

Gynostemma Pentaphyllum Ameliorates Lipid Metabolic Abnormalities in Diabetic Kidney Disease

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Abstract

Background: Patients with diabetic kidney disease (DKD) were often accompanied with dislipidemia. *Gynostemma pentaphyllum* can ameliorate insulin resistance and reduce the synthesis of triglycerides and cholesterol, but the underlying mechanism is still unclear. Therefore, we used the network pharmacologic strategies to evaluate potential therapeutic effects and protective mechanisms of *Gynostemma pentaphyllum* on diabetic kidney disease.

Methods: *Gynostemma pentaphyllum*'s potential targets were predicted using the TCMSP databases. The pathogenic factors involved in DKD and dislipidemia were screened by the OMIM and Gene Cards databases. The common targets of *Gynostemma pentaphyllum*, DKD and dislipidemia were used to establish a protein-protein interaction (PPI) network. Gene Ontology (GO) and Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathway enrichment analysis were used to explore the potential molecular pathways.

Results: The key targets for the therapeutic effects of *Gynostemma pentaphyllum* included IL-6, AKT1, VEGFA, PTGS2, CCL2 and CASP3. Enrichment analysis showed that the underlying mechanism were mainly the involved in inhibition of inflammatory response, negative regulation of apoptotic process and angiogenesis. TNF, PI3K-Akt, and HIF-1 signaling pathways were considered as the key pathways.

Conclusion: *Gynostemma pentaphyllum* played a therapeutic role in DKD complicated with dislipidemia, mainly through influencing inflammation response, apoptosis and angiogenesis.

Background

Nowadays, with the changing of people's lifestyles, diabetic mellitus has become a global problem that mainly affects developing countries. According to epidemiological statistics, diabetics worldwide is expected to reach 366 million by 2030 [1, 2]. Diabetic kidney disease (DKD) is a typical microvascular complication of diabetes and primary cause for end-stage kidney disease, and the number of patients with DKD will exceed 100 million by 2030 [1]. Therefore, an effective strategy for preventing DKD is urgently needed.

In general, patients with DKD often exert a variety of lipoprotein abnormalities [2]. In 1982, Moorhead *et al* [3] found that hyperlipidemia was associated with glomerular capillary injury, and suspected that the continuous filtration of lipids and lipoproteins promoted the development of kidney damage. Studies on animal models indicated that hyperlipidemia was closely related to lipid deposition in mesangium and the progress of early diabetic kidney lesions [4].

Long-term hyperglycemia leads to higher levels of low-density lipoprotein (LDL) cholesterol and lowers levels of high-density lipoprotein (HDL) cholesterol, resulting in abnormal lipid metabolism [5]. Furthermore, Tolomen *et al*, [6] demonstrated that a higher high-density lipoprotein cholesterol level is associated with a lower incidence of chronic kidney disease. The primary cause of diabetic dyslipidemia

is the increased free fatty-acid (FFA) released by fat cells [7–10]. Excessive FFAs can be converted to TG by desertification, which deposit in renal tissues and cause toxicity, aggravating kidney injury [11]. Furthermore, the impaired ability to inhibit FFAs release can enhance the production of very low-density lipoprotein (VLDL) cholesterol in the liver [12]. Dyslipidemia may aggravate renal damage in diabetic patients by affecting the coagulation system and damaging endothelial cells [13].

Hyperlipidemia in diabetic kidney disease is the disease controlled by multiple genes and targets. Drugs with highly selective ligands targeting a single target often cannot change the overall state of the disease and it is difficult to effectively control or prevent the development of the disease. In 2004, Morphy *et al* provided a new thinking for therapy: multi-target therapeutics [14].

Gynostemma pentaphyllum is a creeping plant, belonging to the cucurbit family. As a type of Traditional Chinese Medicine (TCM), *Gynostemma pentaphyllum* is widely used as herbal tea and dietary supplement [15], and contains various chemical ingredients, including saponins, flavonoids, polysaccharides, amino acids and some essential elements [16–19]. *Gynostemma pentaphyllum* has been reported to have multiple protective functions, including anti-microbial [20], anti-oxidant [21–22], anti-cancer [23–24], anti-inflammatory [24–25], anti-diabetic [26–28] and anti-lipidemic [29]. However, no study has systematically explored its efficacy and potential mechanism for diabetic patients with lipotoxic abnormalities.

Due to the complexity of multi-component and multi-target for *Gynostemma pentaphyllum*, it is difficult to accurately elaborate the corresponding relationship between components and targets. However, network pharmacology based on system biology provides us with the possibility of systematic study of drug action network and helps to reveal the potential mechanism of *Gynostemma pentaphyllum* ameliorates lipid metabolic abnormalities in DKD.

Methods And Materials

Target screening

TCMSP (<http://www.tcmspw.com/tcmsp.php>) with the keyword of “*Gynostemmae Pentaphylli Herba*” was employed to collect the active ingredients of *Gynostemma pentaphyllum*. TCM is often clinically used via oral administration. Thus, the active ingredients of *Gynostemma pentaphyllum* were selected based on oral bioavailability (OB) > 30%, drug-likeness > 0.18. The overlapped targets collected from TCMSP and SymMap (<https://www.symmap.org/>) were used to obtain the potential targets of *Gynostemma pentaphyllum*. Genecard (<https://www.genecards.org/>) and DisGeNET (<https://www.disgenet.org/home/>) were used for the related target search with the keywords of “hyperlipidemia”, “hypercholesterolemia”, “hypertriglyceridemia”, “diabetic kidney disease” and “diabetic nephropathy”. The common targets from these two databases were taken to obtain the related targets associated with hyperlipidemia and DKD. Then, the potential targets of *Gynostemma pentaphyllum* were mapped onto the related targets of hyperlipidemia and DKD. Venn diagram for mapped targets was made by Venny2.1.0 (<https://bioinfogp.cnb.csic.es/tools/venny/>).

Network construction

The active ingredients and corresponding targets of *Gynostemma pentaphyllum* were imported into the Cytoscape3.7.2 (<https://cytoscape.org/>) to make the network platform. A protein-protein interaction (PPI) network was established to elucidate the associations between potential targets and other proteins [30]. The disease targets and common targets were imported into STRING tools (<https://string-db.org/>), followed by the construction of PPI network models using Cytoscape3.7.2.

Enrichment analysis

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were performed using the Database for Annotation, Visualization and Integrated Discovery (DAVID) (<https://david.ncifcrf.gov/>), and visualized by an online tool, bioinformatics (<http://www.bioinformatics.com.cn/>). KEGG pathways were made by KEGG Mapper (https://www.genome.jp/kegg/tool/map_pathway2.html).

Molecular docking model

The crystal structures of targets were downloaded from RCSB Protein Data Bank (<http://www.rcsb.org/>). Moreover, receptors were modified by AutoDock Tools1.5.6 (<http://mgltools.scripps.edu/>). Grid box was set to the maximum for docking. The structures of active ingredients were downloaded from TCMSP (<https://tcmssp.com/tcmssp.php>) and modified by AutoDock Tools1.5.6. All ligand and receptor files were converted into pdb format by OpenBabel 3.1.1 (http://openbabel.org/wiki/Main_Page) and visualized by PyMol2.2.0.

Results

To elucidate the mechanisms underlying the inhibition of the abnormal lipid metabolism by *Gynostemma pentaphyllum* in DKD, we performed a series of bioinformatic analyses on several public datasets (Figure 1). Totally, 202 active ingredients of *Gynostemma pentaphyllum* were obtained by TCMSP, of which 24 reached the criteria of OB > 30% and DL > 0.18 (Table 1). Cyclobuxine exhibited the highest bioavailability (84.48%), indicating that it can be utilized by the human body to a high degree. Gypentonoside A_{qt} had the highest DL (0.8), suggesting its high probability for being used as a drug.

Table 1
ADME parameters of active ingredients in gynostemma pentaphyllum

MolID	MolName	OB(%)	DL
MOL000338	3'-methyleriodictyol	51.61	0.27
MOL000351	Rhamnazin	47.14	0.34
MOL000359	sitosterol	36.91	0.75
MOL004350	Ruvoside_qt	36.12	0.76
MOL004355	Spinasterol	42.98	0.76
MOL005438	campesterol	37.58	0.71
MOL005440	Isofucosterol	43.78	0.76
MOL007475	ginsenoside f2	36.43	0.25
MOL000953	CLR	37.87	0.68
MOL000098	quercetin	46.43	0.28
MOL009855	(24S)-Ethylcholesta-5,22,25-trans-3beta-ol	46.91	0.76
MOL009867	4 α ,14 α -dimethyl-5 α -ergosta-7,9(11),24(28)-trien-3 β -ol	46.29	0.76
MOL009877	cucurbita-5,24-dienol	44.02	0.74
MOL009878	Cyclobuxine	84.48	0.7
MOL009888	Gypenoside XXXVI_qt	37.85	0.78
MOL009928	Gypenoside LXXIV	34.21	0.24
MOL009929	Gypenoside LXXIX	37.75	0.25
MOL009938	Gypenoside XII	36.43	0.25
MOL009943	Gypenoside XL	30.89	0.21
MOL009969	Gypenoside XXXV_qt	37.73	0.78
MOL009971	Gypenoside XXVII_qt	30.21	0.74
MOL009973	Gypenoside XXVIII_qt	32.08	0.74
MOL009976	Gypenoside XXXII	34.24	0.25
MOL009986	Gypentonoside A_qt	36.13	0.8
Abbreviations: ADME, absorption, distribution, metabolism, and excretion; MOL, molecule; OB, oral bioavailability; DL, drug-likeness.			

As shown in Figure 2A, quercetin was the active ingredient correlated with most of the target genes. Two types of gypenosides, gypenoside XXVII_{qt} and XXVIII_{qt}, were also connected to some target genes. These results indicated that quercetin and gypenosides might play key roles during the whole process. After overlapping the disease genes derived from Genecard and DisGeNET databases, 289 common genes were obtained, and 267 target interactions were obtained from these overlapped genes. As shown in Figure 2B, INS (insulin), ALB (albumin), IL-6 (interleukin-6), AKT1 (AKT Serine/Threonine Kinase 1), TNF (tumor necrosis factor) and VEGFA (vascular endothelial growth factor A) might play key roles in hyperlipidemia in DKD.

From the 47 common target genes of *Gynostemma pentaphyllum* associated with hyperlipidemia and DKD (Figure 3A), IL-6, AKT1, VEGFA, PTGS2 (prostaglandin-endoperoxide synthase 2), CCL2 (C-C Motif Chemokine Ligand 2) and CASP3 (Caspase 3) showed the highest significance (Figure 3B), indicating their critical potential in drug therapy. GO analysis for these 47 common genes revealed that inflammatory response (GO: 0006954) and signal transduction (GO: 0007165) were significantly enriched (Figure 4A). Furthermore, negative regulation of apoptotic process (GO: 0043066) and angiogenesis (GO: 0001525) were also significantly enriched. KEGG pathway analysis revealed that 80 pathways were involved, including HIF-1 signaling pathway (hsa04066), TNF signaling pathway (hsa04668) and PI3K-Akt signaling pathway (hsa04151) (Figure 4B-C). These pathways correlated with inflammation, apoptosis and angiogenesis, and may contribute to the effect of *Gynostemma pentaphyllum* in ameliorating the lipid metabolic abnormalities in DKD.

Thus, the genes involved in inflammation, apoptosis and angiogenesis, including IL-6, PTGS2, CASP3 and VEGFA, were selected for molecular docking analysis with Rhamnazin and quercetin. As shown in Figure 5, the molecular interaction between VEGFA and quercetin (VEGFA-quercetin) was the closest interaction, indicating its critical role in attenuating the lipid metabolic abnormalities in DKD by *Gynostemma pentaphyllum*.

Discussion

Three significant signaling pathways (hsa04066, hsa04668, hsa04151) were selected as the critical pathways by PPI networks and enrichment analysis of common-targets, which were mainly related to three functional modules including inflammation, apoptosis and angiogenesis. Therefore, this research was focused on these three modules.

It was reported that *Gynostemma pentaphyllum* could remarkably suppress the increase of triglyceride, total cholesterol and LDL-cholesterol in serum caused by high-fat diet [31]. Gao *et al* found that *Gynostemma pentaphyllum* had the function of hypoglycemic and hypolipidemic by the expression of NFE2-related factor 2 signaling in diabetic rats [32].

In some previous studies, lipid accumulation in the kidney was considered to be the key process in DKD [33–34]. And a research reported that quercetin could alleviate lipid accumulation in the kidney of DKD rats by regulating the expression of sterol regulatory element-binding proteins (SREBPs) and LDL receptor protein, which were controlled by Akt [35]. In addition, quercetin could also regulate the expression of insulin receptor substrate and glucokinase and improved the insulin secretion function of β -cells [36]. In terms of blood lipid, a study showed that 1% quercetin diet could reduce the content of FFA in serum of rats and promoted the catabolism of fat [37].

Gypenosides could significantly reduce the insulin resistance parameters and increased the glycogen concentration [38]. As well, gypenosides could effectively prevent hyperlipidemia and atherosclerosis. Some related researches found that theregulation of blood lipid was related to the inhibition of FFA produced by adipocytes and the promotion of neutral fat synthesis [39–40]. Therefore, quercitrin and gypenosides may be key ingredients in the therapy.

The inflammatory model showed that *Gynostemma pentaphyllum* may influence inflammation through HIF-1 signaling pathway and TNF signaling pathway. Pro-inflammatory cytokines IL-6 was the key gene in these two pathways. Senn *et al*/ found that IL-6 could inhibit the insulin signal transduction and insulin action [41]. And another study reported that the release of IL-6 could weaken the function of pancreatic β cells, leading to insulin resistance, insulin secretion dysfunction and the occurrence of metabolic syndrome [42]. Therefore, abnormal secretion of IL-6 may result in the hyperlipidemia in DKD by affecting insulin. In addition, a significant inhibition of IL-6 was observed in the gypenosides concentration of 150 $\mu\text{g/ml}$ and 200 $\mu\text{g/ml}$ [43]. It was reasonable to believe that *Gynostemma pentaphyllum* may inhibit the expression of IL-6, which furthermore would relieve the DKD.

Some inflammatory cytokines, such as TNF- α , could activate the NF- κ B signaling pathway, which would aggravate the inflammatory response and accelerate the development of DKD [44]. And a significant inhibition of TNF- α was observed in the gypenosides concentration range of 100–200 $\mu\text{g/ml}$ [43]. These results indicated that *Gynostemma pentaphyllum* may inhibit the expression of TNF- α to improve inflammation and lipid metabolism in DKD.

HIF-1 signaling pathway and PI3K-Akt signaling pathway acted as the crucial roles in apoptosis and angiogenesis. And HIF-1 α as a transcription factor could stimulate the expression of cytokines related to renal interstitial cell fibrosis, which would aggravate the diabetic kidney injury [45–46]. Some animal studies indicated that HIF could cause the increase of insulin sensitivity as well as the decrease of serum cholesterol levels [47, 48]. The mechanism might be associated with the reduction of metabolite accumulation in glycolysis and TCA cycle in diabetic renal cortical tissue [49]. These results showed that HIF stabilization could ameliorate lipid metabolic abnormalities in DKD. Another study reported that quercetin could scavenge the ROS to reduce the level of HIF-1 α and reduce the level of caspase-9 in H_2O_2 -treated cells, suggesting that quercetin could inhibit the caspase-dependent apoptosis [50]. These results showed that *Gynostemma pentaphyllum* could inhibit the cell apoptosis as a HIF stabilizer, which may be

related to the mechanism that gynostemma pentaphyllum ameliorated the lipid metabolic abnormalities in DKD.

PI3K and the downstream effector Akt belonged to the signal transduction enzymes and was involved in regulating cellular activation and apoptosis [51]. Li *et al* [52] found that the PI3K signaling pathway played a crucial part in autophagy and DKD, of which the mechanism may be related to the podocyte adhesion injury. In terms of tumors, Maurya *et al* reported that quercetin modulated the PI3K-AKT signaling pathway and reduced the Akt and PDK1 phosphorylation [53], which was similar to the mechanism that gynostemma pentaphyllum ameliorated lipid metabolic abnormalities in DKD.

VEGF was a growth factor for endothelial cell, which played a crucial part in the process of angiogenesis [54]. VEGFA was closely related to angiogenesis, which was mediated by VEGF receptor 1 (VEGFR1) and VEGFR2 signaling pathways [55–56]. A study found that quercetin can significantly suppress the activation of VEGFR2 downstream molecules, to inhibit angiogenesis, such as AKT and mTOR[57]. Few studies have reported that gynostemma pentaphyllum improved the symptoms of DKD through increasing the angiogenesis, more research is needed to confirm these points in the future.

Conclusion

In summary, gynostemma pentaphyllum could ameliorate the abnormal lipid metabolism in DKD through influencing inflammation response, apoptosis and angiogenesis. However, the research on specific cell or gene expression is not in-depth enough, which will be further explored in follow-up experiments.

Abbreviations

Abbr.	Full Name
DKD	Diabetic Kidney Disease
PPI	Protein-Protein Interaction
GO	Gene Ontology
KEGG	Kyoto Encyclopaedia of Genes and Genomes
TCMSP	Traditional Chinese Medicine Systems Pharmacology
OMIM	Online Mendelian Inheritance in Man
IL-6	Interleukin-6
AKT1	Protein Kinase B
VEGFA	Vascular Endothelial Growth Aactor A
PTGS2	Prostaglandin Endoperoxide Synthase 2
CCL2	Chemotactic Cytokines Ligand2
CASP3	Cysteine-Containing Aspartate-specific Proteases-3
TNF	Tumor Necrosis Factor
PI3K-Akt	Phosphatidylinositol 3 kinase -Protein kinase B
HIF-1	Hypoxia-Inducible Factor 1
LDL	Low-Density Lipoprotein
HDL	High-Density Lipoprotein
FFA	Free Fatty-Acid
TCM	Traditional Chinese Medicine
OB	Oral Bioavailability
DAVID	Database for Annotation Visualization and Integrated Discovery
STRING	Search Tool for the Retrieval of Interacting Proteins Database
INS	Insulin
ALB	Albumin
NFE2	Nuclear Factor Erythroid-2
SREBPs	Sterol Regulatory Element-Binding Proteins
NF-κB	Nuclear Factor Kappa-B
TCA	Tricarboxylic Acid

ROS	Reactive Oxygen Species
PDK1	3-Phosphoinositide-Dependent Protein Kinase 1
mTOR	Mammalian Target of Rapamycin

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and material

No data deposition for this paper.

Competing interests

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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Authors' contributions

PR Peng and SH Jiao performed bioinformatic analysis and wrote the manuscript. L Sun, HL Sun and CZ Wang revised the manuscript. HY Sun and ZW Zhang designed and wrote the manuscript.

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Figures

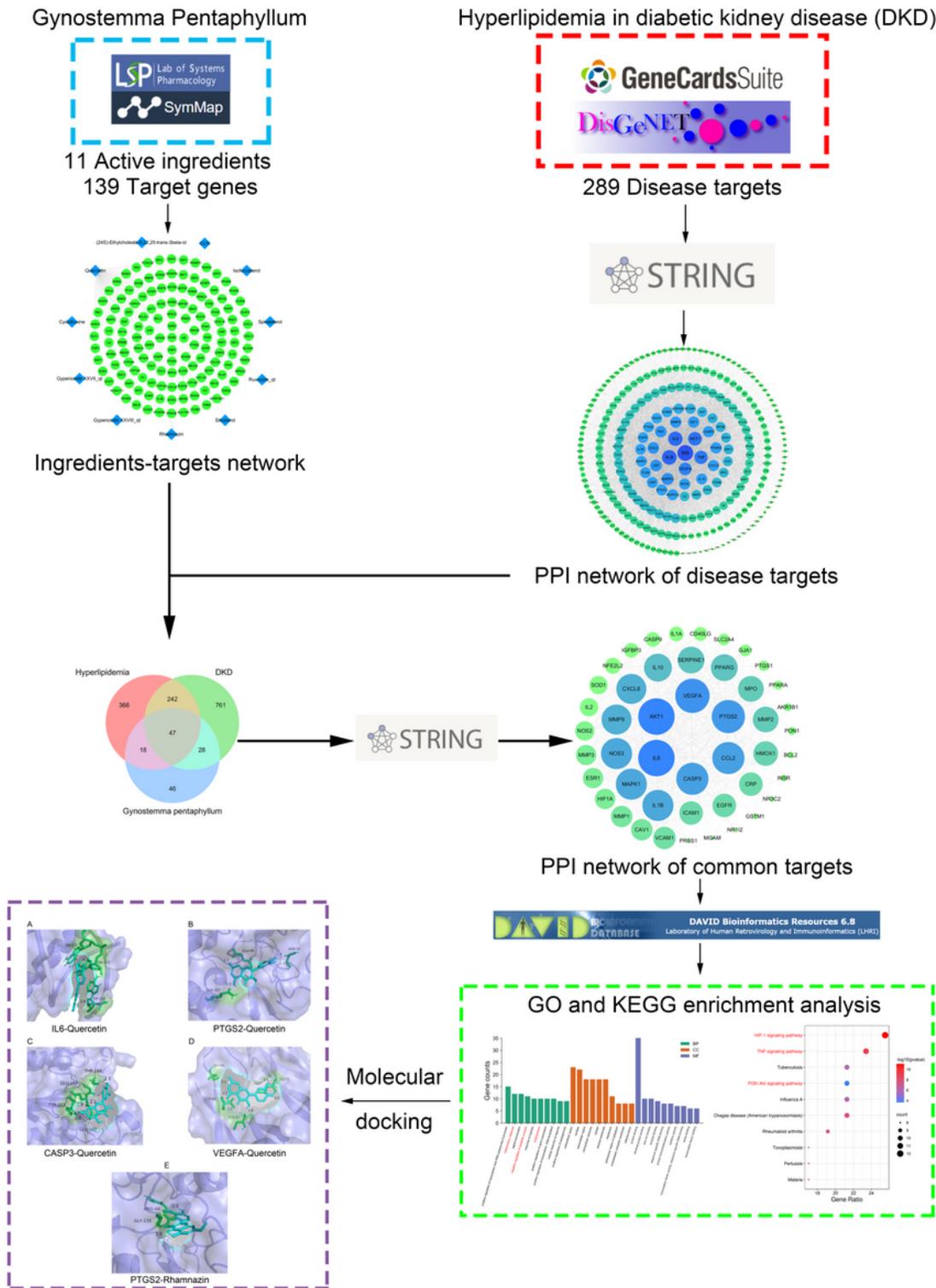


Figure 1

A flowchart for researching therapeutic mechanism of gynostemma pentaphyllum on hyperlipidemia in DKD.

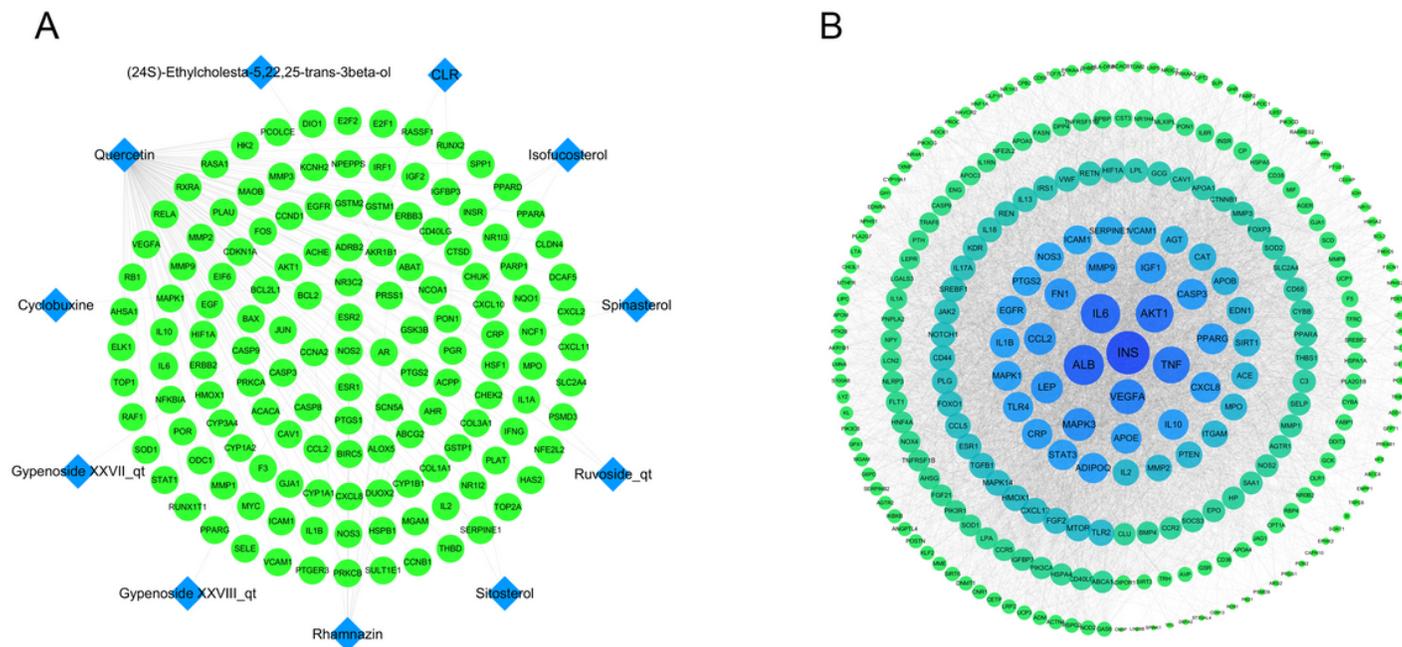
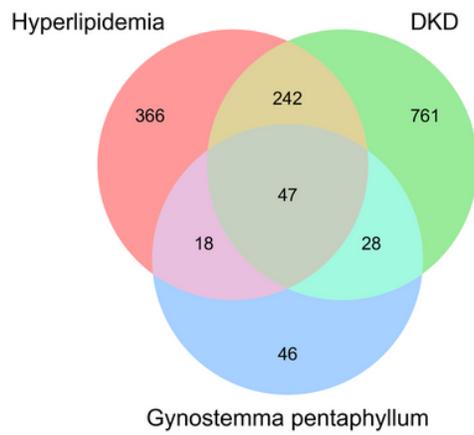
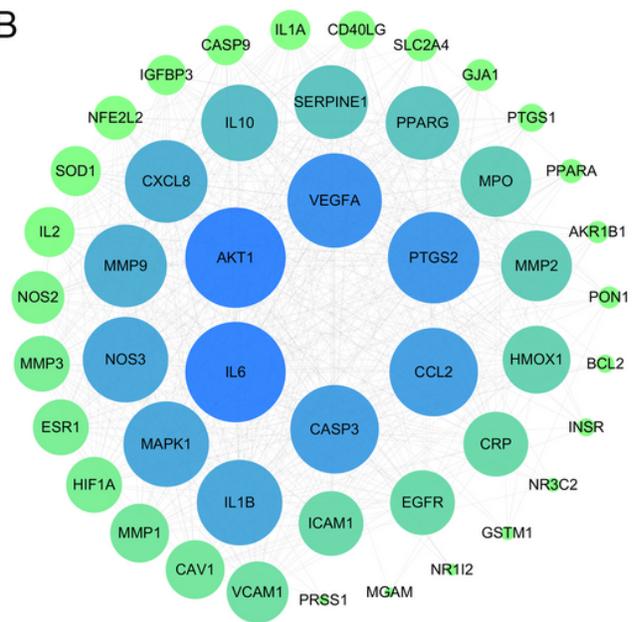


Figure 2

Active ingredient-target relationships and PPI network of disease targets. (A) Active ingredient-target relationships. Blue nodes represent 11 active ingredients. Green nodes represent 139 target genes. The lines represent relationships between the ingredients and target genes. **(B)** PPI network. The nodes are in descending order of degree values with sizes from large to small and colors from blue to green.

A**B****Figure 3**

Venn diagram and PPI network of common targets. (A) Venn diagram for the associated target genes of gynostemma pentaphyllum with hyperlipidemia and DKD. (B) PPI network. The nodes are in descending order of degree values with sizes from large to small and colors from blue to green.

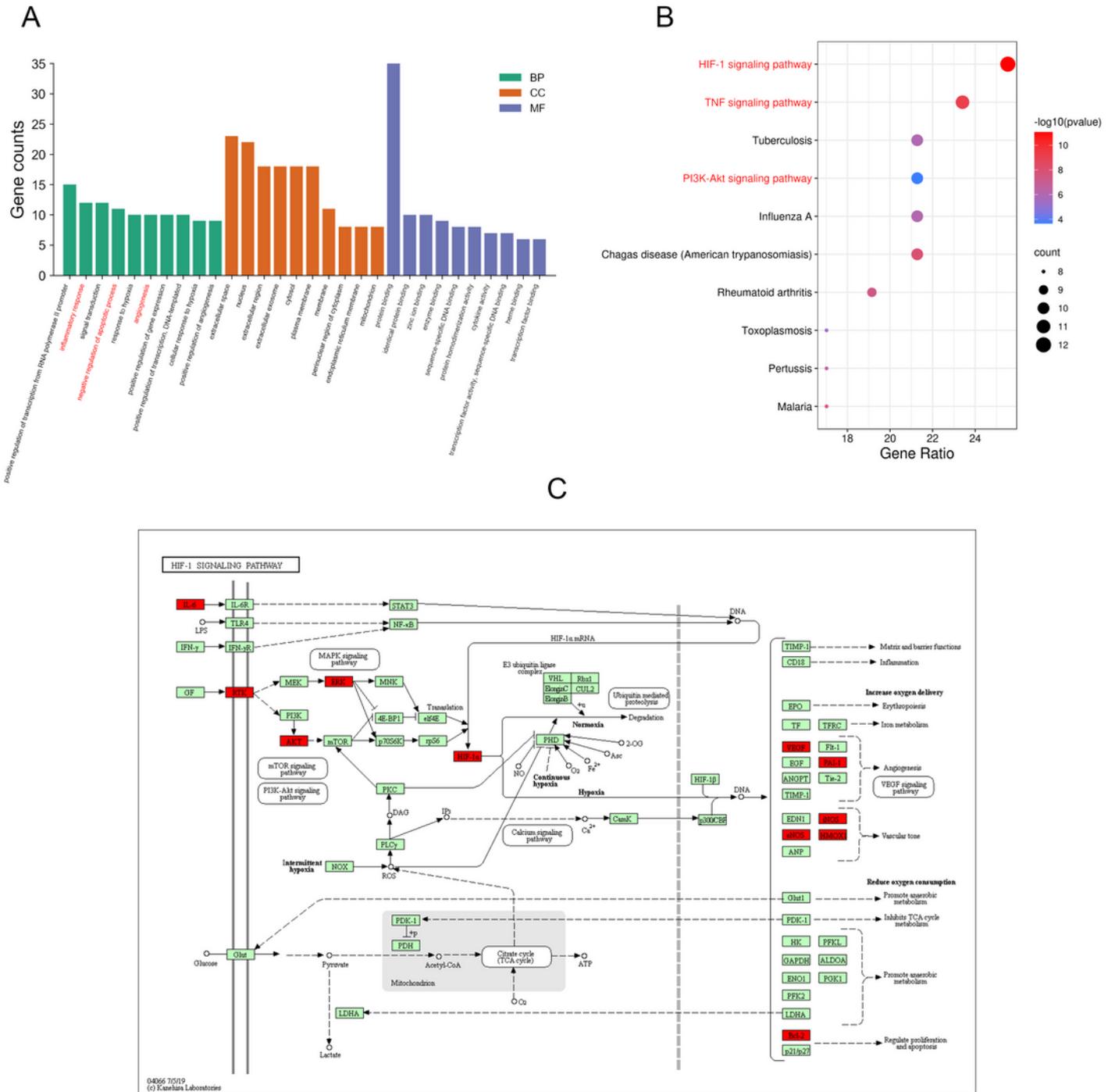


Figure 4

Enrichment analysis and HIF-1 signaling pathway. (A) GO enrichment analysis. The top 10 components for each module are presented. BP: biological processes, CC: cell component, MF: molecular function. The vertical axis number represents the number of gene enrichment sites. **(B)** The top 10 pathways of KEGG pathway analysis. The horizontal axis number represents the number of gene enrichment sites. **(C)** HIF-1 signaling pathway. The red nodes represent overlapping targets.

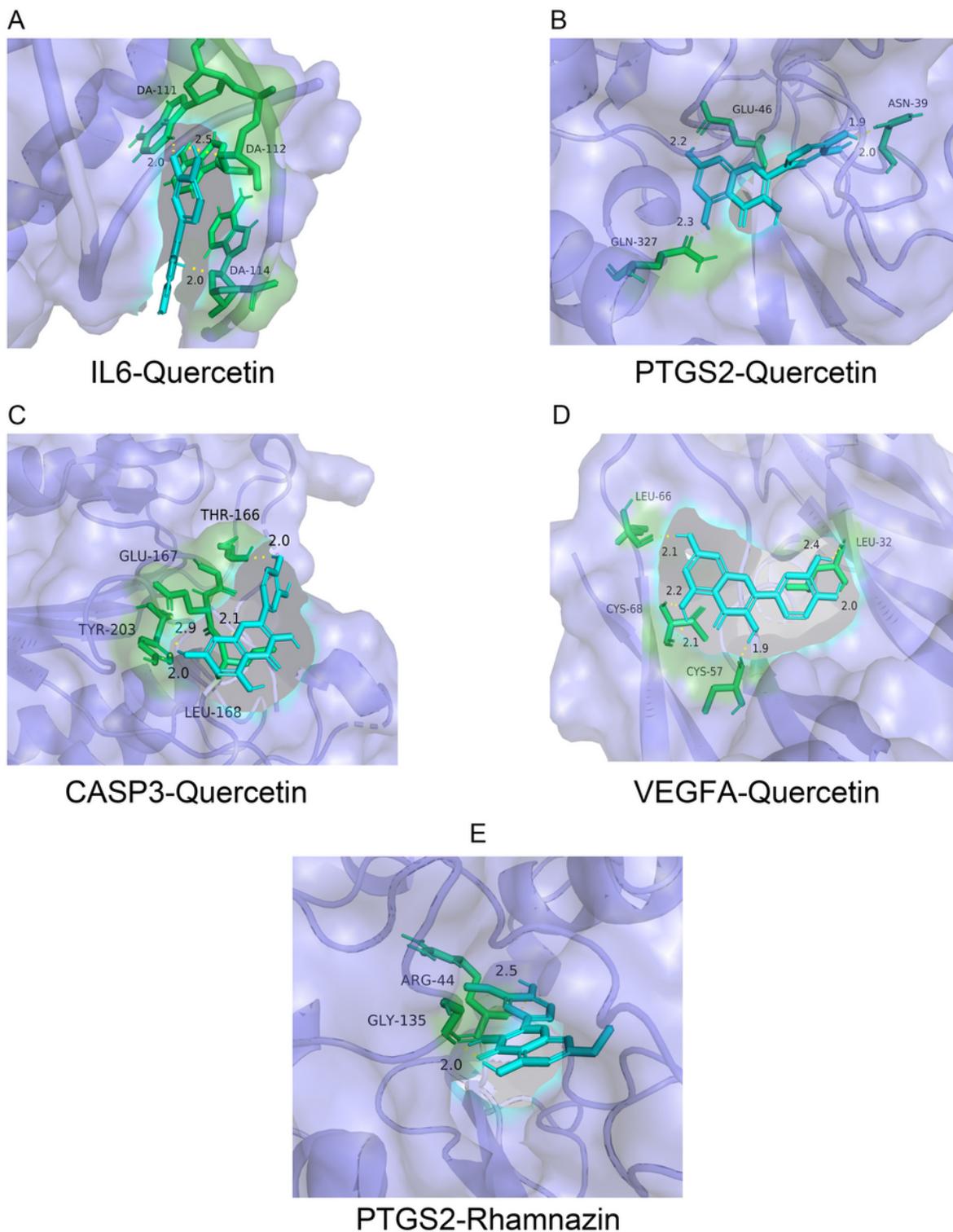


Figure 5

Molecular docking for active ingredients binding to targets. (A) IL-6-Quercetin. **(B)** PTGS2-Quercetin. **(C)** CASP3-Quercetin. **(D)** VEGFA-Quercetin. **(E)** PTGS2-Rhamnazin. The active ingredients are indicated by stick models with blue colored. The surrounding amino acid residues are shown through surface. The amino acids that interact with the compound are shown as stick models with green colored. The yellow

dashed lines represent hydrogen bonds and the numbers beside to the hydrogen bonds represent the interaction distances.