Research Article

Based on Network Pharmacology Tools to Investigate the Molecular Mechanism of Cordyceps sinensis on the Treatment of Diabetic Nephropathy

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Background. Diabetic nephropathy (DN) is one of the most common complications of diabetes mellitus and is a major cause of end-stage kidney disease. Cordyceps sinensis (Cordyceps, Dong Chong Xia Cao) is a widely applied ingredient for treating patients with DN in China, while the molecular mechanisms remain unclear. This study is aimed at revealing the therapeutic mechanisms of Cordyceps in DN by undertaking a network pharmacology analysis.

Materials and Methods. In this study, active ingredients and associated target proteins of Cordyceps sinensis were obtained via Traditional Chinese Medicine Systems Pharmacology Database (TCMSP) and Swiss Target Prediction platform, then reconfirmed by using PubChem databases. The collection of DN-related target genes was based on DisGeNET and GeneCards databases. A DN-Cordyceps common target interaction network was carried out via the STRING database, and the results were integrated and visualized by utilizing Cytoscape software. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were performed to determine the molecular mechanisms and therapeutic effects of Cordyceps on the treatment of DN.

Results. Seven active ingredients were screened from Cordyceps, 293 putative target genes were identified, and 85 overlapping targets matched with DN were considered potential therapeutic targets, such as TNF, MAPK1, EGFR, ACE, and CASP3. The results of GO and KEGG analyses revealed that hub targets mainly participated in the AGE-RAGE signaling pathway in diabetic complications, TNF signaling pathway, PI3K-Akt signaling pathway, and IL-17 signaling pathway. These targets were correlated with inflammatory response, apoptosis, oxidative stress, insulin resistance, and other biological processes.

Conclusions. Our study showed that Cordyceps is characterized as multicomponent, multitarget, and multichannel. Cordyceps may play a crucial role in the treatment of DN by targeting TNF, MAPK1, EGFR, ACE, and CASP3 signaling and involved in the inflammatory response, apoptosis, oxidative stress, and insulin resistance.

1. Introduction

Diabetic nephropathy (DN) is featured as hyperglycemia, hyperfiltration, proteinuria, and progressive renal function decline, which can cause end-stage kidney disease [1]. It accounts for about 40% of chronic kidney disease worldwide and is undoubtedly a medical challenge worldwide [2], in terms of high incidence, multifactorial pathogenesis, and the absence of practical methods in the diagnosis and treatment [3]. Traditional Chinese medicine (TCM) guided by the unique theory provides an effective treatment of complex chronic diseases via multicomponent, multitarget, and multi-pathway [4]. In recent years, Chinese herb medicine has been commonly utilized to alleviate and reverse diabetes and its complications, such as DN in clinical practice and scientific researches, which has been considered a beneficial supplement...
of the drug therapy for DN [4]. Cordyceps, a traditional Chinese herbal medicine, is reported to have multiple health-promoting characteristics, including anti-inflammatory, anti-cancer, anti-diabetic, analgesic, antioxidant, antiallergic, and antiobesity [5]. Also, Cordyceps has been reported to have broad pharmacological effects on DN by inhibiting the epithelial-mesenchymal transition, alleviating oxidative stress, repressing inflammation, modulating gut microbiota dysbiosis, and activating autophagy [6–9]. However, the pharmacological mechanisms of Cordyceps associated with DN only focus on a single chemical molecule. Accordingly, a comprehensive and systematic evaluation of the molecular mechanisms of Cordyceps on DN is indispensable.

This study is aimed at analyzing the pharmacological mechanisms of active ingredients of Cordyceps involved in the progression of DN via using the network pharmacology databases and biological analysis methods. It laid a stable foundation for further research on exploring pharmacological mechanisms of Cordyceps in treating DN. The study framework is showed in Figure 1.

2. Materials and Methods

2.1. Establishment of Active Ingredients and Correlated Target Database. TCMSP (http://lsp.nwu.edu.cn/tcmsp.php), a systematic pharmacological platform that contains the relationships among herbal compounds, related targets, and diseases [10], was applied to identify the chemical constituents of Cordyceps. The active components were selected based on oral bioavailability (OB) and drug likeness (DL) values, and the ingredients were captured when the OB was ≥30% and the DL ≥ 0.18 (a screening threshold of TCMSP database) [10]. The chemical formulas of components were confirmed by PubChem (https://www.ncbi.nlm.nih.gov/pccompound) to double-check the final compounds of Cordyceps [11]. The targets associated with Cordyceps components were further identified based on the TCMSP database and Swiss Target Prediction (http://www.swistargetprediction.ch/), a webserver to accurately identify the targets of active molecules [12]. The genes corresponding to the protein targets were obtained from the UniProt database.

2.2. Network Construction of Active Components-Potential Targets. A comprehensive network was constructed via using Cytoscape software to reflect the intricate relationship between active compounds and putative targets [13]. Nodes represent the components and targets, while edges reveal the interactions between components and targets (Figure 2).

2.3. Selection of Potential DN Targets. The keyword “diabetic nephropathy” was inputted in the GeneCards (https://www.genecards.org/), a human gene compendium with information about genomics, proteomics, and transcriptomics [14], and DisGeNET (https://www.disgenet.org/home/), a comprehensive platform including one of the largest publicly accessible collections of genes, to search for DN-associated targets [15].

2.4. Screening Compound-Disease Overlapping Targets. The screened compound targets and disease targets were imported into Funrich, a software used mainly for functional enrichment and interaction network analysis of genes and proteins for analysis [16]. The common targets of compound-disease were obtained as the potential targets for further analysis (Figure 3).

2.5. Network Construction of Compound-Disease Common Targets. A protein-protein interaction (PPI) network was obtained based on the STRING platform (https://string-db.org/), which covers nearly all functional interactions among the expressed proteins [17]. Target interaction information derived from the STRING database was imported into the Cytoscape (version 3.7.1; https://www.cytoscape.org/) software where the interaction information was integrated and analyzed.

2.6. GO and KEGG Pathway Enrichment Analyses. GO is the most comprehensive and widely used knowledgebase for the classification of gene functions, including the biological process (BP), molecular function (MF), and cell component (CC) [18]. KEGG (http://www.kegg.jp/) is an encyclopedia of genes and genomes; connecting genomic information to higher-order functional information to capture significantly enriched biological pathways is the major objective of the KEGG knowledgebase [19].

In our study, GO functional annotation and KEGG pathway enrichment analysis were carried out via using the ClusterProfiler package of R software, and \( P < 0.05 \) was employed as a screening threshold.

3. Results

3.1. Active Ingredients of Cordyceps. The active chemical components of Cordyceps were selected via the TCMSP databases, and 38 compounds were collected with the thresholds of OB ≥ 30% and DL ≥ 0.18 properties. Finally, 7 candidate ingredients were screened out for further study (Table 1).

3.2. Compound-Target Network Construction. In order to visualize the interaction relationship between the Cordyceps ingredients and corresponding targets, we established a compound-target network (Figure 2). By mapping 7 components to 293 potential targets, the network comprises 300 nodes and 500 edges, in which the red circles correspond to the putative targets and Cordyceps ingredients are in green. Chemical compound arachidonic acid, cerevisterol, betasitosterol, linoleyl acetate, cholesteryl palmitate, CLR, and peroxyerygosterol correspond to 133, 100, 77, 57, 53, 49, and 38 compounds, respectively. The results suggest that these 7 components probably serve significant therapeutic roles in DN.

3.3. Predicting DN-Related Targets. By retrieving the DisGeNET and GeneCards databases, results were integrated to obtain the DN-associated targets. As shown in Figure 3, the potential target genes in Cordyceps were matched to the DN-associated target gene by using the Funrich platform and was shown as a Venn diagram. Finally, 85 putative targets were selected according to the intersection of component
Cordyceps
TCMSP database
OB+DL screening
TCMSP database+Swiss target prediction
Targets of cordyceps

Diabetic nephropathy
DisGeNET and GeneCards databases
Targets of diabetic nephropathy

Compound-target network
Cytoscape
Funrich
Network construction of dordyceps and DN common targets
GO and KEGG pathway enrichment
Functional enrichment analysis

Figure 1: A flow chart based on an integration strategy of network pharmacology.

Figure 2: Compound-target network of putative targets and active Cordyceps ingredients. The green nodes represent the active components of Cordyceps, and nodes in red represent the corresponding targets of the ingredients.
targets and DN-related targets; one target was excluded because it had no interaction with other targets (Table 2).

3.4. Common Target Network. 85 putative genes correlated with DN were imported to the STRING database for analysis and network establishment. The interaction network was based on the selected targets with a medium confidence score of 0.400 (Figure 4). A total of 84 nodes and 767 edges were embodied, and the average node degree is 18.3 after analysis. The results were imported to the Cytoscape software for further analysis and network construction (Figure 5). In this figure, the edges represent the interaction between a pair of potential targets, while the nodes represent the targets, and the degree value indicates the intensity of target interaction.

3.5. GO and KEGG Pathway Enrichment Analyses. After using the ClusterProfiler package for pathway analysis, a total of 1843 biological processes were enriched. The top 10 remarkably enriched BP terms were selected for analysis, including regulation of inflammatory response, response to lipopolysaccharide, response to molecule of bacterial origin, response to nutrient levels, muscle cell proliferation, response to oxygen levels, cellular response to chemical stress, regulation of lipid metabolic process, and lipid localization. Besides, 32 cell components were enriched, and the top 10 entries were screened, consisting of membrane raft, membrane microdomain, membrane region, RNA polymerase II transcription regulator complex, transcription regulator complex, caveola, vesicle lumen, plasma membrane raft, mitochondrial outer membrane, and region of cytosol. Furthermore, a total of 118 molecular functions were enriched; the top 10 entries were selected, containing steroid hormone receptor activity, monooxygenase activity, carboxyl binding, carboxyl binding, fatty acid binding, organic acid binding, steroid binding, nuclear receptor activity, and long-chain fatty acid-binding. These processes are of great significance to further understand the curative mechanism of Cordyceps on the treatment of DN. The results of the GO analysis are illustrated in Figure 6.

In terms of KEGG analysis, a total of 118 pathways were obtained. The top 20 significantly enriched pathways were screened out based on the threshold of $P < 0.05$ (Figure 7). The results indicated that these genes were mainly associated with the inflammatory signaling pathway, apoptosis, oxidative stress, and insulin signaling pathway, including AGE-RAGE signaling pathway in diabetic complications, TNF signaling pathway, apoptosis, IL-17 signaling pathway, PI3K-Akt signaling pathway, and insulin resistance. The results prove that Cordyceps may alleviate DN by regulating insulin resistance, apoptosis, and inflammatory reaction.

To more intuitively demonstrate which pathway each target is involved in, the target pathway data captured from KEGG analysis was uploaded into Cytoscape software for constructing a network graph of target and pathway (Figure 8).

4. Discussion

DN, featured as high incidence, multifactorial pathogenesis, and absence of practical methods for diagnosis and treatment, is undoubtedly a medical challenge worldwide. The etiology of DN is multifactorial; with hyperglycemia, oxidative stress, and advanced glycation end products (AGE) as the leading factors, chronic inflammation and infiltrated immune cells in renal tissue are considered to be the common pathological consequences [20, 21]. Despite current improving therapies, there is still a considerable residual risk of DN onset and progression [22]. Some relevant studies highlight new perspectives of TCM for delaying DN progression and strengthen the therapeutic rationale for TCM on the treatment of DN [23, 24]. Cordyceps contains several active ingredients which affect multiple targets and pathways in the progression of DN and has been used as an adjuvant on the treatment of DN in China for a long time [7, 25]. In this study, we selected 7 active ingredients from Cordyceps based on the OB and DL, including linoleyl acetate, arachidonic acid, cholesteryl palmitate, CLR, beta-sitosterol, cerevisisterol, and peroxyergosterol. Some compounds of Cordyceps were reported to have the effect on ameliorating endocrine and metabolic disorders during the development of DN [26–29]. For instance, it was reported that beta-sitosterol protects the expression of insulin signaling molecules through activating insulin receptor and glucose transporter 4 in the adipose tissue with a high-fat diet, which can slow the development of DN [29]. Arachidonic acid is a strong inducer of insulin secretion and it can attenuate DN by inhibiting the TGF-β/Smad signaling pathway [26, 30]. In addition, arachidonic acid also can facilitate the production of anti-inflammatory lipoxins which were reported to improve insulin sensitivity.
Table 2: Information of putative targets and topological attributes.

| No. | UniProt ID | Protein names                                      | Gene names | Degree |
|-----|------------|----------------------------------------------------|------------|--------|
| 1   | P01375     | Tumor necrosis factor                              | TNF        | 51     |
| 2   | P28482     | Mitogen-activated protein kinase 1                 | MAPK1      | 45     |
| 3   | P01133     | Proepidermal growth factor                         | EGF        | 45     |
| 4   | P00533     | Epidermal growth factor receptor                   | EGFR       | 45     |
| 5   | P42574     | Caspase-3                                          | CASP3      | 45     |
| 6   | P04637     | Cellular tumor antigen p53                         | TP53       | 44     |
| 7   | P35354     | Prostaglandin G/H synthase 2                       | PTGS2      | 41     |
| 8   | P14780     | Matrix metalloproteinase-9                         | MMP9       | 40     |
| 9   | P29474     | Nitric oxide synthase, endothelial                | NOS3       | 40     |
| 10  | P05412     | Transcription factor AP-1                          | JUN        | 40     |
| 11  | P37231     | Peroxisome proliferator-activated receptor gamma   | PPARG      | 37     |
| 12  | P06484     | Phosphatidylinositol 3,4,5-trisphosphate and dual-specificity protein phosphatase | PTEN | 37     |
| 13  | P45983     | Mitogen-activated protein kinase 8                 | MAPK8      | 36     |
| 14  | Q04206     | Transcription factor p65                           | RELA       | 35     |
| 15  | P35968     | Vascular endothelial growth factor receptor 2      | KDR        | 33     |
| 16  | Q16539     | Mitogen-activated protein kinase 14                | MAPK14     | 33     |
| 17  | P08253     | 72 kDa type IV collagenase                         | MMP2       | 32     |
| 18  | P42345     | Serine/threonine-protein kinase mTOR               | MTOR       | 32     |
| 19  | P03372     | Estrogen receptor                                  | ESR1       | 31     |
| 20  | P12821     | Angiotensin-converting enzyme                       | ACE        | 28     |
| 21  | P01137     | Transforming growth factor beta-1 proprotein       | TGFBI      | 27     |
| 22  | P16284     | Platelet endothelial cell adhesion molecule        | PECAM1     | 27     |
| 23  | P42336     | Phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit alpha isoform | PIK3CA | 26     |
| 24  | P08254     | Stromelysin-1                                       | MMP3       | 25     |
| 25  | Q14790     | Caspase-8                                           | CASP8      | 25     |
| 26  | P09619     | Platelet-derived growth factor receptor beta       | PDGFRB     | 23     |
| 27  | Q00987     | E3 ubiquitin-protein ligase Mdm2                  | MDM2       | 23     |
| 28  | P19438     | Tumor necrosis factor receptor superfamily member 1A | TNFRSF1A | 23     |
| 29  | P55211     | Caspase-9                                           | CASP9      | 22     |
| 30  | Q06124     | Tyrosine-protein phosphatase nonreceptor type 11   | PTPN11     | 22     |
| 31  | P41235     | Hepatocyte nuclear factor 4-alpha                  | HNF4A      | 20     |
| 32  | P30556     | Type-1 angiotensin II receptor                     | AGTR1      | 20     |
| 33  | Q07869     | Peroxisome proliferator-activated receptor alpha   | PPARA      | 20     |
| 34  | P11511     | Aromatase                                           | CYP19A1    | 16     |
| 35  | P35228     | Nitric oxide synthase, inducible                  | NOS2       | 16     |
| 36  | P49327     | Fatty acid synthase                                | FASN       | 16     |
| 37  | O14920     | Inhibitor of nuclear factor kappa-B kinase subunit beta | IKKKB  | 16     |
| 38  | P25116     | Proteinase-activated receptor 1                    | F2R        | 15     |
| 39  | P17252     | Protein kinase C alpha type                         | PRKCA      | 15     |
| 40  | P07148     | Fatty acid-binding protein, liver                  | FABP1      | 15     |
| 41  | P15090     | Fatty acid-binding protein, adipocyte              | FABP4      | 15     |
| 42  | P16109     | P-selectin                                         | SELP       | 14     |
| 43  | P11473     | Vitamin D3 receptor                                | VDR        | 14     |
| 44  | P29350     | Tyrosine-protein phosphatase nonreceptor type 6    | PTPN6      | 14     |
| 45  | Q05477     | Phospholipid-transporting ATPase ABCA1             | ABCA1      | 14     |
| 46  | P25101     | Endothelin-1 receptor                              | EDNRA      | 13     |
| 47  | P55851     | Mitochondrial uncoupling protein 2                 | UCP2       | 12     |
Table 2: Continued.

| No. | UniProt ID | Protein names | Gene names | Degree |
|-----|------------|---------------|------------|--------|
| 48  | P48736     | Phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit gamma isoform | PIK3CG | 12     |
| 49  | P05771     | Protein kinase C beta type | PRKCB | 12     |
| 50  | Q13464     | Rho-associated protein kinase 1 | ROCK1 | 12     |
| 51  | P20333     | Tumor necrosis factor receptor superfamily member 1B | TNFRSF1B | 12     |
| 52  | P15121     | Aldo-keto reductase family 1 member B1 | AKR1B1 | 11     |
| 53  | P21554     | Cannabinoid receptor 1 | CNR1 | 11     |
| 54  | P04629     | High affinity nerve growth factor receptor | NTRK1 | 11     |
| 55  | P22894     | Neutrophil collagenase | MMP8 | 11     |
| 56  | Q99814     | Endothelial PAS domain-containing protein 1 | EPAS1 | 10     |
| 57  | P10415     | Apoptosis regulator Bcl-2 | BCL2 | 10     |
| 58  | P32246     | C-C chemokine receptor type 1 | CCR1 | 8      |
| 59  | P11413     | Glucose-6-phosphate 1-dehydrogenase | G6PD | 8      |
| 60  | P08123     | Collagen alpha-2 | COL1A2 | 8      |
| 61  | P23219     | Prostaglandin G/H synthase 1 | PTGS1 | 8      |
| 62  | Q13133     | Oxysterols receptor LXR-alpha | NR1H3 | 8      |
| 63  | O75116     | Rho-associated protein kinase 2 | ROCK2 | 8      |
| 64  | Q03181     | Peroxisome proliferator-activated receptor delta | PPARD | 7      |
| 65  | P25105     | Platelet-activating factor receptor | PTAFR | 7      |
| 66  | P27169     | Serum paraoxonase/arylesterase 1 | PON1 | 7      |
| 67  | P17706     | Tyrosine-protein phosphatase nonreceptor type 2 | PTPN2 | 7      |
| 68  | O75469     | Nuclear receptor subfamily 1 group I member 2 | NR1H2 | 6      |
| 69  | P28223     | 5-Hydroxytryptamine receptor 2A | HTR2A | 6      |
| 70  | P12104     | Fatty acid-binding protein, intestinal | FABP2 | 6      |
| 71  | P02753     | Retinol-binding protein 4 | RBP4 | 5      |
| 72  | P39900     | Macrophage metalloelastase | MMP12 | 5      |
| 73  | P08235     | Mineralocorticoid receptor | NR3C2 | 5      |
| 74  | P11597     | Cholesteryl ester transfer protein | CETP | 5      |
| 75  | P07477     | Trypsin-1 | PRSS1 | 4      |
| 76  | Q07973     | 1,25-Dihydroxyvitamin D | CYP24A1 | 4      |
| 77  | P30542     | Adenosine receptor A1 | ADORA1 | 4      |
| 78  | P18054     | Polyunsaturated fatty acid lipoygenase ALOX12 | ALOX12 | 4      |
| 79  | O00763     | Acetyl-CoA carboxylase 2 | ACCB | 4      |
| 80  | P04278     | Sex hormone-binding globulin | SHBG | 3      |
| 81  | P80365     | Corticosteroid 11-beta-dehydrogenase isozyme 2 | HSD11B2 | 3      |
| 82  | P13866     | Sodium/glucose cotransporter 1 | SLC5A1 | 2      |
| 83  | P35610     | Sterol O-acyltransferase 1 | SOAT1 | 1      |
| 84  | P05091     | Aldehyde dehydrogenase, mitochondrial | ALDH2 | 1      |
and may prevent the development of diabetes [27]. Besides, several arachidonic acid metabolites, including PGE2, PG12, and LXA4, PGE2 and PG12 can alleviate insulin resistance and improve insulin sensitivity of pancreatic cells [28]; LXA4 can inhibit the production of IL-6, TNF-α, and ROS, thus alleviating inflammation, and has an antidiabetic effect [31, 32].

We found that many targets can be regulated by multiple compounds from the compound-target network, such as CYP19A1, NOS2, NR1H3, SHBG, and PTGS2. When it comes to core targets, TNF (degree = 51), MAPK1 (degree = 51), EGFR (degree = 45), and CASP3 (degree = 45) played an important role in the process of Cordyceps in DN treatment. TNF and MAPK1 are correlated with inflammation response and deterioration of renal function [33, 34]. CASP3 is known to have an important role in the promotion of apoptotic cell death [35]. AG1478 can block EGFR signaling and inhibit oxidative stress and endoplasmic reticulum stress markers in diabetic mice [36]. In addition, inhibition of EGFR activation is associated with improved DN and insulin resistance in type 2 diabetes mouse models [37]. Therefore, the candidate targets are mainly enriched for oxidative stress, insulin resistance, apoptosis, and inflammation.

We also selected 85 common targets between the components of Cordyceps and DN for performing GO enrichment, consisting of biological processes, molecular function, and cellular components, which is aimed at predicting the mechanism of Cordyceps in treating DN. We found that the candidate targets are involved in multiple biological processes, such as regulation of inflammatory response, response to lipopolysaccharide, response to
nutrient levels, response to oxygen levels, cellular response to chemical stress, regulation of lipid metabolic process, and lipid localization. The active targets such as TNF, PPARG, MAPK1, EGFR, and TGF-β1 mainly participate in the biological processes of inflammatory response, oxidative stress, and lipid metabolic process [36, 38, 39]. Thus, the molecular processes of several targets are relatively consistent with the pathogenesis and mechanism of clinical DN. In addition, cellular components constitute membrane raft, membrane microdomain, membrane region, RNA polymerase II transcription regulator complex, and transcription regulator complex. It indirectly illustrates the complexity of the pathogenesis of DN and its damage to several cellular components, and Cordyceps may have a function in regulating these cellular components, eventually improving DN. Besides, molecular functions are mainly enriched in steroid hormone-related activity and steroid-binding, and many studies reported that steroid hormones were closely related to DN in patients with diabetes [40, 41]. It reveals that Cordyceps might target steroid hormone in DN treatment.

Using network pharmacological analysis and performing KEGG enrichment, we found that these Cordyceps components may relieve the symptoms of DN through the action of targets in various signaling pathways and multiple biological processes, including AGE-RAGE signaling pathway, TNF signaling pathway, apoptosis, IL-17 signaling pathway, PI3K-Akt signaling pathway, and insulin resistance. Activation of the receptor for AGEs or RAGE (receptor for advanced glycation end-products) is associated with the development of DN [42], which evokes oxidative stress and chronic inflammation in renal tissues, ending up in losses in kidney function by activating various intracellular signalings like PI3K/Akt/mTOR, NF-κB, MAPK/ERK, and TGF-β/Smad [43–45]. Additionally, it is believed that reactive oxygen species (ROS) can regulate PI3K/Akt/mTOR signaling and play an essential role in the development of DN, including epithelial-mesenchymal transition (EMT). During EMT,
epithelial cells lose their primary epithelial properties, such as epithelial- (E-) cadherin, while acquiring characteristics typical of mesenchymal cells such as α-SMA, ending up with renal interstitial fibrosis [46]. Moreover, PI3K/Akt/mTOR signaling can promote high glucose-induced podocyte apoptosis, which contributes to the pathogenesis of DN [47].

TNF signaling is characterized as a well-known inflammatory cytokine associated with renal injury [48]. Once stimulated by TNF-α, NF-κB moves from the cytoplasm to the nucleus and activates the transcription of VCAM-1, ICAM-1, IL-6, and IL-8, which will result in endothelial inflammatory and DN pathological process acceleration [49]. Furthermore, NF-κB can be activated by several cytokines, which in turn induces the production of TNF-α, resulting in diabetic renal damage [50].

Insulin resistance is a critical process and one of the main symptoms in the initiation and progression of DN, which is closely related to microalbuminuria [51, 52]. High levels of insulin can cause insulin receptor degradation and drive early podocyte insulin resistance, and both the insulin receptor and nephrin are needed for full insulin sensitivity of podocytes. Thus, it explains why individuals with nephropathy caused by type 2 diabetes are commonly hyperinsulinaemic in the early stage of their disease [53]. Moreover, there are lots of mediators of insulin resistance that participate in driving renal function decline, including TGF-β1, blood pressure, inflammation, TNF-α, IL-6, and oxidative stress [54–56].

Therefore, these results of network pharmacological analyses not only verify that our screened targets are consistent with previous literature reports but also indicate that Cordyceps play a significant therapeutic role in DN by regulating several signaling pathways, including inflammatory response, insulin resistance, oxidative stress, apoptosis, and other pathways with unclear mechanisms. It will provide a novel methodology for further study of the therapeutic mechanism of Cordyceps in alleviating DN.

5. Conclusion

In summary, our network pharmacological analyses have shown that Cordyceps play an indispensable supplementary role in the treatment of DN, which is consistent with previous studies. Moreover, the biological functions of active chemical molecules and their corresponding targets of Cordyceps analyzed by network pharmacological methods provide a unique and innovative path for the
study of TCM and further reveal the molecular biological mechanism of Cordyceps in treating DN. However, in vivo and in vitro experiments should be undertaken to validate the relationship between key targets and pathways of Cordyceps for the treatment of DN. Despite the limitations of this study, the results of this study provide new evidence and theoretical basis which will be used in subsequent theoretical and clinical research studies of Cordyceps.

**Data Availability**

The data used to support the findings of this study are included within the article.
Conflicts of Interest
The authors declare that they have no competing interests.

Authors’ Contributions
Yan Li and Lei Wang contributed equally to this work. All authors have contributed to this study and approve its submission.

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