichment analysis, TW treating DKD was mainly related to oxidative stress, apoptotic process, cell proliferation, and

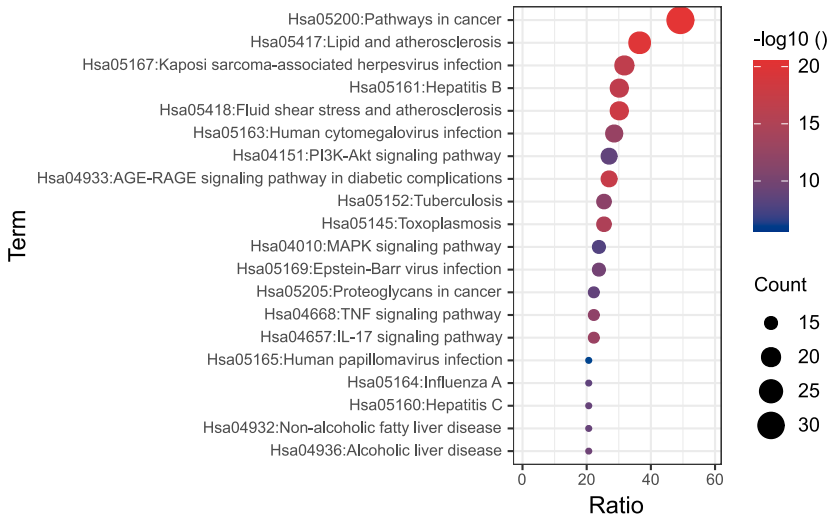

Fig. 6. KEGG pathway enrichment analyses of DKD-TW common targets. The color scale indicates the adjusted p-value, and the dot size represents the gene count in each term.

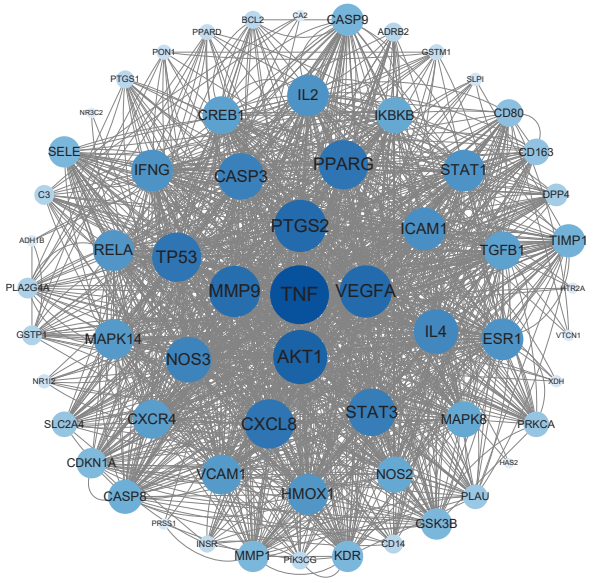

Fig. 7. PPI network of TW-DKD common targets. The larger the node is, the darker the color, the greater the node degree value is, and the more likely it is to exert a vital function via the target.

Table 2
The core targets of TW in the treatment of DKD.

Target gene	Degree	Betweenness Centrality	Closeness Centrality
TNF	104	0.092327525	0.861111111
AKT1	94	0.050121689	0.805194805
PTGS2	90	0.061768532	0.784810127
VEGFA	90	0.030331384	0.775
MMP9	88	0.070644353	0.765432099
PPARG	84	0.033489176	0.746987952
CXCL8	84	0.02769564	0.746987952
TP53	84	0.032698555	0.756097561
STAT3	80	0.016994447	0.738095238
CASP3	78	0.011656447	0.720930233

Table 3
The core targets of TW in treating DKD.

Target	PDB ID	Compound	Binding Energy/(kcal/mol)
TNF	2AZ5	kaempferol	−6.73
TNF	2AZ5	beta-sitosterol	−8.58
TNF	2AZ5	triptolide	−8.2
TNF	2AZ5	nobiletin	−6.42
TNF	2AZ5	stigmasterol	−9.35
AKT1	3CQW	kaempferol	−6.49
AKT1	3CQW	beta-sitosterol	−9.37
AKT1	3CQW	triptolide	−7.68
AKT1	3CQW	nobiletin	−6.26
AKT1	3CQW	stigmasterol	−8.65

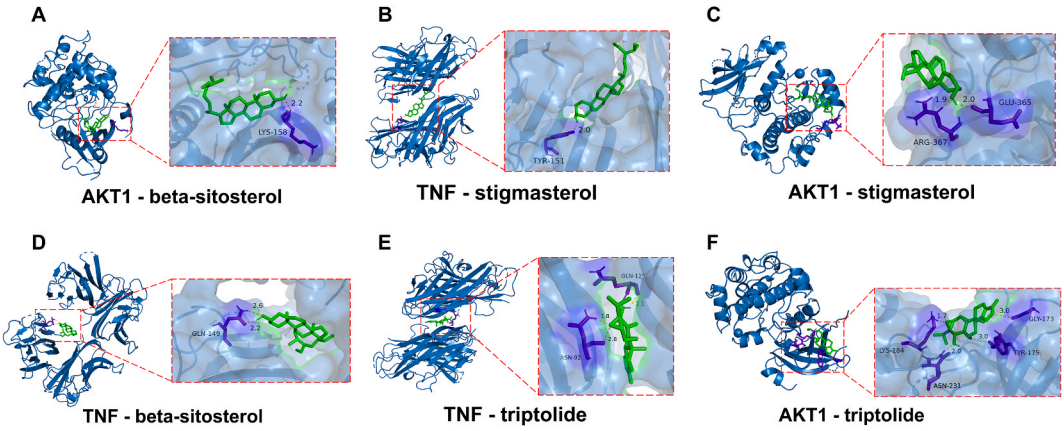

Fig. 8. Six best molecular docking results. (A) AKT1 to beta-sitosterol; (B) TNF to stigmasterol; (C) AKT1 to stigmasterol; (D) TNF to beta-sitosterol; (E) TNF to kaempferol; (F) AKT1 to kaempferol.

inflammatory response. Combined with the current research, KEGG analysis suggested that the screening targets were mostly associated with three categories, namely: Lipid and atherosclerosis, the AGE-RAGE pathway, and the PI3K-Akt pathway. The kidney is most susceptible to lipotoxicity, and kidney injury is related to abnormal lipoprotein/lipid metabolism. Dyslipidemia is suggested as an important risk factor during DKD. Abnormal serum lipid is related to kidney diseases among DM cases, and, additionally, lipid accumulation causes apoptosis and dysfunction of podocytes within DKD [44,45]. The PI3K/Akt pathway is a vital pathway associated with glucose metabolism, which is a downstream insulin pathway. Over-activation of this pathway may induce damage to podocyte structure and functions, causing proteinuria [46]. Studies have demonstrated that attenuating podocyte apoptosis and restoring podocyte autophagy were closely associated with the inhibition of the PI3K/AKT pathway [47,48]. The AGE-RAGE pathway within DKD has been widely studied. A hyperglycemic state accelerates the response and AGE aggregation within tissues, and AGE binding to RAGE (the specific receptor) can generate cytotoxicity in the kidney, leading to the development of DKD. Suppressing the AGE-RAGE axis is a novel therapeutic strategy [49,50]. TW may treat DKD by regulating the lipid and atherosclerosis, AGE-RAGE, and PI3K-Akt pathways for alleviating lipids and inflammation.

Twenty-nine active compounds in TW met the OB $\geq$ 30% and DL $\geq$ 0.18 threshold, which meant that they could be absorbed into the blood. Based on the analysis of this study, critical bioactive components in TW included kaempferol, beta-sitosterol, nobiletin triptolide, and stigmasterol. Kaempferol, a natural polyflavonol, has lipolysis, anti-inflammatory, anti-oxidative, and anti-fibrosis effects [51,52]. Beta-sitosterol is recognized as a possible herbal nutraceutical used to treat DKD since it shows anti-inflammatory, lipid-lowering, anti-oxidative, and anti-diabetic effects [53]. Triptolide, specifically the diterpenoid trioxide, is the metabolite that shows the highest abundance and pharmacological activity detected from TW extracts, which has anti-glomerular sclerosis, anti-fibrosis, regulating oxidative stress and anti-inflammatory effects [54, 55]. Nobiletin has biological effects, including lowering blood lipids, lowering blood pressure, anti-oxidative effects, and anti-inflammatory effects [56]. Stigmasterol can suppress the Ang II-mediated proliferation of aortic smooth muscle cells by arresting the cell cycle and also enhances ROS production and cell apoptosis [57]. According to pharmacological analysis, such bioactive components potentially protect individuals from DKD.

In order to reveal TW's potential mechanism for treating DKD, MD was carried out on key targets (TNF and AKT1) and the related bioactive compounds. We found that beta-sitosterol, stigmasterol, and triptolide have a strong binding capability with AKT1 and TNF, while kaempferol and nobiletin have a good binding ability with the key targets. TNF is a pro-inflammatory factor that substantially accelerates the emergence of DKD and causes injury to the glomerular filtration barrier [58]. Additionally, TNF contributes to activating NF-κB, promotes infiltration of inflammatory cells, and also accelerates renal fibrosis leading to kidney injury [59]. As verified

in previous research, TNF expression is remarkably upregulated in DKD cases, showing a positive relation to disease course [60]. AKT possesses 3 isoforms, including AKT1, AKT2, and AKT3. AKT1 represents the serine/threonine kinase that is a vital factor related to the PI3K/AKT pathway, which significantly affects protein biosynthesis, lipid metabolism, glucose homeostasis, and apoptosis [61,62]. Compared with normal rats, AKT1 mRNA and protein levels within renal tissues from DKD rats are significantly elevated [63]. In the study, we also found that both TNF and AKTI are related to the lipid and atherosclerosis and AGE-RAGE pathways, among other diabetic complications.

According to evidence-based research in medicine, TW can effectively reduce blood urea nitrogen (BUN) contents, serum creatinine, proteinuria and can also improve the response rate [64]. Its toxic effect restricts its application in clinical settings. According to reports, TW is toxic to the digestive, liver, nervous, and reproductive systems [65]. It is urgent, therefore, that bioactive substances are found and that their structures are modified: purifying their extracts and combining them with other drugs to reduce the toxicity of TW could enable its use in clinical settings.

This research has some limitations. To begin, we only analyze DKD genes with two or more databases; while these disease genes are more reliable, this may result in a gene collection that is not comprehensive. Second, the results must be validated through experiments.

5. Conclusion

To summarize, the network pharmacology method was used to uncover the chemical basis and investigate the mechanism of action of TW on DKD. At first, five TW active compounds (kaempferol, beta-sitosterol, triptolide, nobiletin, and stigmasterol) and two TW core target genes (AKT1 and TNF) against DKD were identified. Then, as indicated by GO and KEGG analyses, lipid, atherosclerosis, AGE-RAGE, and PI3K-Akt pathways were found to be closely associated with the protective effect of TW on DKD. It provided a theoretical foundation as well as a clue for the study of pharmacological mechanisms.

Author contribution statement

Meiqi Lu: Performed the experiments; Wrote the paper.
Juanjuan Ou: Analyzed and interpreted the data.
Xiaoqi Deng, Yixuan Chen: Contributed reagents, materials, analysis tools or data.
Qing Gao: Conceived and designed the experiments.

Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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