



Analysis of Traditional Chinese Medicine Syndromes and Treatment Laws of Diabetic Kidney Disease and the Action Mechanism of High-Frequency Chinese Herbs in the Treatment of Diabetic Kidney Disease Based on Real-World Study

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Abstract

Objective Our objective was to analyze the traditional Chinese medicine (TCM) syndrome and treatment laws of diabetic kidney disease (DKD) and the action mechanism of high-frequency Chinese herbs in the treatment of DKD based on real-world study.

Methods The data of patients with DKD who had been treated in the First Hospital Affiliated to Henan University of Chinese Medicine from January 1, 2014 to December 31, 2021 were retrospectively analyzed through the hospital information management system. The contents of the cases were statistically analyzed using IBM SPSS Statistics 25 software, and the laws of DKD treatment were summarized. Network pharmacology and molecular docking were used to analyze the action mechanism of high-frequency Chinese herbs in the treatment of DKD.

Results The data of a total of 1,201 patients with DKD were included, involving 72 kinds of TCM syndromes. Nine disease nature elements and six disease location elements were extracted, involving 405 Chinese herbs. The top five high-frequency Chinese herbs were Baizhu (*Atractylodis Macrocephalae Rhizoma*), Fuling (*Poria*), Huangqi (*Astragali Radix*), Chuanxiong (*Chuanxiong Rhizoma*), and Danshen (*Salviae Miltiorrhizae Radix et Rhizoma*). Thirty kinds of Chinese herbs with the frequency of ≥ 100 were mainly deficiency-tonifying herbs and blood-activating and stasis-eliminating herbs. The medicinal properties were mainly warm and mild, and the medicinal flavors were sweet and bitter mostly. For the meridian tropism, the main meridian tropism of these herbs is spleen meridian and lung meridian. The clustering method

Keywords

- ▶ diabetic kidney disease
- ▶ laws of syndromes and treatment
- ▶ high-frequency Chinese Herbs
- ▶ real-world study
- ▶ TCM
- ▶ network pharmacology
- ▶ molecular docking
- ▶ diabetes mellitus
- ▶ action mechanism

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aggregated the 30 commonly used Chinese herbs into six categories. A total of 58 effective active ingredients of high-frequency Chinese herbs and 164 related targets were screened based on the traditional Chinese medicine systems pharmacology database and analysis platform (TCMSP); 1,434 targets of DKD and 90 potential targets of high-frequency Chinese herbs for DKD were obtained. “Active ingredient-potential target” network topology analysis indicated that quercetin, luteolin, 7-O-methylisoxitol, hederagenin, and 4-methylene miltirone were the five core chemical components of high-frequency Chinese herbs in the treatment of DKD. Protein-protein interaction network topology analysis indicated that protein kinase B1, interleukin-6, tumor necrosis factor, vascular endothelial growth factor A, tumor protein P53 were the core protein targets. Twenty signaling pathways were obtained by Kyoto Encyclopedia of Genes and Genomes enrichment analysis. Molecular docking showed that luteolin, 4-methylene miltirone, and osthole had strong binding to AKT1.

Conclusion The differentiation and treatment of DKD with TCM follows the principles of “taking kidney as the root, considering both the liver and spleen, tonifying qi and nourishing yin, promoting blood circulation and resolving blood stasis.” High-frequency Chinese herbs for the treatment of DKD have multicomponent, multitarget, and multipath characteristics.

Introduction

Diabetic kidney disease (DKD) is a chronic kidney disease caused by diabetes mellitus (DM). The clinical characteristics of DKD are that the estimated glomerular filtration rate (eGFR) is less than $60 \text{ ml}\cdot\text{min}^{-1}\cdot(1.73\text{m}^2)^{-1}$ or persistent albuminuria.¹ The incidence of DKD in diabetic patients worldwide is as high as 40%, which significantly increases the mortality of diabetic patients.² As far as the clinical status is concerned, Western medicine treatment of DKD cannot prevent the development process of the disease.³ In recent years, it has been found that traditional Chinese medicine (TCM) plays an increasingly obvious role in alleviating symptoms such as fatigue, edema, lumbar soreness, reducing urinary protein content, protecting kidney function, and improving prognosis in DKD patients and is gradually recognized and valued.⁴ At present, it lacks high-level evidence-based medical research on the efficacy of DKD, and its mechanism needs to be further clarified.

Randomized controlled trials, clinical observation analysis, and cohort study analysis are the main methods of TCM clinical trials based on the current medical model, and TCM clinical trials have their own relative perfect theoretical systems from ancient times to the present. Therefore, the direct application of modern medical clinical research methods in TCM clinical trials will inevitably lead to some problems. Real-world study (RWS) is the process of collecting relevant information about patients in the actual medical environment and analyzing the benefits and risks of herbs. The adaptability of the two has changed to some extent. RWS evaluates the effects of herbs on large numbers of users in a real medical environment. This study aims to combine real clinical data with bio-information technology, study evidence and treatment strategies, analyze the characteristics

of Chinese medicine in treating DKD, clarify the action mechanism of high-frequency Chinese herbs, and provide standard treatment guidelines for DKD.

Retrospective Study on Traditional Chinese Medicine Syndromes and Treatment Laws of Diabetic Kidney Disease Based on Real-World Study

Materials and Methods

Sources of Materials

The First Hospital Affiliated to the Henan University of Chinese Medicine provided experimental data for this study. The electronic medical records of DKD patients who had received treatment in the department of endocrinology from January 1, 2014 to December 31, 2021 were read through the hospital information management system to obtain the case data of patients with the first visit. The ethics committee of the First Hospital Affiliated to Henan University of Chinese Medicine approved this experimental study (ethical batch No: 022HL-080-01).

Diagnostic Criteria

Western medicine diagnostic criteria: cases with a history of DM were searched according to the *Chinese Guidelines for the Clinical Diagnosis and Treatment of Diabetic Nephropathy*⁵ and the *Chinese Guidelines for the Prevention and Treatment of Diabetic Nephropathy*,⁶ and DKD could be diagnosed if one of the following conditions was met: (1) The urinary albumin creatinine rate (UACR) or urinary albumin excretion rate (UAER) was detected three times within 3 to 6 months, and $\text{UACR} \geq 30 \text{ mg/g}$ or $\text{UAER} \geq 30 \text{ mg/24 h}$ occurred at least twice. (2) The kidney biopsy was consistent with the pathological

changes of DKD, such as nodular glomerulosclerosis and diffuse glomerulosclerosis. (3) eGFR < 60 ml·min⁻¹·(1.73m²)⁻¹ for more than 3 months. It can be considered as DKD with liver and kidney yin deficiency and blood stasis syndrome if three of the following five items, together with item 6 from TCM diagnostic criteria are met: according to liver and kidney yin deficiency and blood stasis syndrome in the *Internal Medicine of Chinese Medicine (Zhong Yi Nei Ke Xue)*,⁷ the *Development and Application of TCM Syndrome Differentiation Guidelines for Diabetic Nephropathy*,⁸ and the *2011 Edition of TCM Prevention and Treatment Guidelines for Diabetic Nephropathy*: (1) turbid urine and frequent and profuse urination; (2) fatigue, shortness of breath, and laziness to speak; (3) dry throat and mouth, feverish palms, and soles; (4) insomnia and dreaminess; (5) palpitation and restlessness; (6) dark or red thin tongue, or tortuous sublingual veins, petechia and ecchymosis, and deep pulse.

Inclusion Criteria

Inclusion criteria were (1) cases conforming the above diagnostic criteria; (2) relatively complete cases in the system data (such as definite and confirmed diseases); (3) mainly Chinese medicine compounds and certain Western medicine prescriptions used; and (4) the treatment outcome (such as recovery or death) clearly reflected in the case information.

Exclusion Criteria

Exclusion criteria were (1) pregnant, lactating, and menstruating women; (2) concomitant with other serious organic diseases (such as cancer); (3) concomitant with other primary or secondary renal disease and systemic disease; (4) various recent emergencies and infections occurred during data acquisition; and (5) patients with mental disorders and allergies.

Data Standardization and Database Establishment

The syndrome patterns of DKD are standardized by referring to the *Guidelines for Combination of Diabetes and Nephropathy*,⁹ such as classifying the “Qi Yin Kui Xu Zheng (qi and yin deficiency syndrome)” as the “Qi Yin Liang Xu Zheng (deficiency syndrome of both qi and yin).” According to the syndrome patterns of DKD included in the clinical data in *Science of Syndrome Elements Syndrome Differentiation (Zheng Su Bian Zheng Xue)*,¹⁰ the involved syndrome patterns are structurally divided according to “syndrome pattern = disease location element + disease nature elements.” The names, efficacies, flavors, and meridian tropism of Chinese herbs involved in the administration of herbs by patients were standardized through the 2020 edition of the *Pharmacopoeia of the People's Republic of China*.¹¹ For example, the specifications of “Xueshen” and “Honggen” were “Danshen (*Salviae Miltiorrhizae Radix et Rhizoma*)”, “Huaishan” was “Shanyao (*Dioscoreae Rhizoma*)”, “Erhua” and “Shuanghua” were “Jinyinhua (*Lonicerae Japonicae Flos*)”, and “Jinbuchang” was “Sanqi (*Notoginseng Radix et Rhizoma*)”. In the Excel 2016 software database, components were input by individual independent retrieval and checked by two people and double computers. The data processing software IBM SPSS Statistics 25 was used

Table 1 Distribution of the top seven TCM syndromes of DKD

Syndrome patterns	Frequency/ times	Frequency/%
Syndrome of both spleen and kidney deficiency	403	23.04
Syndrome of blood stasis obstructing the collaterals	382	21.84
Syndrome of dampness accumulation and blood stasis	353	20.18
Syndrome of both qi and yin deficiency	235	13.43
Syndrome of turbid toxin and blood stasis	77	4.40
Syndrome of both yin and yang deficiency	39	2.22
Syndrome of Liver and kidney yin deficiency	19	1.08

Abbreviations: DKD, diabetic kidney disease; TCM, traditional Chinese medicine.

to perform statistical analysis of the case contents and draw corresponding charts as required.¹²

Results

Distribution Analysis of Syndrome Patterns

According to the screening and processing of previous case data, a total of 1,201 patients with DKD were obtained, including 72 TCM syndrome patterns, with a total frequency of 1,749 times. DKD includes deficiency syndrome and excess syndrome. Spleen and kidney deficiency syndrome is the most common,¹³ complicated with the most frequent phlegm-dampness, blood stasis, and turbid toxin, see ► **Table 1**.

Analysis of Disease Nature Element and Disease Location Element

According to the analysis, nine disease nature elements were extracted, including two deficiency syndrome elements (qi deficiency and yin deficiency) and seven excess syndrome elements (blood stasis, dampness accumulation, turbid toxin, phlegm and blood stasis, heat toxin, dampness turbidity, and qi stagnation), with a total frequency of 1,643 times. The proportion of qi deficiency and yin deficiency in the deficiency syndrome elements was relatively high; blood stasis was the most frequent evidence of excess syndrome element, followed by dampness accumulation and turbid toxicity, see ► **Table 2**. Six disease location elements were extracted, with a total frequency of 916 times. The disease location element with the highest frequency was the kidney, followed by the spleen, liver and lung, see ► **Table 3**.

Frequency Analysis of Herbs

Through the extraction and correction of experimental data, a total of 1,201 prescriptions were included, involving 405 Chinese herbs. The cumulative frequency of the use of Chinese herbs was 11,366 times. The top five high-frequency

Table 2 Analysis of disease nature element of DKD

Disease nature element	Frequency/times	Frequency/%
Blood stasis	850	51.73
Dampness accumulation	354	21.54
Qi deficiency	261	15.88
Turbid toxin	77	4.69
Phlegm and blood stasis	41	2.49
Heat toxin	28	1.70
Yin deficiency	19	1.16
Damp turbidity	10	0.60
Qi stagnation	3	0.18

Abbreviation: DKD, diabetic kidney disease.

Table 3 Analysis of disease location elements of DKD

Disease location element	Frequency/times	Frequency/%
Kidney	435	47.48
Spleen	420	45.85
Liver	25	2.72
Lung	15	1.63
Stomach	12	1.31
Heart	9	0.98

Abbreviation: DKD, diabetic kidney disease.

Chinese herbs were Baizhu (*Atractylodis Macrocephalae Rhizoma*; 435 times), Fuling (*Poria*; 415 times), Huangqi (*Astragali Radix*; 403 times), Chuanxiong (*Chuanxiong Rhizoma*; 278 times), and Danshen (*Salviae Miltiorrhizae Radix et Rhizoma*; 265 times), see ►Table 4.

Analysis of Efficacy Categories and Meridian Tropism of the Properties and Flavors of the Commonly Used Chinese Herbs

Through screening and analysis of the 30 commonly used Chinese herbs with a frequency of ≥ 100 times, the cumulative frequency of use was 5,585 times. The efficacy categories of Chinese herbs were mainly deficiency tonifying herbs (2,083 times, 40.44%), blood-activating, and stasis-dispelling herbs (899 times, 17.45%); the herb properties were mainly warm (9 times, 29.03%) and mild (7 times, 22.58%); the herbs of sweetness (16 times, 34.78%) and bitterness (13 times, 28.26%) were the most common. As to meridian tropism, the main meridian tropism was the spleen meridian (19 times, 19.00%) and lung meridian (14 times, 14.00%), see ►Figure 1.

Clustering of Commonly Used Chinese Herbs

Combined with the characteristics of the syndromes of DKD and clinical practice, the top 30 high-frequency Chinese herbs include Baizhu (*Atractylodis Macrocephalae Rhizoma*), Fuling (*Poria*), Huangqi (*Astragali Radix*), Chuanxiong (*Chuanxiong Rhizoma*), Danshen (*Salviae Miltiorrhizae Radix et Rhizoma*),

Gancao (*Glycyrrhizae Radix et Rhizoma*), Chenpi (*Citri Reticulatae Pericarpium*), Danggui (*Angelicae Sinensis Radix*), Guizhi (*Cinnamomi Ramulus*), Baishao (*Paeoniae Alba Radix*), Yiyiren (*Coicis Semen*), Chishao (*Paeoniae Rubra Radix*), Qingbanxia (*Pinelliae Rhizoma Praeparatum cum Alumine*), Shanyao (*Dioscoreae Rhizoma*), Chuanniuxi (*Cyathulae Radix*), Dilong (*Pheretima*), Huanglian (*Coptidis Rhizoma*), Sharen (*Amomi Fructus*), Dahuang (*Rhei Radix et Rhizoma*), Jixuecao (*Centellae Herba*), Zhishi (*Aurantii Fructus Immaturus*), Maidong (*Ophiopogonis Radix*), Houpo (*Magnoliae Officinalis Cortex*), Taizhishen (*Pseudostellariae Radix*), Shuizhi (*Hirudo*), Honghua (*Carthami Flos*), Dangshen (*Codonopsis Radix*), Zexie (*Alismatis Rhizoma*), Gegen (*Puerariae Lobatae Radix*), Shudihuang (*Rehmanniae Radix Praeparata*). According to the average intergroup connection, the 30 commonly used Chinese herbs were reasonably clustered into six categories, see ►Figure 2 and ►Table 5.

Research on the Action Mechanism Based on Network Pharmacology

Materials and Methods

Screening of Active Ingredients of High-Frequency Herbs and the Related Targets

The chemical composition of herbs (high-frequency herbs) with a frequency of ≥ 100 times was retrieved by using the TCM systems pharmacology database and analysis platform, TCMS (TCMS, <http://tcmsp.com/tcmsp.php>), and the effective components and the action targets of Chinese herbs were screened under the conditions of oral bioavailability $\geq 30\%$ and herblikeness (DL) ≥ 0.18 . The target gene names were standardized through the UniProt (<https://sparql.uniprot.org>) online platform.

Target Screening of Diabetic Kidney Disease

The GeneCards database (<http://www.genecards.org>), DisGeNET database (<https://www.disgenet.org/>), and online mendelian inheritance in man (OMIM, <https://www.omim.org/>) were searched with the keyword “diabetic kidney disease” to obtain the disease target and finally get the collection of disease targets.

"Active Ingredients–Potential Targets" Network and Protein–Protein Interaction Network Construction

The intersections of the targets of high-frequency Chinese herbs and the disease targets of DKD were achieved by using the Venn online network platform (<http://bioinfo.cnb.csic.es/tools/venny/>), and the intersection targets were the potential targets of high-frequency Chinese herbs for the treatment of DKD. The potential targets and their corresponding active ingredients were input into Cytoscape 3.7.2 software to construct the “active ingredient–potential target” network graph, and the core chemical composition was screened by topological analysis. The potential targets were imported into STRING database (<http://cn.string-db.org>), protein–protein interaction (PPI) network was constructed, and key targets were screened by topological analysis.

Table 4 Top 15 Chinese herbs in the prescriptions for the treatment of DKD treatment

Chinese herb	Frequency/ times	Frequency/ %	Classifications	Efficacy
Baizhu (<i>Atractylodis Macrocephalae Rhizoma</i>)	435	3.83	Qi-tonifying herb	Invigorating the spleen and benefiting qi, drying dampness and promoting diuresis
Fuling (<i>Poria</i>)	415	3.65	Diuresis-promoting and dampness-diffusing herbs	Promoting diuresis and diffusing dampness, strengthening the spleen
Huangqi (<i>Astragali Radix</i>)	403	3.55	Qi-tonifying herb	Tonifying qi and promoting yang, benefiting defensive phase and consolidating the exterior
Chuanxiong (<i>Chuanxiong Rhizoma</i>)	278	2.45	Blood-activating and pain-relieving herbs	Promoting blood and qi circulation, dispelling wind and relieving pain
Danshen (<i>Salviae Miltiorrhizae Radix et Rhizoma</i>)	265	2.33	Blood-activating and menstruation-regulating herbs	Promoting blood circulation and removing blood stasis, dredging meridians and relieving pain
Gancao (<i>Glycyrrhizae Radix et Rhizoma</i>)	260	2.29	Qi-tonifying herb	Tonifying the spleen and qi, relieving spasm and pain
Chenpi (<i>Citri Reticulatae Pericarpium</i>)	256	2.25	Qi-regulating herb	Regulating qi and harmonizing the middle jiao, dry dampness and resolving phlegm
Danggui (<i>Angelicae Sinensis Radix</i>)	240	2.11	Blood-nourishing herb	Tonifying blood and promoting blood circulation, regulating menstruation and relieving pain, moistening intestine and promoting bowel movement
Dangshen (<i>Codonopsis Radix</i>)	192	1.69	Qi-tonifying herb	Tonifying the middle jiao and benefiting qi, promoting body fluid generation and nourishing blood
Zexie (<i>Alismatis Rhizoma</i>)	181	1.59	Diuresis-promoting and dampness-diffusing herbs	Promoting diuresis and diffusing dampness, purging heat
Guizhi (<i>Cinnamomi Ramulus</i>)	167	1.47	Pungent-warming exterior-relieving herb	Promoting sweating and expelling pathogenic factors from the muscles, warming and dredging meridians, promoting yang and transforming qi
Baishao (<i>Paeoniae Alba Radix</i>)	165	1.45	Blood-nourishing herb	Nourishing blood, regulating menstruation, astringing yin, relieving sweating, softening liver and relieving pain
Yiyiren (<i>Coicis Semen</i>)	162	1.42	Diuresis-promoting and dampness-diffusing herbs	Promoting diuresis and diffusing dampness, strengthening the spleen and stopping diarrhea, clearing heat and excreting pus

Abbreviation: DKD, diabetic kidney disease.

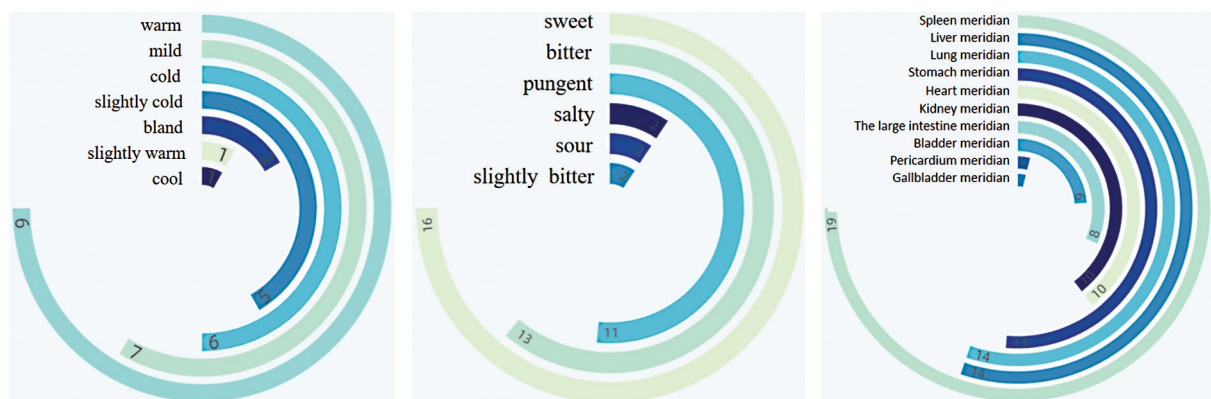


Fig. 1 The properties, flavors, and meridian tropism of commonly used Chinese herbs (frequency ≥100 times).

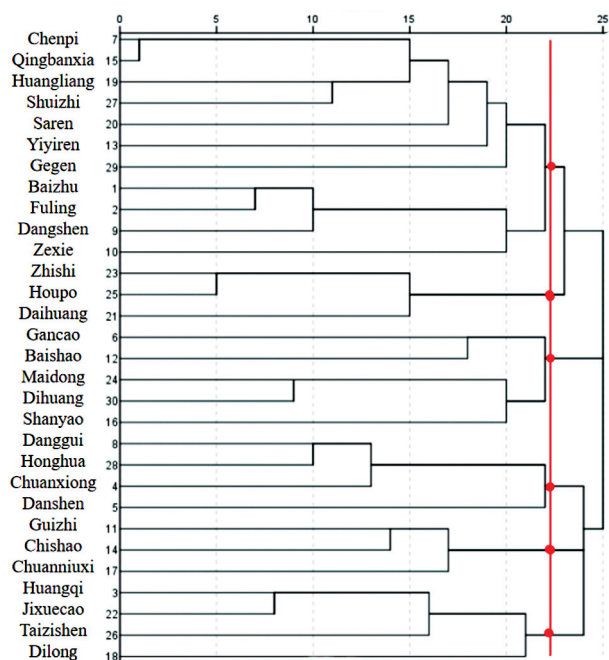


Fig. 2 Clustering analysis of the top 30 Chinese herbs in frequency.

Analysis of Signal Pathway Enrichment in Kyoto Encyclopedia of Genes and Genomes

The intersection targets were input into the Metascape database (<https://metascape.org>) for Kyoto Encyclopedia of Genes and Genomes (KEGG) signaling pathway enrichment analysis.

Validation of Docking Between Key Active Ingredients and Key Target Protein Molecules

The key proteins in the top three of the number of nodes in the PPI network were molecularly interfaced with the core com-

ponents in the top five of the network degree of the “active ingredient-potential target” by using AutoDock software.

Results

Prediction Results of the Active Ingredients and Action Targets of the High-Frequency Herbs

Based on the screening of TCMSP, 58 active ingredients, including Baizhu (*Atractylodis Macrocephalae Rhizoma*), Fuling (*Poria*), Huangqi (*Astragali Radix*), Chuanxiong (*Chuanxiong Rhizoma*), and Danshen (*Salviae Miltiorrhizae Radix et Rhizoma*) and 164 herb action targets were obtained.

Target Screening of Diabetic Kidney Disease

According to the databases of GeneCards, OMIM, and DisGeNET, etc., 2,253 DKD targets were obtained, and a total of 1,434 targets were obtained after integration and deduplication.

“Active Ingredients–Potential Target” Network and Protein–Protein Interaction Network Construction

Using the Venn online network platform, the intersections of high-frequency Chinese medicine targets and DKD targets were selected, and 90 intersection targets were screened out, which were potential targets of high-frequency Chinese herbs for the treatment of DKD. The “active ingredient-potential target” network graph includes 222 nodes (58 active ingredients and 164 target genes). Topological analysis indicated that quercetin, luteolin, 7-O-methylisoxitol, hederagenin, and 4-methylene miltirone were the five core chemical components of high-frequency Chinese herbs for the treatment of DKD, as shown in ►Fig. 3. Ninety potential targets were input into the STRING database and the PPI network was plotted, as shown in ►Fig. 4. The network topology analysis suggested that the top five target proteins were protein kinase B1 (AKT1),

Table 5 Clustering analysis results of top 30 high-frequency Chinese herbs

No.	Composition	Treatment method
C1	Baizhu (<i>Atractylodis Macrocephalae Rhizoma</i>), Fuling (<i>Poria</i>), Dangshen (<i>Codonopsis Radix</i>), Zexie (<i>Alismatis Rhizoma</i>), Chenpi (<i>Citri Reticulatae Pericarpium</i>), Qingbanxia (<i>Pinelliae Rhizoma Praeparatum cum Alumine</i>), Huanglian (<i>Coptidis Rhizoma</i>), Shuizhi (<i>Hirudo</i>), Sharen (<i>Amomi Fructus</i>), Yiyiren (<i>Coicis Semen</i>), Gegen (<i>Puerariae Lobatae Radix</i>)	Tonifying qi and strengthening spleen, promoting diuresis and diffusing dampness
C2	Dahuang (<i>Rhei Radix et Rhizoma</i>), Zhishi (<i>Aurantii Fructus Immaturus</i>) and Houpo (<i>Magnoliae Officinalis Cortex</i>)	Promoting blood circulation and removing blood stasis, drying dampness and resolving phlegm
C3	Dihuang (<i>Rehmanniae Radix</i>), Shanyao (<i>Dioscoreae Rhizoma</i>), Gancao (<i>Glycyrrhizae Radix et Rhizoma</i>), Baishao (<i>Paeoniae Alba Radix</i>), Maidong (<i>Ophiopogonis Radix</i>)	Invigorating the spleen and kidney, nourishing yin and promoting body fluid production
C4	Danggui (<i>Angelicae Sinensis Radix</i>), Honghua (<i>Carthami Flos</i>), Chuanxiong (<i>Chuanxiong Rhizoma</i>), Danshen (<i>Salviae Miltiorrhizae Radix et Rhizoma</i>)	Tonifying blood and promoting blood circulation, dredging the meridians and relieving pain
C5	Chishao (<i>Paeoniae Rubra Radix</i>), Chuanniuxi (<i>Cyathulae Radix</i>), Guizhi (<i>Cinnamomi Ramulus</i>)	Activating blood circulation and dredging the meridians, dissipating blood stasis and relieving pain
C6	Huangqi (<i>Astragali Radix</i>), Jixuecao (<i>Centellae Herba</i>), Taizishen (<i>Pseudostellariae Radix</i>), Dilong (<i>Pheretima</i>)	Tonifying qi and promoting body fluid generation, promoting diuresis and dredging the collaterals

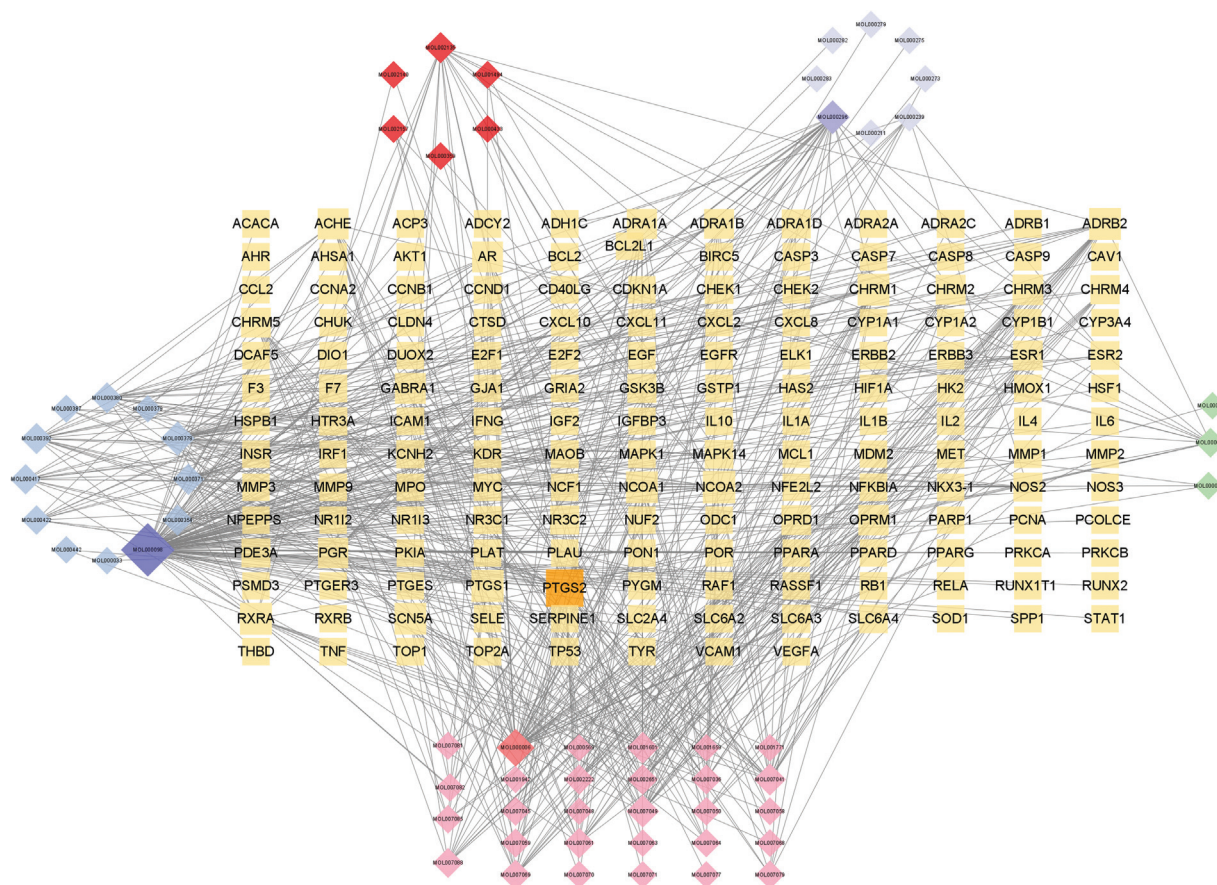


Fig. 3 “Active ingredient-potential target” network.

interleukin-6 (IL-6), tumor necrosis factor (TNF), vascular endothelial growth factor A (VEGFA), and tumor protein P53 (TP53).

Analysis of Kyoto Encyclopedia of Genes and Genomes Pathway Enrichment

The enrichment analysis of KEGG showed that the key targets were mainly enriched in 20 signaling pathways including tumor-associated pathways (pathways in cancer, proteoglycans in cancer, and transcriptional misregulation in cancer), viral infection-associated pathways (human cytomegalovirus infection, malaria, and epithelial cell signaling in helicobacter pylori infection) and glucose metabolism-associated signaling pathways (AGE-RAGE signaling pathway in diabetic complications, NK-kappa B signaling pathway, diabetic cardiomyopathy, and viral myocarditis), as shown in ▶ **Fig. 5**.

Verification of Docking Between the Key Active Ingredients of High-Frequency Herbs and Core Protein Molecules

It was found that luteolin, 4-methylene neoketone, and hederagenin had strong binding to AKT1 with binding energies of $-6.72 \text{ kJ/mol}^{-1}$, -7.2 kJ/mol^{-1} , and $-8.13 \text{ kJ/mol}^{-1}$, respectively. The potential binding sites were mainly the amino acid residues of TRP-99, LYS-39, GLU-40, GLU-91, LYS-8, TRP-11, GLU-95, HIS-13, as shown in ▶ **Fig. 6**. Through analysis, it was discovered that luteolin,

4-methylene neoketone, and hederagenin all had multiple active groups, and their chemical structures were highly similar, forming hydrogen bonds with amino acids with an average of 2.45, suggesting that the hydrogen bond distance was short, and it also reflected that the three chemical components could well match the active pocket of AKT1 core protein, see ▶ **Fig. 7**.

Discussion

There is no clear record of the name of DKD in TCM classics and the literature. Through clinical symptom analysis, DKD can be classified into the categories of “Xiao Ke or Xiao Dan” (consumptive thirst), “edema”, “kidney consumption”, and “urine turbidity.”¹⁴ It is recorded in *Formulas from Benevolent Sages Compiled during the Taiping Era (Tai Ping Sheng Hui Fang)* that “Three Xiaos (consumptions) are originated from kidney deficiency”. The kidney stores essence and is the innate foundation of human beings. In ancient times, it is generally believed that consumptive thirst is related to kidney deficiency and blood stasis in collaterals.¹⁵ The liver governs the free flow of qi. As early as given in *Ling Shu:Wubian (Spiritual Pivot:Five Changes)*, it has the description of consumptive thirst caused by emotional disturbance, and this is an early record of “consumption caused by emotions”. In the *Treatise on the Three Consumption (San Xiao Lun)*, it believes that the disease is caused by excessive five emotions and led by excessive anger

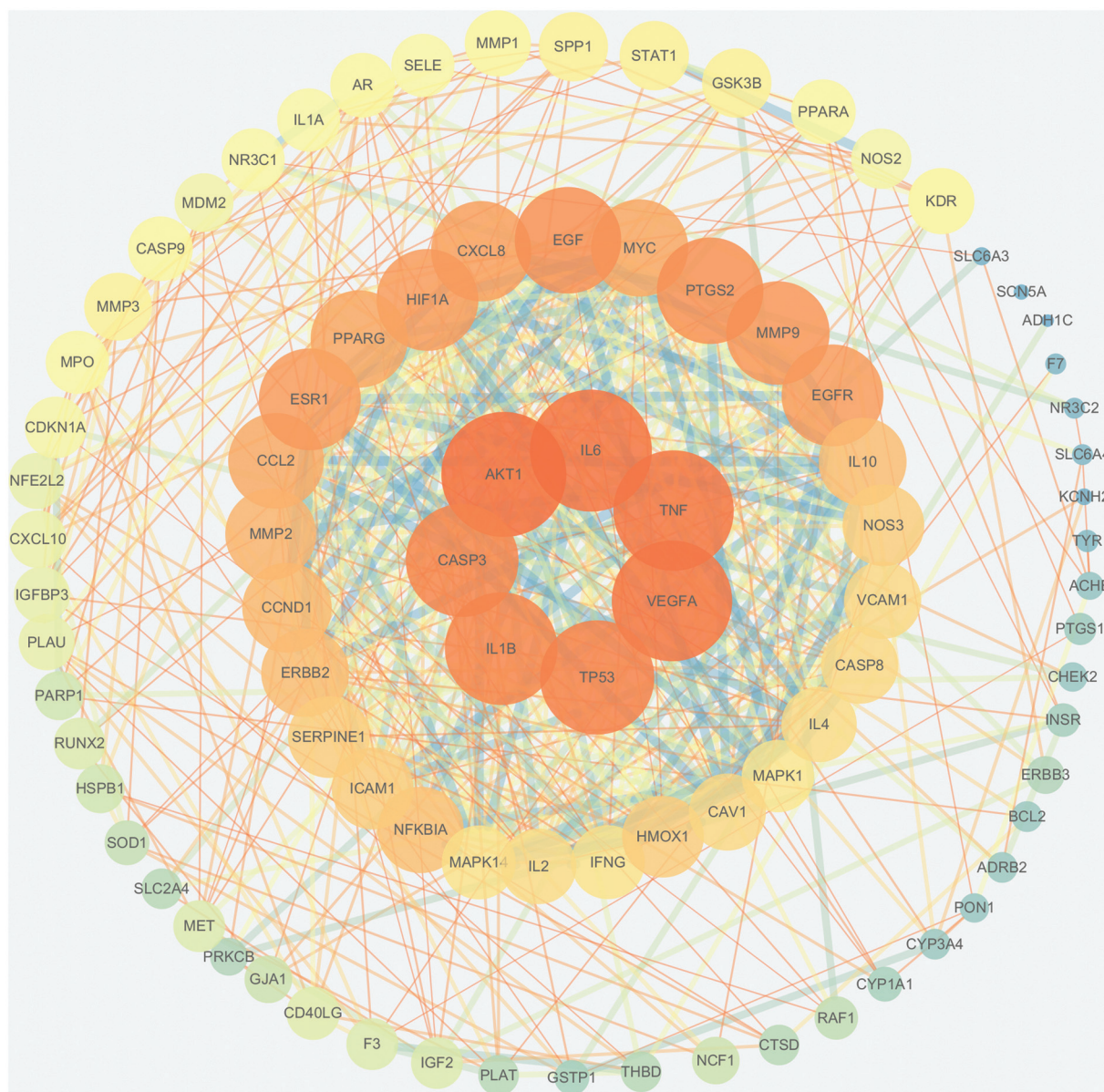


Fig. 4 PPI network diagram.

which will lead to endogenous internal fire and results in consumptive thirst.¹⁶ As the saying goes in *Jingui Zhenyan Lun* (*Discussion on the Important Ideas in the Golden Chamber of Huangdi's Cannon of Internal Medicine*), the abdomen belongs to yin, and the yin within yin is kidney; the abdomen belongs to yin, and the yang within yin is the liver. Both the liver and kidney are situated in the lower jiao, with one pertaining to yang and the other yin. The kidney governs storage and the liver governs the free flow of qi. They work together to dominate the generation of essence and blood so as to nourish the meridians of the whole body. The spleen is the root of the acquired constitution among the five internal organs, which governs qi and blood production and dominates transportation and transformation.¹⁷ According to the characteristics of different stages of DKD, the great TCM master Boshou Xue emphasized the importance of spleen-strengthening, kidney-nourishing, dampness-dissolving, and turbidity-purging in the

treatment of DKD.¹⁸ Through real-world data analysis, it discovered that in DKD, spleen and kidney deficiency syndrome, high-frequency syndrome patterns of syndrome of blood stasis obstructing the collaterals, and the disease location elements mainly include the kidney, liver, spleen, etc., while the commonly used herbs mainly pertain to the kidney meridian, liver meridian, and spleen meridian. It can be seen that in the clinical syndrome differentiation and treatment of DKD, the kidney should be taken as the root, liver and spleen should be regulated, the kidney should be nourished and the liver should be softened, so as to achieve kidney-spleen harmony and liver-kidney cotreatment.

Baizhu (*Atractylodes Macrocephalae Rhizoma*), Fuling (*Poria*), Huangqi (*Astragalus Radix*), Chuanxiong (*Chuanxiong Rhizoma*), and Danshen (*Salviae Miltiorrhizae Radix et Rhizoma*) are the top five high-frequency Chinese herbs in clinical application. Huangqi Powder can slow down the development

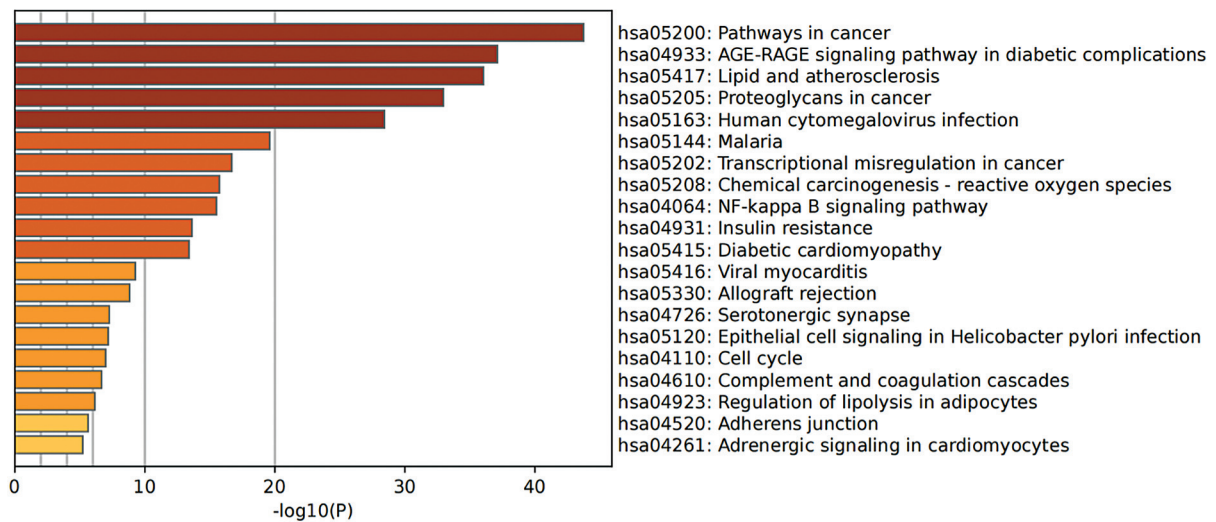


Fig. 5 Analysis of KEGG pathway enrichment.

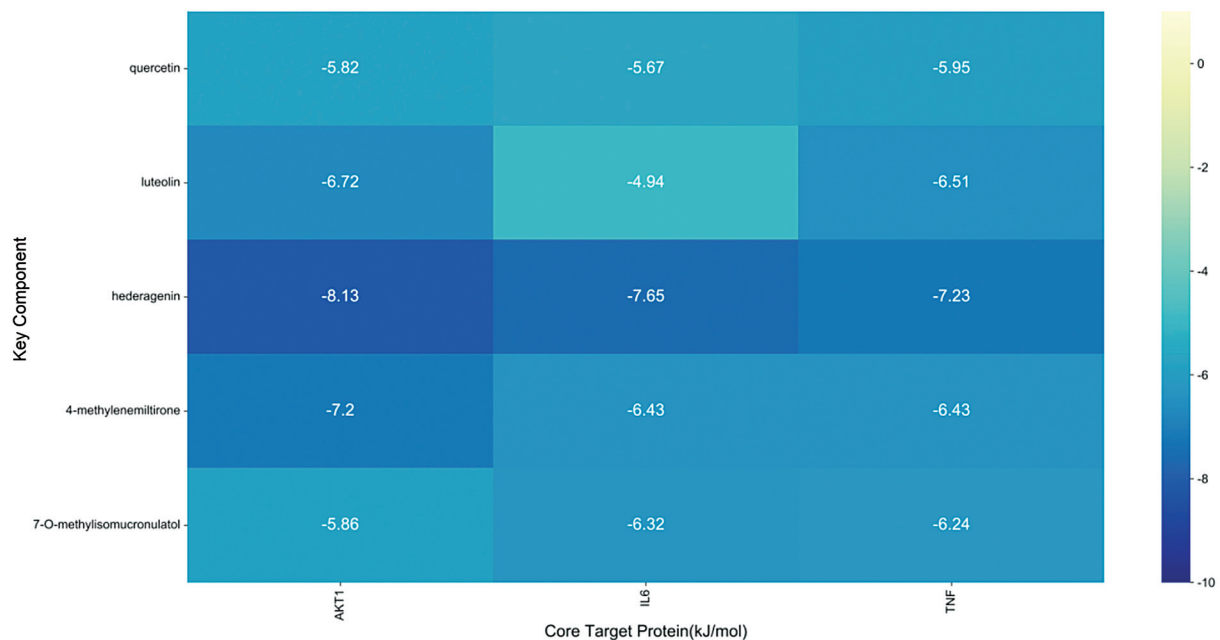


Fig. 6 Binding energy thermogram of main components of high-frequency Chinese herbs with AKT1, IL-6, and TNF (kcal/mol). Notes: The darker the color is, the lower the binding energy is; number represents binding energy.

of DKD by inhibiting the signaling pathways of phosphatidylinositol 3-kinase/protein kinase B (AKT)/mammalian target of rapamycin (mTOR).¹⁹ Huangqi (Astragali Radix), Baizhu (Atractylodis Macrocephalae Rhizoma), and Fuling (Poria) in the Supplemented Qiwei Baizhu Powder have the effects of supplementing qi and nourishing yin and have good curative effects on the clinical treatment of DKD.²⁰ It also shows that although there are many causes of DKD, they are generally summarized as the imbalance between yin and yang, disorder of qi and blood, deficiency, and excess. Huangqi (Astragali Radix), Fuling (Poria) plus Danshen (Salviae Miltiorrhizae Radix et Rhizoma), and Chuanxiong (Chuanxiong Rhizoma) have the effects of removing dampness and relieving swelling, and simultaneously promoting blood circulation and resolving

blood stasis, promoting qi circulation and relieving pain.²¹ Qiqi Dihuang Pill was found to have a significant protective effect on DKD, which may be related to the reduction of inflammatory response and oxidative stress damage in the body.²² To sum up, in the treatment of DKD, the Chinese herbs with the effects of supplementing qi and nourishing yin, promoting blood circulation, and resolving blood stasis should be well and skilfully used so that the herbs can directly reach the disease sites to exert their curative effects.

The data analysis showed that DKD is mainly treated with warm and sweet herbs. The most widely used herbs are deficiency-invigorating herbs, diuresis-promoting and dampness-diffusing herbs, blood-activating and stasis-dispelling herbs, etc., which correspond to the blood stasis,

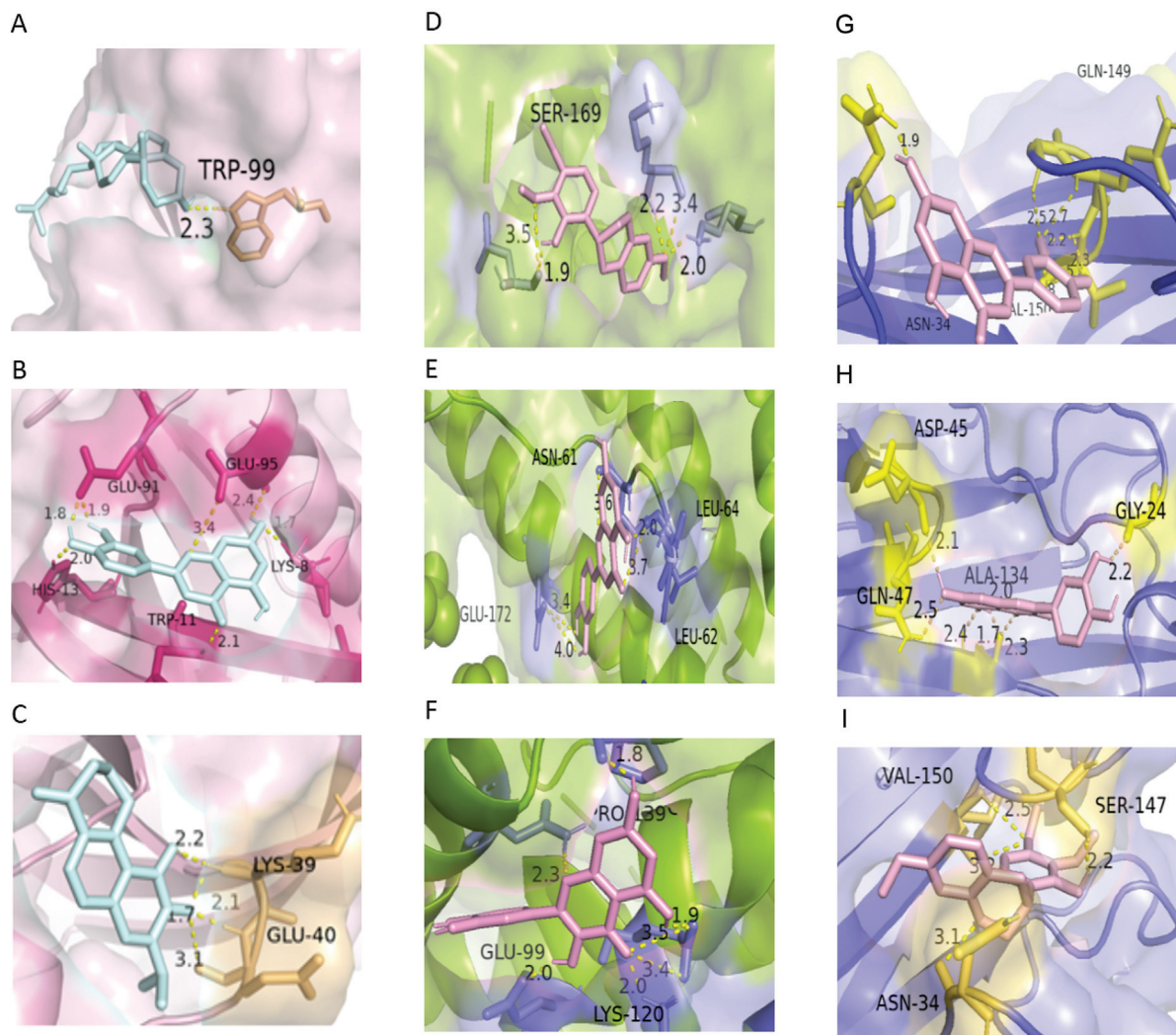


Fig. 7 Molecular docking diagram of "key active ingredient-core protein". (A) AKT1-hederagenin, (B) AKT1-4-methylenemiltirone, (C) AKT1-luteolin, (D) IL-6-7-O-methylisomucronulat, (E) IL-6-hederagenin, (F) IL-6-quercetin, (H) TNF-luteolin, (I) TNF-quercetin, and (J) TNF-7-O-methylisomucronulat.

dampness accumulation, qi deficiency, etc., in the disease nature elements. Through literature study combined with the *Chinese Guidelines for the Prevention and Treatment of Type 2 Diabetes (2020 edition)*, the *Chinese Clinical Guidelines for the Prevention and Control of Type 2 Diabetes in the Elderly (2022 edition)* and cluster analysis results,^{23–25} it was indicated that "promoting blood circulation and resolving blood stasis" and "reducing mass and dredging the collaterals" were the therapeutic principles of DKD. Through clinical studies, Dan Tang found that Chinese herbs with functions of promoting blood circulation and resolving blood stasis, reducing mass, and dredging the collaterals have remarkable effects on DKD.²⁶ To sum up, in the clinical treatment of DKD, we should pay attention to the overall "harmony" in the compatibility of the herbs in the prescription.

The high-frequency Chinese herbs found in this study are Baizhu (*Atractylodis Macrocephalae Rhizoma*), Fuling (*Poria*), Huangqi (*Astragali Radix*), Chuanxiong (*Chuanxiong Rhizoma*), Danshen (*Salviae Miltiorrhizae Radix et Rhizoma*), etc., which have a high coincidence with the Chinese medi-

cine composition of Zhenhua Li's Yishen Tongluo Prescription. Both of them reflect the principle of tonifying the kidney and dredging the collaterals. Through clustering and implicit structure analysis, it showed that the Chinese herbs with effects of tonifying the kidney and dredging the collaterals have good clinical effects on DKD. Quercetin, luteolin, 7-O-methylisoxitol, hederagenin, and 4-methylene neoketone were the key active ingredients of the above-mentioned herbs. Quercetin is a flavanol compound widely existing in the plant world. It can promote the release of inflammatory factors of IL-1, IL-8, etc., by regulating the renal NF- κ B signaling pathway and reduce inflammation and ease the development process of DKD; it can also protect the damage caused by DKD by intervening TGF- β 1 and Smad7 pathways by alleviating oxidative stress response in the body^{27,28}; Huangqi Guizhi Wuwu Decoction can reduce the complications of DKD.²⁹ Luteolin is a flavonoid in Danshen (*Salviae Miltiorrhizae Radix et Rhizoma*), which can delay the development of DKD.³⁰ Yao et al found that the glomerular basement membrane hyperplasia and fat

inflammation of diabetic mice were well relieved after the use of hederagenin through three omics studies.³¹ AKT1, IL-6, TNF, VEGFA, and TP53 are important potential action targets of high-frequency Chinese herbs of Baizhu (*Actinidia chinensis* Radix), Fuling (*Poria*), Huangqi (*Astragalus Radix*), Chuanxiong (*Chuanxiong Rhizoma*), and Danshen (*Salvia miltiorrhiza* Radix et Rhizoma) in the treatment of DKD. It was found that Huangqi (*Astragalus Radix*) (quercetin and hederagenin) can regulate autophagy through negative regulation of AKT1/mTOR signaling pathway in the development of DKD.³² Bupi Yishen Huoxue Decoction can delay the development of DKD by reducing the levels of IL-6 and TNF- α .³³

Conclusion

Based on the real-world data, this paper makes a preliminary study on the syndrome laws of DKD and the action mechanism of high-frequency Chinese herbs through retrospective analysis, network pharmacology, molecular docking, etc. It has found that the differentiation and treatment of DKD with TCM follows the principles of “taking kidney as the root, considering both the liver and spleen, tonifying qi and nourishing yin, promoting blood circulation and resolving blood stasis.” High-frequency Chinese herbs for the treatment of DKD have multicomponent, multitarget, and multipath characteristics. This can provide a basis for the clinical treatment of DKD. However, the proper integrative treatment by Chinese medicine and Western medicine needs to be effectively discussed in combination with the actual clinical situation.

CRedit Authorship Contribution Statement

Keyi Ji: Writing -original draft, methodology, validation. **Genlin Li:** Methodology, project administration. **Suhui Wu:** Resources, supervision. **Jiayao Yuan:** Formal analysis, supervision. **Zheng Du:** Data curation, investigation. **Hanbing Li:** Conceptualization, supervision. **Jiangyan Xu:** Funding acquisition resources, writing-review & editing.

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Conflict of Interest

The authors declare no conflict of interest.

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