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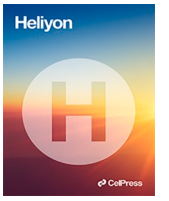

Modular characteristics and mechanism of action of herbs for type 2 diabetes treatment in Chinese medicine

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ABSTRACT

Type 2 diabetes (T2D) has emerged as a global epidemic, and conventional treatment approaches often face limitations in achieving long-term glycemic control and preventing complications. Traditional Chinese Medicine (TCM) offers a valuable alternative for managing T2D, with a long history of effectively using herbal formulations in clinical practice. However, the modular characteristics of these herbs and their specific mechanisms of action remain poorly understood. To comprehensively investigate the modular characteristics and mechanisms of Chinese herbs in treating T2D, as well as explore the synergistic interactions among different herbs and their modular components, we employed data mining, systematic pharmacology, and molecular docking. Our aim was to gain a comprehensive understanding of the potential therapeutic targets and pathways involved in herbal T2D treatment. In this study, a total of 1114 studies investigating the effects of TCM interventions in the treatment of T2D in adults were included. The analysis revealed 170 distinct types of Chinese herbs, 118 active components, and 238 common targets shared between the medicine and T2D. Additionally, this study identified six hub proteins (TNF, MMP2, PTGS, CASP3, CASP8, and CASP9) and two key chemicals (Diosgenin and Formononetin) found in TCM-mediated T2D suppression. It was observed that these proteins could bind with the ingredients. The MMP2-Diosgenin interaction exhibited the lowest binding free energy (−13.05 kJ/mol) and was primarily driven by hydrogen bonds with ALA-165. TNF-Diosgenin (−10.5 kcal/mol) showed three hydrogen bonds with LEU-37, ARG-82, and ASN-30. PTGS2 and Diosgenin (−8.71 kJ/mol) demonstrated a hydrogen bond with HIS-214. Furthermore, CASP9-Formononetin (−6.53 kcal/mol) exhibited the lowest binding free energy and hydrogen bonds with GLU-261 and SER-339 as the primary forces involved. CASP3-Formononetin (−6.07 kcal/mol) displayed three hydrogen bonds with ASN-342, TRP-348, and GLU-379. Lastly, CASP8 and Formononetin (−6.06 kJ/mol) formed a hydrogen bond with THR-390, TYR-392, and TYR-334. Moreover, critical therapeutic pathways, such as the immune inflammatory response, AGE-RAGE, and IL-17 signaling pathway, were found to be associated with T2D Chinese herb therapy. In conclusion, this study sheded light on the modular characteristics and mechanism of

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action of herbs used in Chinese Medicine for the treatment of T2D, which provided valuable insights for both researchers and practitioners in the field of Chinese Medicine, offering potential avenues for improved treatment strategies and personalized approaches to address the complex nature of T2D.

1. Introduction

Diabetes is a serious public health epidemic and is considered one of the fastest-growing health crises in the world in the 21st century. Currently, approximately 537 million people worldwide are affected by diabetes, and this number is expected to increase to 643 million by 2030 and 783 million by 2045, according to the International Diabetes Federation's 10th Global Diabetes Map [1]. The prevalence and rate of growth of Type 2 Diabetes (T2D), which accounts for over 90% of all cases of diabetes and is characterized by hyperglycemia and insulin resistance (IR), puts a significant strain on healthcare resources [2]. There are already a growing number of options for treating T2D, including oral anti-diabetic medications, lifestyle changes (such as adopting a diabetic diet and engaging in daily physical exercise), and various insulin injections [1,3,4]. However, medications such as metformin, which is considered the first-line medicine for T2D treatment, have been reported to have side effects such as digestive intolerance, particularly diarrhea and nausea [5]. When a single anti-diabetic treatment is ineffective, people often turn to various anti-diabetic medication combinations or insulin, which carries a significant risk of causing hypoglycemia [1,3]. Due to poor levels of compliance and confidence, many clinical patients struggle constantly with significant obstacles, such as when or how to administer those medications or insulin, and how to prevent hypoglycemia or digestive intolerance [3]. Research and development efforts are therefore actively focused on finding an alternative effective medicine that is more appealing to patients.

Traditional Chinese medicine (TCM), an intangible cultural heritage of China, provides abundant factual knowledge for current clinical treatment and fundamental medical research. T2D, also known as "thirst disease" or Xiaoke in Chinese, was originally mentioned in the Huangdi Neijing. Chinese herbs have been used to treat T2D since ancient times, and their use remains prevalent today despite the rapid advancement of Western medicine. Moreover, more than 18.7% of Chinese patients who have used both Western medicine and TCM report that TCM is their preferred method of treatment [6]. The discovery of Chinese herbs offers significant potential advantages for treating T2D. There is still a lack of understanding about which Chinese herbs are the most commonly used in T2D treatment, the common characteristics of these herbs, and the main active ingredients and molecular mechanisms of these commonly used drugs.

The primary objective of this study is to systematically investigate the modular characteristics of herbs used in Chinese Medicine for T2D treatment. By categorizing these herbs into distinct modules based on their therapeutic properties and biochemical composition, we aim to provide a comprehensive understanding of their functional roles in managing T2D. Furthermore, this study seeked to elucidate the mechanism of action underlying the therapeutic effects of these herbs. Through additional analyses, we will explore the biochemical pathways targeted by the modular components of these herbs and their interactions between key components and targets.

2. Material and methods

2.1. Collection of Chinese prescription and herbs

The research framework of this study is illustrated in Fig. 1A. To construct the dataset of Chinese herbal medicines, we searched both English and Chinese literature. The search methods were as follows: we used Chinese databases such as China National Knowledge Internet (CNKI: http://www.cnki.net), Wanfang Database (http://www.wanfangdata.com.cn/index.html), and China Science and Technology Journal Database (VIP: http://www.cqvip.com), as well as English databases such as PubMed (https://www.ncbi.nlm.nih.gov) and Web of Science (Clarivate) databases, to search for Chinese prescriptions for the treatment of type 2 diabetes (T2D). The publication type considered was original research updated until March 11, 2022. We used Endnote (version x9.3.3), Zotero (version 6.0.10), and Excel (version 2019) tools to analyze and collect relevant data. We used various search terms either individually or in combination, such as "Chinese medicine", "traditional medicine", "herbal medicine", "oriental medicine", "herb", "plant", "prescription", "decoction", and "diabetes", "type 2 diabetes", "diabetes mellitus", "T2D", among others. We considered all types and formulations of herbal medicines, including extracts, decoctions, pills, and natural compounds. We excluded outcomes of clinical studies, and only selected complete drug records for prescriptions intended for diabetes treatment. We evaluated formulations of pills, powders, and decoctions, and extracted the name of each herbal formula, as well as its composition of medicinal herbs, its origin (name of article or ancient literature), author, publication year, and internal/external application. We excluded studies that did not fulfill the inclusion requirements, such as animal tests, case reports, reviews, incomplete herb compositions, identical prescription work, and so on. Two different researchers processed the prescription collections, while another researcher summarized information to verify correctness and dependability. Note that according to the literature, identical prescription formulations were only considered once. The method of literature searching is presented in Fig. 1B.

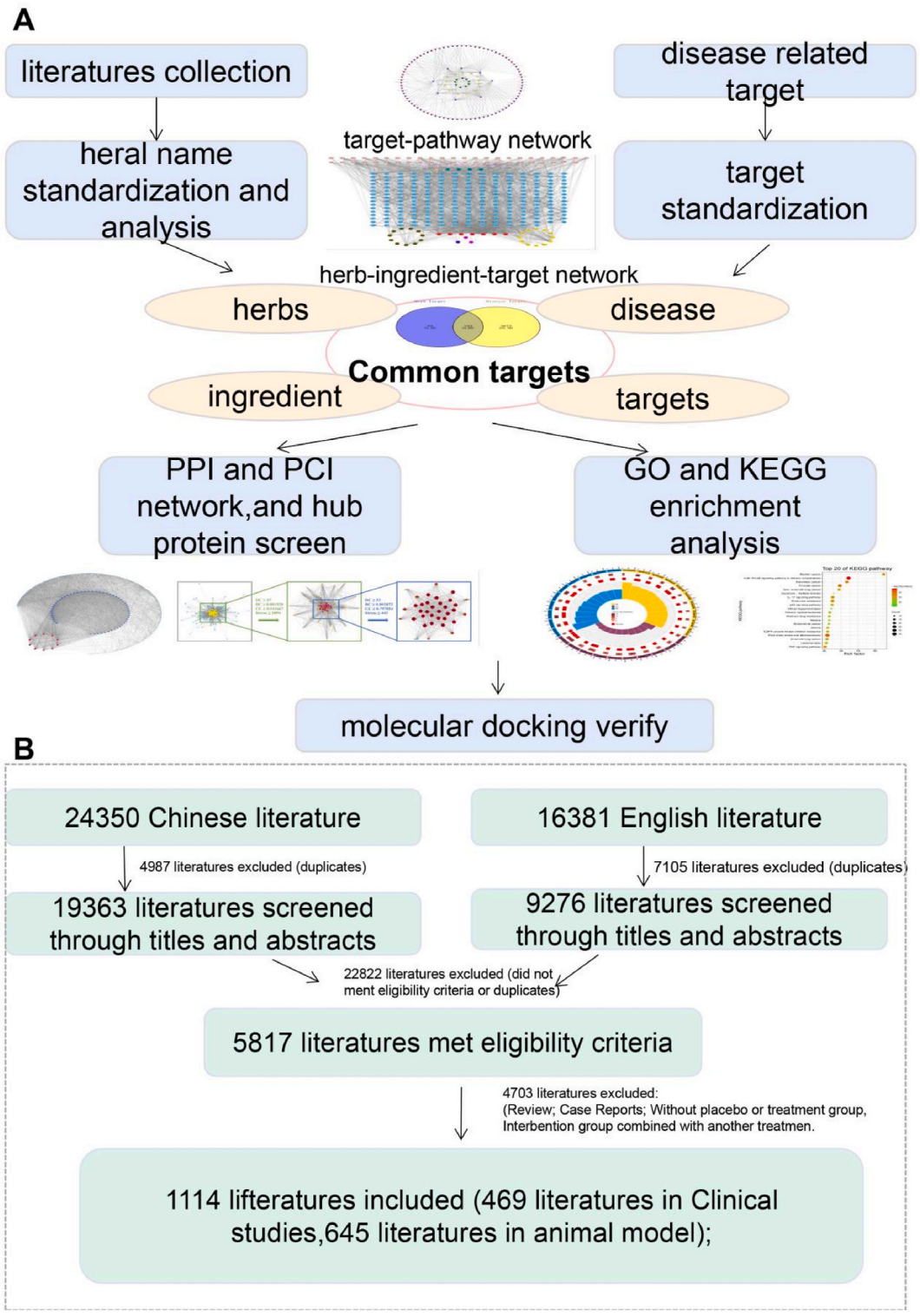


Fig. 1. This study's flow chart and process for gathering documents. (A) This study's flow chart. (B) Process for gathering documents.

2.2. Herbal name standardization and analysis

The names of 170 single herbs were standardized to their official names based on the People's Republic of China Pharmacopoeia (2020 edition), Traditional Chinese Medicine "13th Five-Year Plan" textbook, and the Chinese Clinical Medicine Dictionary. Unless specified otherwise in the China Pharmacopoeia, herb processing procedures such as "Honey-made-HuangQi" for "HuangQi" and "Wine-made-GanCao" for "GanCao" were ignored. We evaluated and summarized the following variables: frequency, family, genus, classification, effectiveness, features, flavor, and channel tropism. Based on their frequency of usage, a total of 20 herbs were selected for this research.

2.3. Herbal components acquisition

To identify the bioactive components of the herbs, researchers used the TCM Systems Pharmacology Database (http://lsp.nwu.edu.cn/tcmsp.php), ETCM (http://www.nrc.ac.cn:9090/ETCM/), and relevant literature. In addition, absorption, distribution, metabolism, and excretion (ADME) characteristics were considered, and filters were set based on oral bioavailability (OB) of $\leq$30%, drug-likeness (DL) of $\leq$0.18, and half-life of $\leq$4. However, some Chinese herbs such as MuLi were not included in this study due to a lack of information on their insect species in the TCMSP database. The chemical structure of the bioactive components was obtained from the NCBI PubChem database (https://www.ncbi.nlm.nih.gov/).

2.4. Herbal ingredients-targets analysis

The likely targets for each bioactive component were identified by combining data from the TCMSP (http://lsp.nwu.edu.cn/tcmsp.php), DrugBank (https://go.drugbank.com/), and BATMAN-TCM (http://bionet.ncpsb.org.cn/batman-tcm/index.php/Home/Index/index) databases. The targets were then classified according to their target sites, and redundant targets and components were removed. If more than 20 components targeted the same site, these targets and components were discarded. The Metascape database (https://metascape.org/gp/index.html#/main/step1) was then used for GO and KEGG enrichment analysis. Finally, the herbal-ingredients-target-pathway-disease network was created using Cytoscape 3.8.0 software (http://cytoscape.org/).

2.5. Common targets analysis

To predict T2D-related targets, researchers used the GeneCard database (https://www.genecards.org/). Then, they identified the common targets between the pharmacological targets and T2D-related targets by removing duplicates and taking the intersection. This was visualized using Venn diagrams. The targets were then standardized using the UniProt Database (https://www.uniprot.org/). For GO and KEGG pathway enrichment analysis, the Metascape database was used. GO keywords were divided into three categories to represent gene properties: biological process (BP), molecular function (MF), and cellular component (CC). The KEGG database was used to represent the interaction routes of proteins. Bioinformatics and R Software were used to generate the visualizations (version 3.4.0).

2.6. HCCT, PPI and PCI network construction

The researchers used Excel to locate similar compounds and plants based on shared goals using the F (lookup) function. The common targets, related ingredients, and herbs were then put into the Cytoscape 3.8.2 program to form a network of herbs-components-common targets (HCCT). This allowed them to identify the most common herb compounds and the components linked to the most common targets as the most likely core constituents for T2D treatment. To gain a better understanding of how those common proteins interact with one another, PPI networks were acquired from the STRING database version 11.5 and the Protein-chemical interaction (PCI) network was obtained from the STITCH database version 5.0. Both databases had "Homo sapiens" as the chosen organism. An interaction score greater than 0.9 was classified as moderate confidence in the STRING database. In STITCH, the maximum number of interactors was limited to 20. The raw data obtained from both databases were visualized using the Cytoscape 3.8.0 software. The Molecular Complex Detection (MCODE) plugin and the cytohubba plugin in the Cytoscape program were used to perform the crucial network and hub gene of biological networks, respectively.

2.7. Molecular docking

Molecular docking is a computational technique used to predict the binding affinity and orientation of a ligand (in this case, a component of the herbs) with a target protein. The Autodock 4 software uses a grid-based approach to calculate the binding energy between the ligand and the protein receptor. The results of the docking simulation are then analyzed using PyMOL 2.5 software to visualize the binding conformation and calculate the binding energy. The binding energy is an indicator of the strength of the interaction between the ligand and the receptor, with lower binding energies indicating stronger binding interactions.

2.8. Statistical analyses

We ensured an adequate sample size for statistical analysis and utilized a computational systems pharmacology method and

statistical analysis to determine the associated molecular mechanisms. These methods have been professionally certified and widely employed in the field for an extended period. Additionally, two researchers independently replicated the study to ensure the robustness of the results. We effectively utilized appropriate graphs, plots, and figures to visually represent our data, and all results were carefully interpreted within the context of our research question. A value of $p < 0.05$ is defined as statistically significant.

3. Results

3.1. The details and characteristics of therapeutic Chinese herbs for T2D

TCM uses syndrome distinction as the basis for diagnosing and treating T2D. Different prescriptions were used to address the symptoms of each kind of syndrome. For our study, we selected 1114 relevant pieces of literature, from which we retrieved 1114 prescriptions and 170 different types of TCM herbal remedies (Supplementary Table S1). Due to the large number of included literature, we are only displaying a random sample of 20 pieces for reference [7–18]. Table 1 displays the top 20 herbs based on their clinical usage frequency. Understanding the characteristics of Chinese herbs, such as their effects, quality, flavor, and channel tropism,

Table 1
The details of top 20 of herbs with frequency.

Chinese name	Family	Genus	Frequency	Frequency (%)	Herb meridian Tropism	Herb Property	Herb Flavor	Herb effect
HUANG QI	Leguminosae	Astragalus aaronii (Eig) Zohary	1045	6.811367488	Lung meridian, Spleen meridian	Mildly warm	Sweet	Tonifying Qi herb
SHAN YAO	Dioscoreaceae	Dioscorea oppositifolia L.	900	5.866249511	Lung meridian, Spleen meridian, Kidney meridian	Even	Sweet	Tonifying Qi herb
DI HUANG	Scrophulariaceae	Rehmannia glutinosa (Gaertn.) DC.	860	5.605527311	Heart meridian, Liver meridian, Kidney meridian	Cool	Sweet, Bitter	Clearing heat herb
DAN SHEN	Lamiaceae	Salvia miltiorrhiza Bunge	778	5.0710468	Liver meridian, Heart meridian	Mildly warm	Bitter	Activating blood and dispelling stasis herb
MAI DONG	Liliaceae	OPhiopogon japonicus (L.f) Ker-Gawl.	699	4.556120454	Lung meridian, Stomach meridian, Heart meridian	Mildly cold	Sweet, Mildly bitter	Nourishing yin herb
GE GEN	Leguminosae	Pueraria lobata (Willd.) Ohwi	617	4.021639943	Lung meridian, Spleen meridian, Stomach meridian	Cool	Sweet, Pungent	Relieving exterior syndrome herb
HUANG LIAN	Ranunculaceae	Coptis chinensis Franch.	597	3.891278842	Large intestine meridian, Gallbladder meridian, Liver meridian, Spleen meridian, Stomach meridian, Heart meridian	Cold	Bitter	Clearing heat herb
FU LING	Polyporaceae	Poria cocos (Schw.) Wolf	565	3.682701082	Lung meridian, Spleen meridian, Kidney meridian, Heart meridian	Even	Bland, Sweet	Dissipating dampness
XUAN SHEN	Scrophulariaceae	Scrophularia ningpoensis Hemsl.	541	3.526267762	Lung meridian, Kidney meridian, Stomach meridian	Mildly cold	Sweet, Bitter,Salty	Clearing heat herb
TIAN HUA FEN	Cueurbitaceae	Trichosanthes kirilowii Maxim.	493	3.213401121	Lung meridian, Stomach meridian	Mildly cold	Sweet, Mildly bitter	Clearing heat herb
SHAN ZHU YU	Cornaceae	Cornus officinalis Sieb. et Zucc.	452	2.946160866	Liver meridian, Kidney meridian	Mildly warm	Astringent, Sour	Nourishing yin herb
WU WEI ZI	Magnoliaceae	Schisandra chinensis (Turcz.) Baill.	427	2.78320949	Lung meridian, Kidney meridian, Heart meridian	Warm	Sweet, Sour	Nourishing yin herb
GOU QI ZI	Solanaceae	Lycium harharum L.	384	2.502933125	Liver meridian, Kidney meridian	Even	Sweet	Nourishing yin herb

(*continued on next page*)

Table 1 (*continued*)

Chinese name	Family	Genus	Frequency	Frequency (%)	Herb meridian Tropism	Herb Property	Herb Flavor	Herb effect
ZHI MU	Liliaceae	Anemarrhena asphodeloides Bge.	325	2.118367879	Lung meridian, Kidney meridian, Stomach meridian	Cold	Sweet, Bitter	Clearing heat herb
CANG ZHU	Compositae	AtrActylodes lancea (Thunb.) DC.	312	2.033633164	Spleen meridian, Stomach meridian	Warm	Bitter, Pungent	Dissipating dampness
BAI ZHU	Compositae	Atractylodes macrocephala Koidz.	287	1.870681789	Spleen meridian, Stomach meridian	Warm	Sweet, Bitter	Tonifying Qi herb
MU DAN PI	Ranunculaceae	Paeonia suffruticosa Andr.	284	1.851127624	Liver meridian, Kidney meridian, Heart meridian	Mildly cold	Bitter, Pungent	Clearing heat herb
DANG GUI	Apiaceae	Angelica sinensis (Oliv.) Diels	280	1.825055403	Liver meridian, Spleen meridian, Heart meridian	Warm	Sweet, Pungent	Hematinic herb
HUANG JING	Liliaceae	Poygonautun kingianum Coll. et Hemsl	202	1.316647113	Lung meridian, Spleen meridian, Kidney meridian	Even	Sweet	Tonifying Qi herb
ZE XIE	Alismataceae	Alisma orientalis (Sam.) Juzep.	199	1.297092947	Bladder meridian, Kidney meridian	Cold	Bland, Sweet	Dissipating dampness

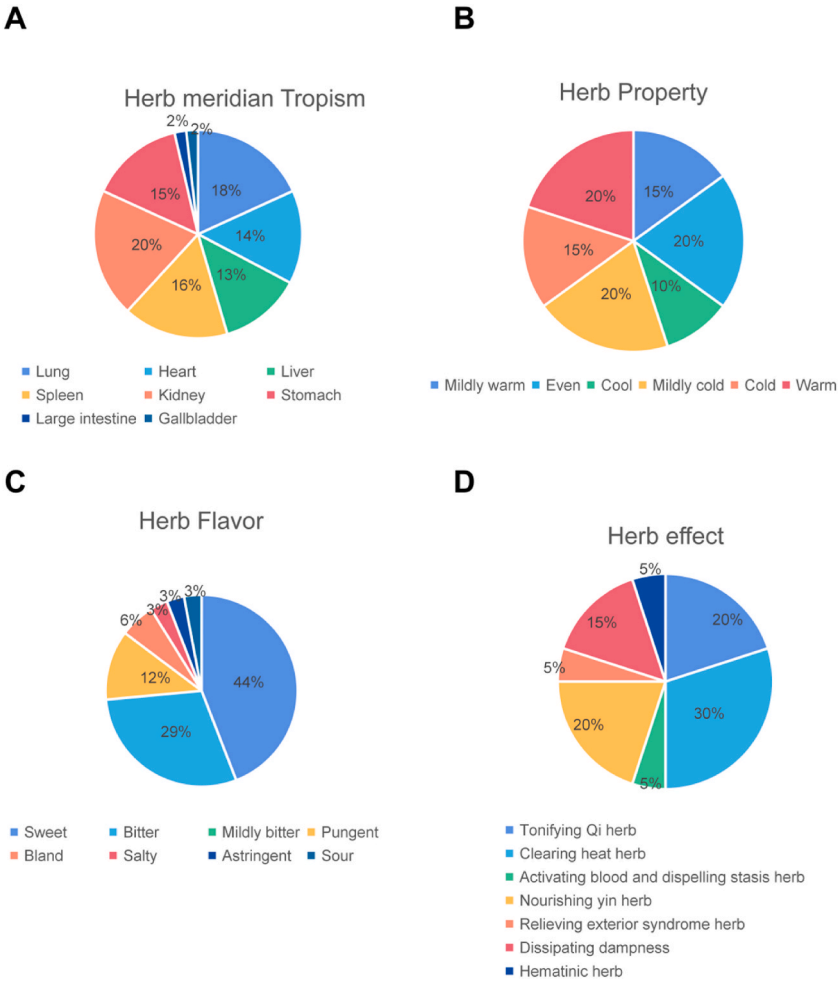


Fig. 2. The characteristics of Chinese herb. (A) The channel tropism of herbs. (B) The properties of herbs. (C)The flavor of herbs. (D)TThe effect of herbs.

Table 2
The details of the 10 random components.

Mol ID	Molecule Name	OB (%)	DL	Half-life
MOL000211	Mairin	55.38	0.78	8.87
MOL000239	Jaranol	50.83	0.29	15.5
MOL001559	piperlonguminine	30.71	0.18	8.66
MOL001736	(−)-taxifolin	60.51	0.27	14.37
MOL000359	sitosterol	36.91	0.75	5.37
MOL000449	Stigmasterol	43.83	0.76	5.57
MOL007058	formyltanshinone	73.44	0.42	24.12
MOL007059	3-beta-Hydroxymethyllenetanshiquinone	32.16	0.41	22.51
MOL002904	Berlambine	36.68	0.82	7.33
MOL002907	Corchoroside A	104.95	0.78	6.68

are the best way to quickly grasp their properties. Our analysis revealed that the majority of these herbs belong to the categories of "Tonifying Qi herb (30%)," "Nourishing yin herb (20%)," and "Clearing heat herb (20%)" (Fig. 2D), with most of them affecting the kidney (20%) and lung meridians (18%) (Fig. 2A), having a sweet flavor (44%) (Fig. 2C), and having no obvious emphasis on their property (Fig. 2B). These findings are consistent with the categorization of diabetic syndrome as "Qi and Yin deficit.

3.2. The active ingredients of Chinese herbs

Due to the complexity of herbal components, we selected the top ten Chinese herbs with the highest usage frequency for further analysis in this study (Supplementary Table S1). Our exploration of existing databases yielded 1371 small compounds in total. When TCM herbs are orally administered, hundreds of chemical components can enter the human body in a single therapy. However, the majority of these molecules are unable to reach the target area due to their weak chemical properties. The success or failure of a medicine in this regard is determined by its ADME properties (absorption, distribution, metabolism, and excretion), which are a valuable statistic for evaluating medications.

To create the candidate molecule database, we screened compounds that fulfilled the essential ADME parameters of oral bioavailability (OB) $\geq$ 30%, druglike characteristics (DL) $\geq$ 0.18, and half-life concentration $\geq$ 4. After removing duplicates and compounds with poor data quality, 118 active compounds were finally filtered and included in the candidate ingredient database of the core prescription, as shown in Supplementary Table S2. Table 2 displays the random distribution of the ten active components.

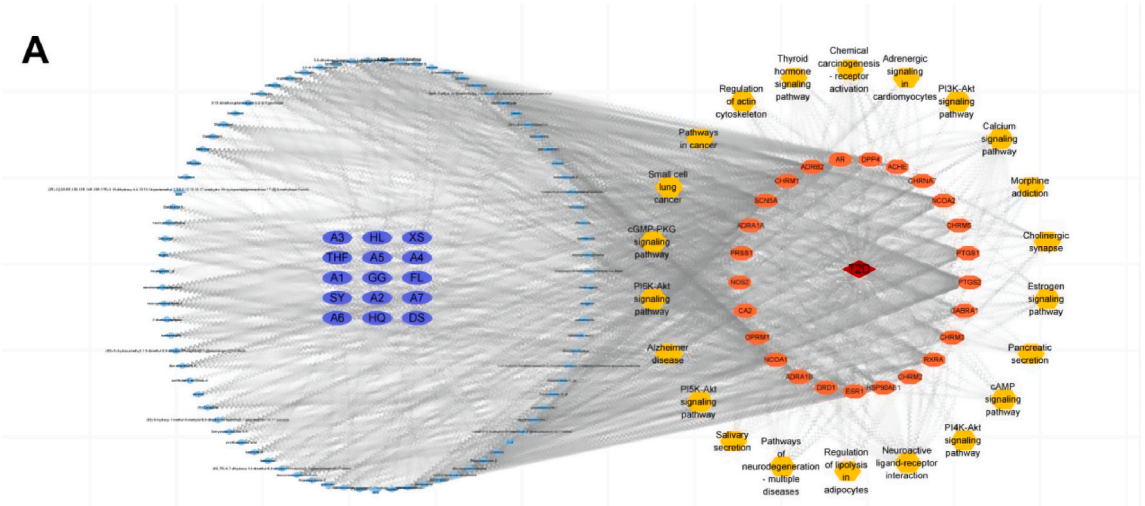
3.3. The herb-ingredient-target-pathway-disease network

This research focused on identifying the therapeutic targets and pathways of plants. After removing duplicates, a total of 247 ingredient targets were discovered (Supplementary Table S3). To obtain more accurate targets and pathways, we selected 24 target sites with at least 20 components for KEGG pathway enrichment analysis *($P < 0.05$)*. The herb-ingredient-target-pathway-disease network was then constructed using cytoscape software (Fig. 3A). The compositions are represented by the outer blue nodes on the left, the Chinese herbs by the inner purple nodes, the pathway by the outer yellow nodes on the right, and the targets by the center orange nodes. Notable terms included PTGS1, PTGS2, HSP90AB1, as well as the P13K-Akt, P16K-Akt, and cGMP-PKG signaling pathways.

In addition, Fig. 3B displays the molecular structures of the top nine components with OB values based on their ADME characteristics. However, it should be noted that these effects are only predicted and further confirmation is needed to establish whether they are the primary components, targets, or pathways of the drug for T2D therapy.

3.4. The common targets across drug targets and T2D related targets

We identified the core target of Chinese herbs for T2D therapy by intersecting disease targets with medication targets, and the resulting targets were referred to as "common targets." After removing duplicates, we retrieved over 13,131 T2D-related targets from various databases including GeneCards, OMIM, TTD, DisGeNET, and DrugBank (Supplementary Table S4). The Venn diagram analysis revealed 238 common targets between pharmaceutical and illness targets (Fig. 4A, Supplementary Table S4), which we focused on in the subsequent research. To begin, utilizing those common targets, the corresponding components and herbs were found. 93 components were related to these common targets (Fig. 4B). 12 constituents are derived from HuangQi (HQ), 47 constituents are derived from DanShen (DS), 11 constituents are derived from ShanYao (SY), 8 constituents are derived from HuangLian (HL), 1 constituent is derived from GeGen (GG), 2 constituents are derived from TianHuaFen (THF), 4 constituents are derived from FuLing (FL), 1 constituent is derived from XuanShen (XS), and (A1-A7). The blue circle nodes in the centre of the diagram indicate common objectives, and the hexagonal nodes with varied colors above and below represent individual elements. The blue circle nodes in the centre of the diagram indicate common objectives, and the hexagonal nodes with varied colors above and below represent individual elements. The fact that these components are the same hue indicates that they are derived from the same Chinese herbs. Quercetin, hederagenin, beta-sitosterol, sitosterol, Stigmasterol, sugiol, and formononetin were all found in numerous plants (Table 3). Meanwhile, formononetin, one of the top nine elements presented in Fig. 3B, can be considered the main components for further investigation.



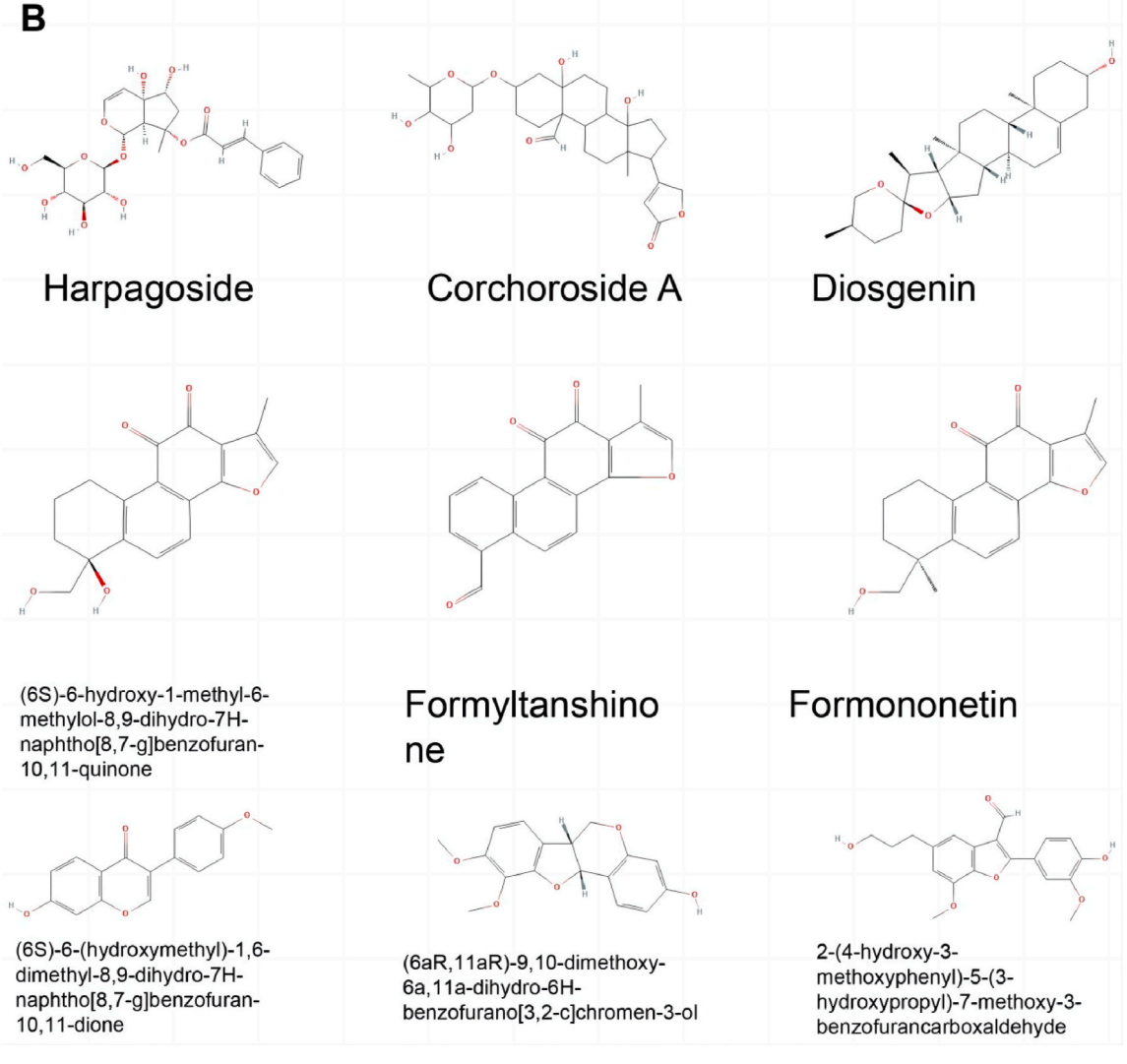


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Fig. 3. The herb-ingredient-target-pathway-disease network and the structure of the active ingredient. (A) The herb-ingredient-target-pathway-disease network. (B) The structure of the active ingredient.

3.5. The biological process and pathways of those common targets

Despite obtaining 238 common targets, the specific biological processes and pathways associated with these targets remain unknown. Thus, GO and KEGG pathway enrichment analyses of the common targets were performed to uncover additional potential mechanisms for treating T2D with Chinese herbs. Results showed that 219 KEGG pathways (Supplementary Table S5) and 2571 GO terms were enhanced (Supplementary Table S6). Fig. 5A depicts the top 10 GO terms from each of the three categories, indicating that the targets such as "immune system process" and "inflammatory response" were relevant to the physiological and pathological processes of T2D. Additionally, the pathways "AGE-RAGE signaling pathway in diabetic complications," "IL-17 signaling pathway," "p53

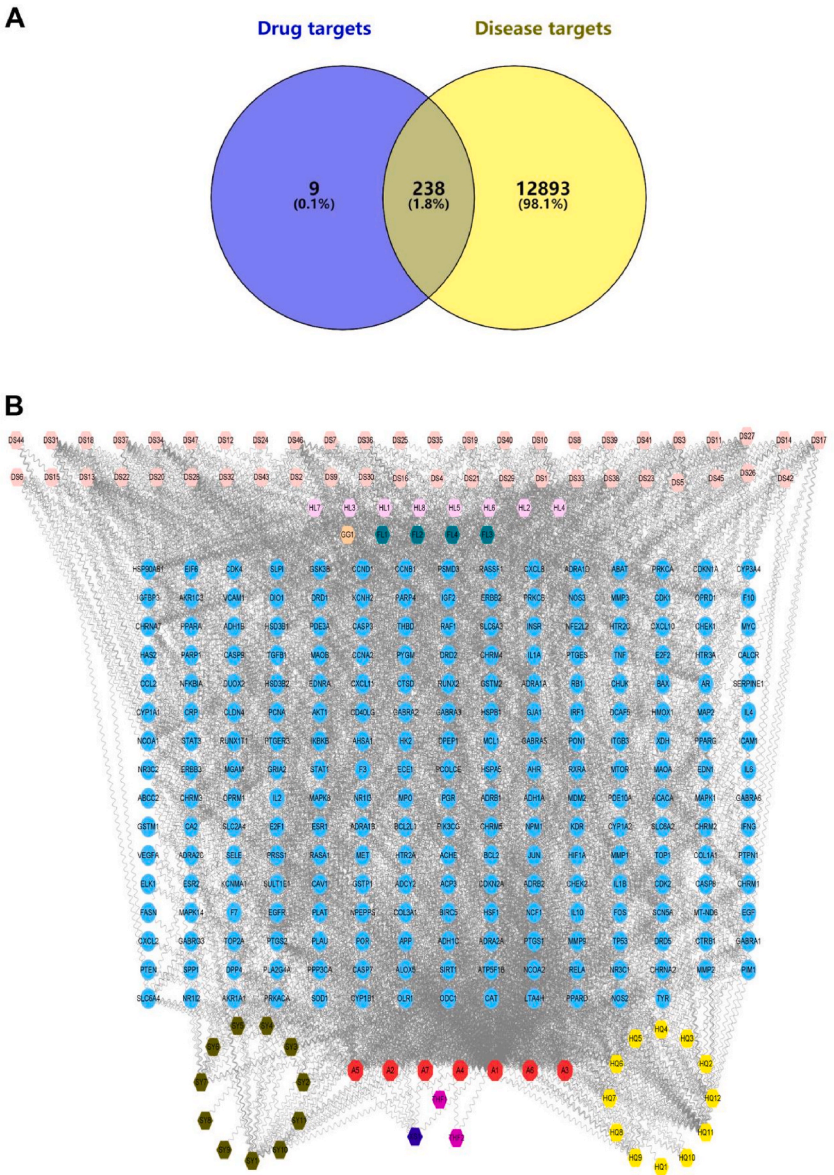

Fig. 4. Herb-ingredient-common target network. (A) Venn diagram of common targets between T2D-related targets and drug targets. (B) herb-ingredient-common target network. The blue circle nodes in the centre of the diagram indicate common objectives, and the hexagonal nodes with varied colors above and below represent individual elements. The blue circle nodes in the centre of the diagram indicate common objectives, and the hexagonal nodes with varied colors above and below represent individual elements. The fact that these components are the same hue indicates that they are derived from the same Chinese herbs.

Table 3
The most common component related to multiple herbs for T2D treatment.

Symbol	Mol ID	Ingredient	Herbs
A1	MOL000098	quercetin	HuangQi, HuangLian
A2	MOL000296	hederagenin	HuangQi, FuLing
A3	MOL000358	beta-sitosterol	XuanShen, GeGen
A4	MOL000359	sitosterol	XuanShen, DiHuang
A5	MOL000392	formononetin	HuangQi, GeGen
A6	MOL000449	Stigmasterol	MaiDong, ShenDiHuang,ShanYao
A7	MOL002222	sugiol	XuanShen, DanShen

signaling pathway," and "TNF signaling pathway" could be involved in treating T2D (Fig. 5B). Further investigation of the AGE-RAGE and IL-17 pathways using the KEGG database (https://www.kegg.jp/kegg/kegg1.html) revealed that the "AGE-RAGE signaling pathway in diabetic complications" was closely related to other metabolism-related pathways, such as "PI3K-Akt signaling pathway" and "MAPK signaling pathway," which were also the most enriched pathways in the common target (Fig. 6A). Importantly, it is also linked to the inflammatory process mediated by NF-kB, IL-1, IL-6, TNF-α, and other targets. Moreover, the IL-17 signaling pathway is associated with immunological inflammatory processes mediated by inflammatory targets (Fig. 6B). In conclusion, the GO and KEGG enrichment data suggest that treating T2D with Chinese medicines may be closely related to the immunological inflammatory process.

3.6. The core therapeutic ingredient and protein

Although we have identified prospective targets and components of Chinese herbs for the treatment of T2D and investigated their potential therapeutic processes, determining which component is the most important remains a work in progress. Therefore, we conducted protein-protein interaction (PPI) and protein-chemical interaction (PCI) analyses.

3.7. PPI network

PPI serves critical functions in various biological processes. Most proteins carry out their functions by interacting with a large number of other proteins. Degree Centrality (DC), Closeness Centrality (CC), and Betweenness Centrality (BC) are prominent tools for analyzing network structures. In our experiment, these common proteins have intricate interactions in the PPI network (Fig. 7A). We first computed the BC, CC, and DC values for all proteins (Supplementary Table S7), and then independently sorted them. Next, we screened the top 100 (Fig. 7B), top 50 (Fig. 7C), and top 10 protein targets (Fig. 7D). Specifically, we ranked the DC value, CC value, and BC value individually and then chose the best 100, 50, and 10 for intersection, accordingly. Thus, only seven proteins were in the top ten for DC, CC, and BC values. In other words, the seven proteins (TNF, TP53, IL6, JUN, AKT1, EGFR, ESR1) satisfied not only the top ten of DC but also the top ten of BC and CC.

3.8. PCI network

We identified the top nine components (Fig. 3A) and common components in the previous section (Table 3). To further predict the interaction between these components and proteins, we selected Formononetin (Fig. 3A and Table 3) and the top three ingredients (Harpagoside, Corchoroside A, Diosgenin) for investigation. These components were entered into the STITCH database to confirm the protein-chemical interaction. We only examined 20 chemicals that interacted with the two chemical components. As a result, Harpagoside and Corchoroside A did not attach to the protein, but Formononetin and Diosgenin interacted with 11 additional proteins each (Fig. 8A and B). Interestingly, TNF was also among the top 10 core proteins predicted by PPI (Fig. 7D), while MMP2, PTGS2, and CASP3 were among the top 50 core proteins predicted by PPI (Fig. 7C). PPI predicted CASP8 and CASP9 as the top 100 core proteins (Fig. 7B). Therefore, Formononetin and Diosgenin were identified as potential therapeutic components for T2D, while TNF, MMP2, PTGS, CASP3, CASP8, and CASP9 were the most promising proteins for T2D treatment.

3.9. The molecular docking between core therapeutic ingredient and protein

We performed molecular docking to evaluate the binding affinity and binding sites of the small molecules and proteins mentioned earlier. Only two compounds (Diosgenin and Formononetin) and six proteins (MMP2, PTGS2, TNF, CASP3, CASP8, CASP9) were selected for further molecular docking in this study. The lower the binding energy, the more stable the binding between the ligand and the receptor. Our results showed that the binding energy of Diosgenin with the three core proteins was all less than −8 kJ/mol, with MMP2-Diosgenin (−13.05 kJ/mol) having the lowest binding free energy and the primary forces involved were hydrogen bonds with ALA-165 (Fig. 9A1). TNF-Diosgenin (−10.5 kcal/mol) came next (Fig. 9A2), and there were three hydrogen bonds with LEU-37, ARG-82, and ASN-30. Fig. 9A3 shows PTGS2 and Diosgenin (−8.71 kJ/mol) as well as the hydrogen bond with HIS-214.

Furthermore, the binding free energies of Formononetin to its three proteins were all less than −6 kJ/mol, with CASP9-Formononetin (−6.53 kcal/mol) having the lowest binding free energy and the primary forces involved were hydrogen bonds with GLU-261 and SER-339 (Fig. 9B1). CASP3-Formononetin (−6.07 kcal/mol) appeared next (Fig. 9B2), and there were three hydrogen bonds with ASN-342, TRP-348, and GLU-379. Fig. 9B3 depicted CASP8 and Formononetin (−6.06 kJ/mol), as well as the hydrogen

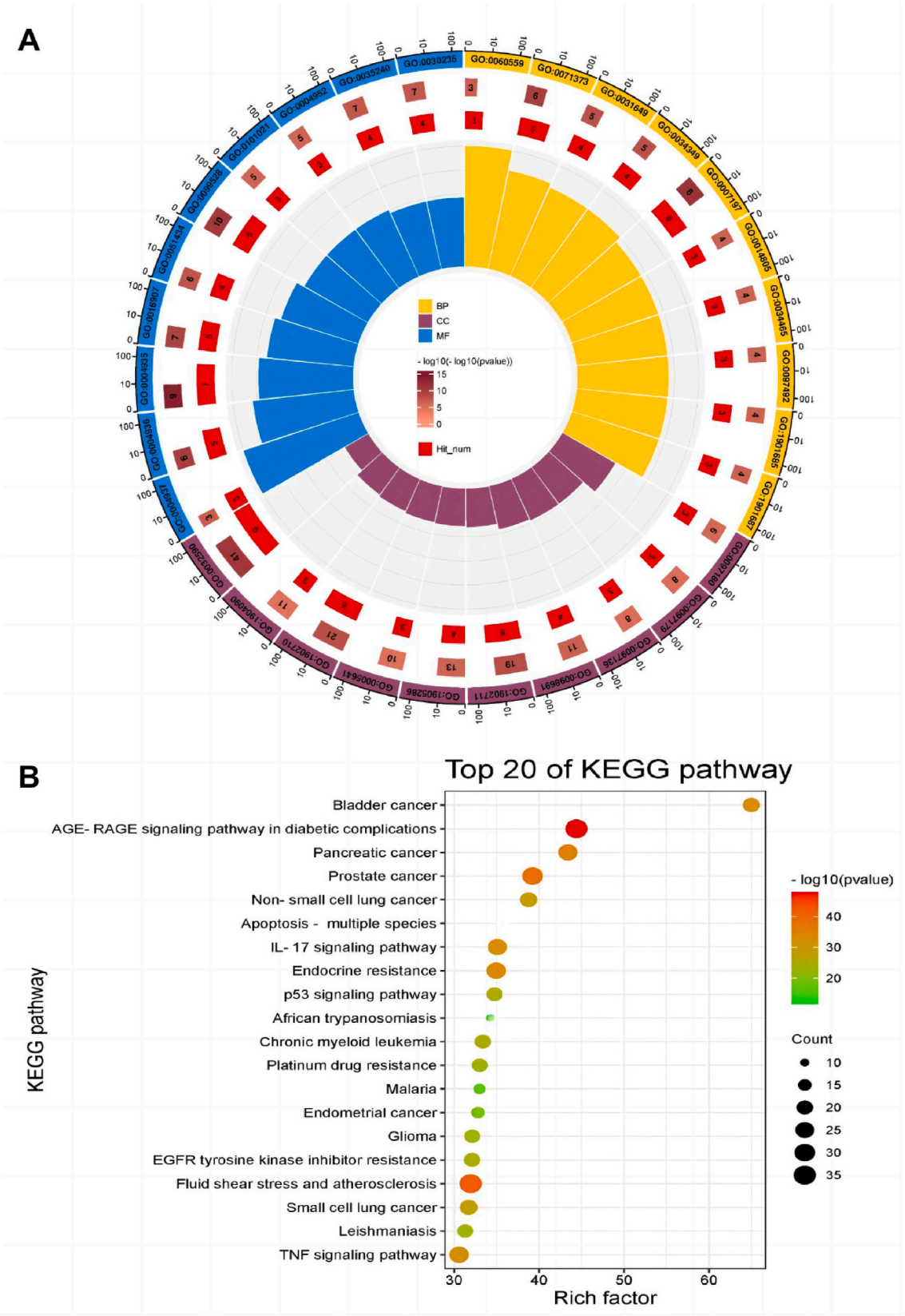

Fig. 5. The GO and KEGG enrichment of common targets. (A) The top 10 GO term of each of three categories. (B) The top 20 of KEGG pathway.

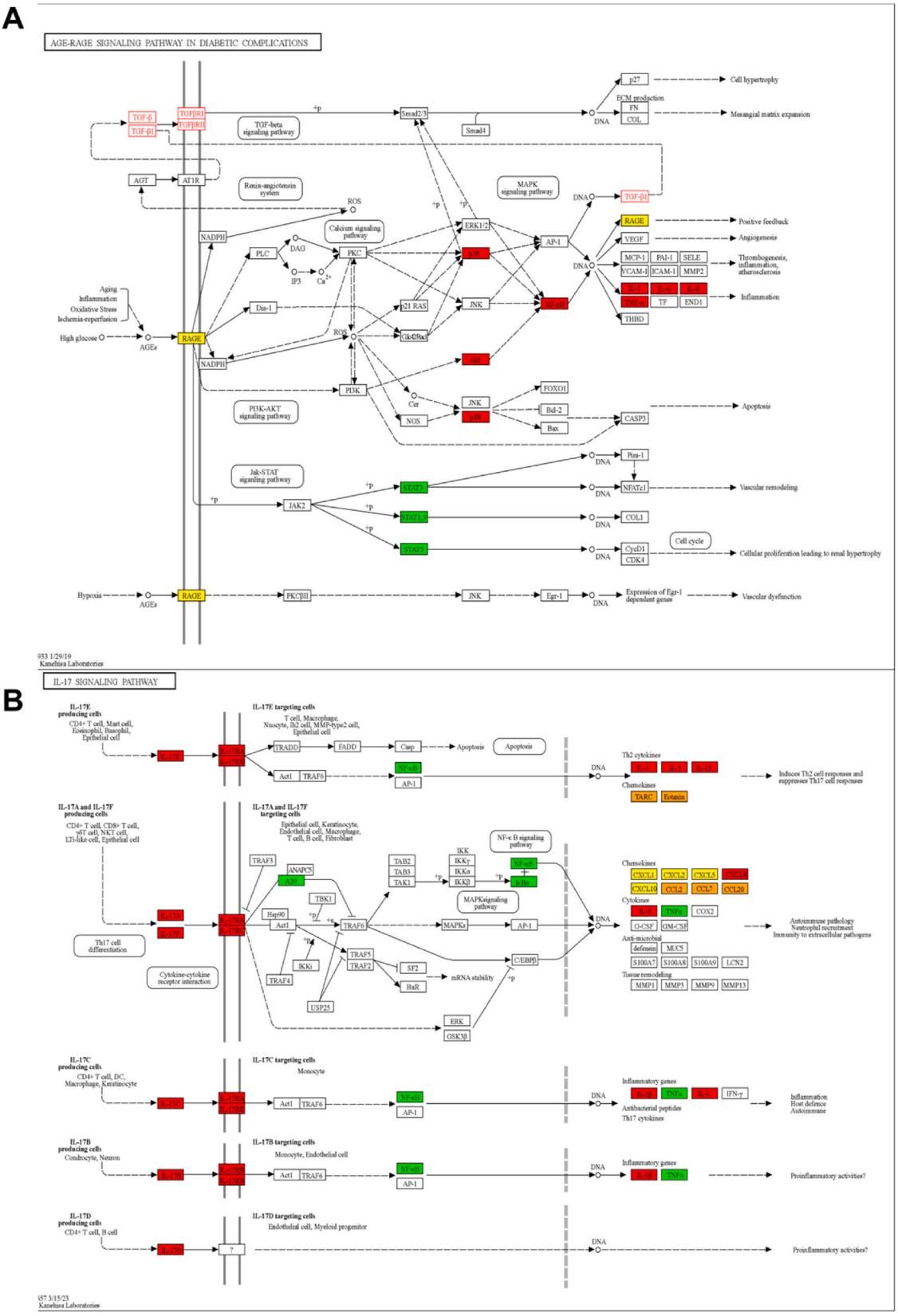

Fig. 6. The detail of pathways. (A) The detail of AGE-RAGE signaling pathway in diabetic complications. (B) The detail of IL-17 signaling pathway.

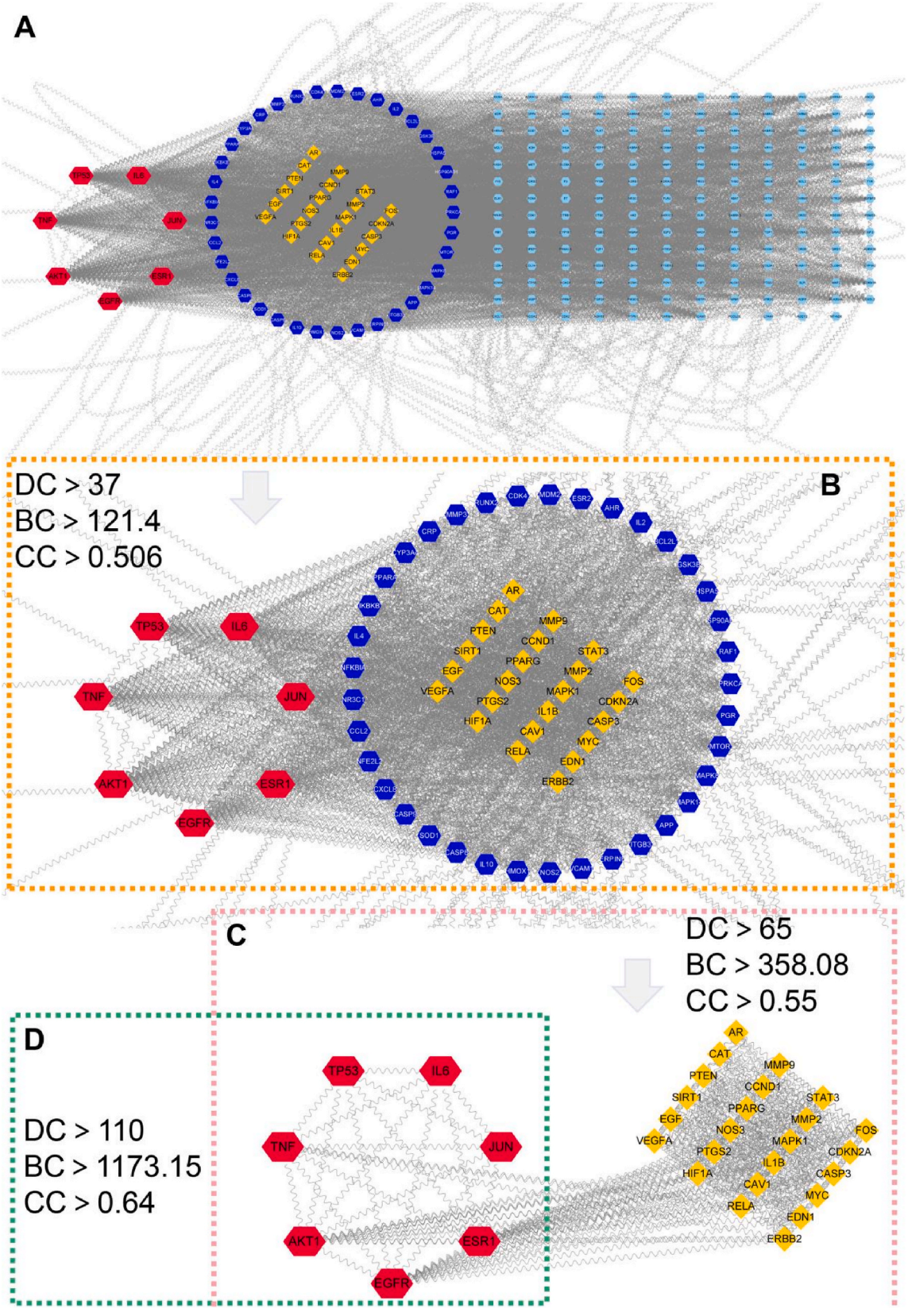

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Fig. 7. PPI network and hub proteins. (A) PPI network. (B) The top 100 proteins ranked by BC, CC, and DC.(C) The top 50 proteins ranked by BC, CC, and DC. (D) The top 10 proteins ranked by BC, CC, and DC.

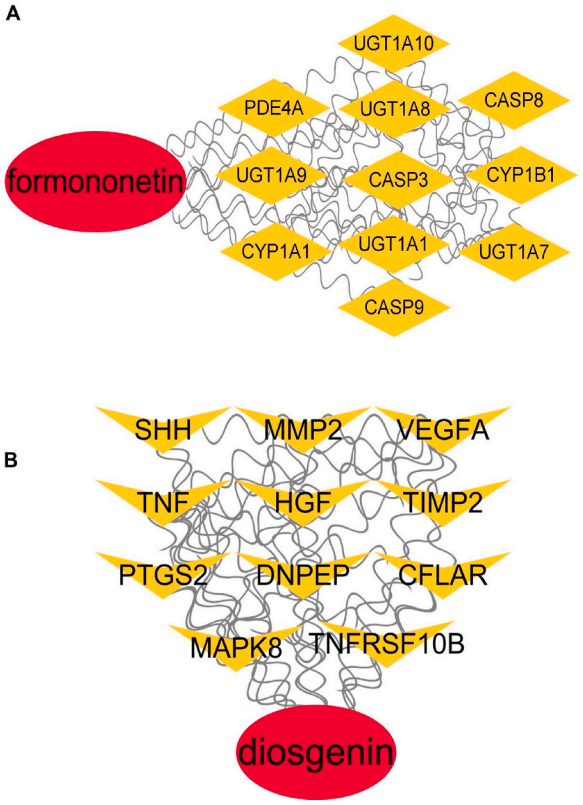

Fig. 8. PCI network. (A) Formononetin and protein interaction network. (B). Diosgenin and protein interaction network.

bond with THR-390, TYR-392, and TYR-334.

4. Discussion

The application of TCM in diabetes has a history of several thousand years. Even with the rapid development of modern medicine, its use has not decreased. Currently, there is little literature on the characteristics and molecular mechanisms of TCM in the treatment of diabetes. Therefore, a comprehensive study was conducted using data mining, network pharmacology, and molecular docking technology to uncover the potential of TCM in treating diabetes.

The study produced two results: (1) Six proteins (TNF, MMP2, PTGS, CASP3, CASP8, and CASP9) and two of the most basic compounds found in TCM herbs (Diosgenin and Formononetin) were identified as potential targets and components related to Type 2 diabetes. Systematic delivery of these active components may offer valuable information for future combination therapy of Type 2 diabetes. (2) The functional modules supporting the mechanism of action of Chinese herbs in the treatment of Type 2 diabetes are related to immune inflammatory response. This complex bio-pathway network depends on two signaling pathways: the AGE-RAGE signaling pathway in diabetic complications and the IL-17 signaling pathway.

4.1. Diosgenin for T2D treatment

Diosgenin is a naturally occurring steroidal sapogenin of the spirostanol class that may be isolated from Dioscorea tubers by hydrolysis, fermentation, and extraction. Diosgenin has been shown in recent studies to increase glucose tolerance, influence the production of proinflammatory cytokines, and have a therapeutic impact on diabetic cardiomyopath via inhibiting apoptosis, boosting the activities of antioxidant enzymes (SOD and GPX), decreasing serum cardiotoxicity indicators and ROS, TBARS and NF-kB expression, decreasing body weight, blood glucose, insulin resistance and modulating lipid profile in T2D rats [19–21]. As a consequence, diosgenin has the potential to treat diabetes, which is consistent with the findings of this study.

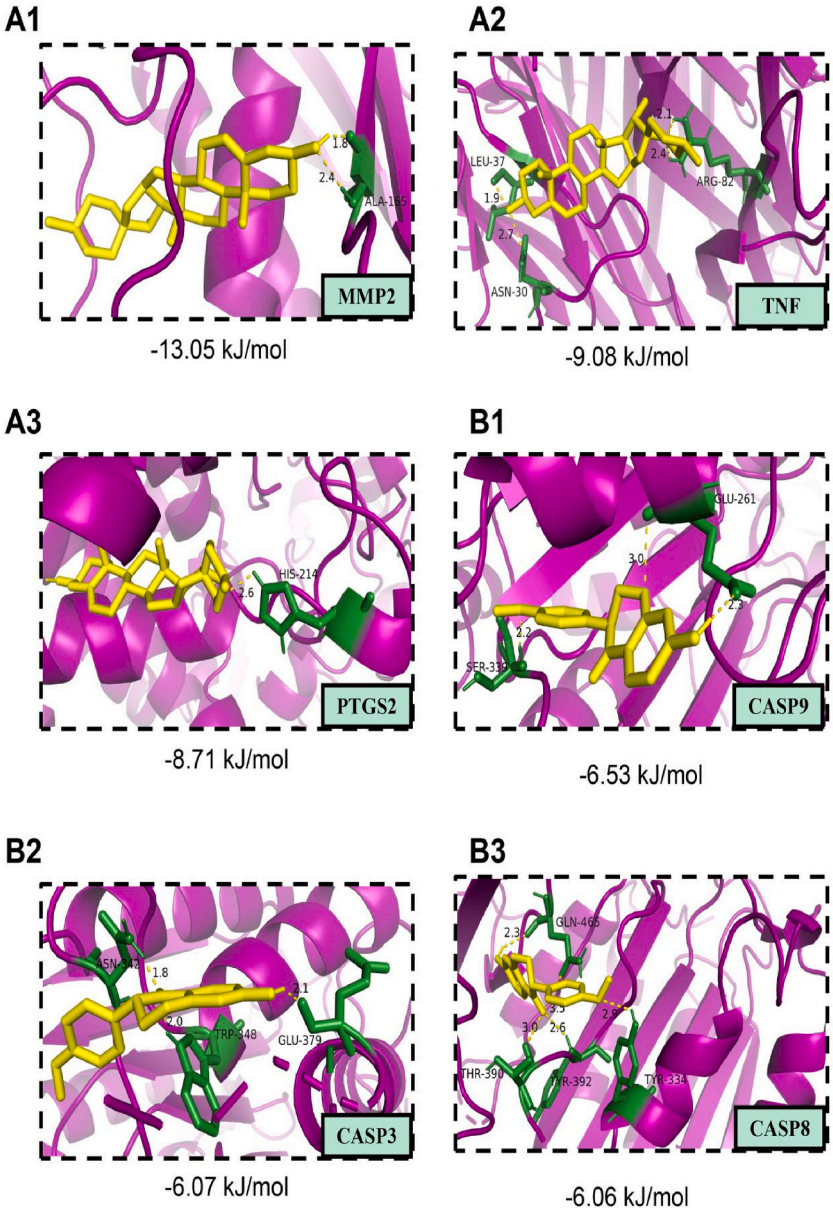

Fig. 9. The detail of molecular docking. (A1) MMP2-Diosgenin (−13.05 kJ/mol). (A2) TNF-Diosgenin (−10.5 kcal/mol). (A3) PTGS2 and Diosgenin (−8.71 kJ/mol). (B1) CASP9-Formononetin (−6.53 kcal/mol). (B2) CASP3-Formononetin (−6.07 kcal/mol). (B3) CASP8 and Formononetin (−6.06 kJ/mol).

4.2. Formononetin for T2D treatment

As an isoflavone from the phytoestrogen family, Formononetin plays a crucial role due to its vast array of biological capabilities, including antioxidant, anticancer, and anti-inflammatory properties. Numerous studies have demonstrated the ability of Formononetin to mitigate hyperlipidemia and obesity, prevent or treat liver damage, provide antioxidant and neuroprotective benefits, ameliorate hepatic steatosis, and regulate nephropathy in type 2 diabetes [22–27]. As a result, formononetin is a potential component for T2D treatment, which is consistent with the findings of this study.

4.3. Targets in T2D

At present, there are several reports on therapeutic targets and techniques for diabetes. TNF, a prominent inflammatory factor, is crucial in diabetes management. Another important group of proteins are MMPs, which are a diverse set of calcium-dependent zinc-

containing endopeptidases involved in a variety of physiological and pathological processes. Among them, MMP2, a type IV collagenase with a molecular weight of 72 kDa, is generated in various cells and secreted as latent proenzymes (pro-MMPs). A recent study has suggested that MMP2, despite being a diabetic susceptibility gene, could be a promising therapeutic target for diabetes [28], pro-MMP2 can directly affect cell function by promoting angiogenesis through the stimulation of vascular endothelial growth factor secretion, and increased expression of hMMP2 may attenuate diabetes severity by protecting islet-cells from apoptosis [21,28]. Therefore, MMP2 could offer diabetic patients an innovative and successful therapy alternative. Inflammatory mediator prostaglandin-endoperoxide synthase 2 (PTGS2) is a rate-limiting enzyme that catalyzes prostaglandin (PG) formation from AA, which has a pro-inflammatory function and disrupts the internal environment balance [28,29]. Caspases are a group of cysteine-dependent endoproteases that hydrolyze substrates following specific aspartic acid residues. They are an evolutionary conserved family of cysteine proteases that play critical roles in cell death and inflammation. Apoptotic caspases are classified as initiator (caspases 8, 9, and 10) or effector (caspases 3, 6, and 7). Caspases' vital involvement in cell death and inflammatory signaling makes them appealing candidates for therapeutic intervention in a wide range of human illnesses [30,31]. All of the aforementioned findings suggest that the targets identified in this study could be viable targets for diabetic medication therapy. Future studies could conduct appropriate experimental investigations around these pharmacological targets.

4.3.1. Immune inflammatory process and pathway in T2D

Several recent studies have offered conclusive evidence that T2D is a chronic, low-grade inflammatory illness [32,33]. Inflammatory cytokines were out of balance, with more pro-inflammatory cytokines and fewer anti-inflammatory factors, indicating chronic inflammation [32,34]. Furthermore, chronic tissue inflammation has been found in the adipose tissue, liver, and pancreas of T2D patients, indicating the potential benefits of targeting inflammation or related targets [33,35]. Since 1876, an increasing number of clinical studies on anti-inflammation medication in persons with type 2 diabetes have been conducted. In that year, the first clinical evidence supporting the therapeutic impact of anti-inflammatory medications in diabetes was published [33,36]. Meanwhile, immune cells are a major generator of inflammatory cytokines. Diabetes-related disease is promoted by long-term innate immune activation. Specifically, research has demonstrated that innate immune mechanisms regulate β-cell function. Long-term innate immune system activation restricts insulin generation and action, resulting in chronic inflammation and the consequences of diabetes [37]. Inflammation-targeting therapy for T2D is anticipated to be the first treatment for metabolic syndrome to address causal pathways. Furthermore, hyperglycemia is the primary cause of advanced glycation endproduct (AGE). The major AGE receptor is the advanced glycation end products receptor (RAGE or AGER), an immunoglobulin superfamily member and pattern recognition receptor [38]. Diabetes can be triggered by the AGE/RAGE signaling pathway through activating a series of intracellular signaling pathways, such as NADPH oxidase and protein kinase C [39]. Interleukin 17 (IL-17) is a highly adaptable pro-inflammatory cytokine that is required for a variety of functions, including host defense, tissue healing, and the pathogenesis of inflammatory disorders [40]. This cytokine has a considerable influence on inflammation, but it also regulates cellular and organismal metabolism [41]. Indeed, metabolic control is involved in both the physiological and pathologic aspects of IL-17 responses. As a result, targeting IL-17 is expected to bring up new therapy options for a number of metabolic illnesses [42].This study also demonstrated that the major mechanism of Chinese herbs in T2D therapy may influence the immune inflammatory process by modulating AGE-RAGE signaling pathway in diabetic complications and the IL-17 signaling pathway.

5. Conclusion

In conclusion, this study sheds light on the modular characteristics and mechanism of action of herbs used in Chinese Medicine for the treatment of T2D. Through systematic investigation, we have identified 170 different types of Chinese herbs, 118 active components, and 238 common targets across medicine and T2D. Six hub proteins (TNF, MMP2, PTGS, CASP3, CASP8, and CASP9), two of the most basic chemicals found in TCM herbs (Diosgenin and Formononetin) and critical therapeutic pathways including immune inflammatory response, AGE-RAGE, and IL-17 were discovered in TCM-mediated T2D suppression. Additionally, our findings highlight the importance of considering synergistic interactions among different herbs and their modular components in optimizing the overall efficacy of herbal treatments. By providing a comprehensive understanding of the potential therapeutic targets and pathways involved in herbal T2D treatment, this research contributes to the growing body of evidence supporting Chinese Medicine approaches for managing this chronic condition. However, further experimental validation and clinical studies are warranted to fully establish the clinical translational potential of these findings.The knowledge gained from this study serves as a solid foundation for future research and development of herbal treatments for T2D. It opens up avenues for exploring novel combinations and formulations of herbs, paving the way for evidence-based approaches that can enhance long-term glycemic control and reduce the risk of complications in individuals with T2D. Overall, the modular characteristics and mechanisms of action uncovered in this study provide valuable insights for both researchers and practitioners in the field of Chinese Medicine, offering potential avenues for improved treatment strategies and personalized approaches to address the complex nature of T2D.

Author contribution statement

Chan Yang: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper. </p>

Hanyu Liu: Performed the experiments; Analyzed and interpreted the data; Wrote the paper. </p>

Xinqiong Li: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

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Xi Peng, Guocheng Rao: Performed the experiments; Analyzed and interpreted the data. </p>
Ziyan Xie: Performed the experiments; Wrote the paper. </p>
Qiangfei Yang: Analyzed and interpreted the data; Wrote the paper. </p>
Lian Du: Contributed reagents, materials, analysis tools or data. </p>
Chunguang Xie: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.
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Data availability statement

Data will be made available on request.

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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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e20106.

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