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Weighted gene co-expression network analysis of key targets and interventional mechanism of Milkvetch root in diabetic nephropathy

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Abstract. – OBJECTIVE: This work aimed to explore the key targets and intervention mechanisms of Huangqi (*Astragalus membranaceus*) in diabetic nephropathy using weighted gene co-expression network analysis (WGCNA). The findings will provide references for identifying critical therapeutic targets for diabetic nephropathy.

MATERIALS AND METHODS: The GSE1009 dataset was selected from the Gene Expression Omnibus (GEO) database of the National Center for Biotechnology Information (NCBI) for analysis. WGCNA network was constructed to identify differentially expressed genes (DEGs). Gene ontology (GO) and pathway enrichment analysis were performed on the DEGs.

RESULTS: There were 752 downregulated DEGs and 1,547 upregulated DEGs in the diabetic nephropathy samples. Genes such as *PLCE1, CLIC5, PTPRO, HSPA12A, AIF1, GMDS,* and *SEMA5A* were significantly suppressed in the diabetic nephropathy samples, while genes such as *CEP152, LUNAR1,* and *SLC9A1* were significantly upregulated. The optimal soft threshold for the WGCNA network was determined as 12. Hierarchical clustering analysis was conducted to detect co-expression modules with corresponding color assignments, and a total of 9 modules were identified. Clinical characteristics showed a high correlation with the gray, blue, green, and brown modules of the WGCNA. GO analysis and KEGG pathway enrichment analysis revealed that the blue module DEGs were mainly enriched in immune response, inflammatory response, signal transduction, plasma membrane, extracellular region, cell surface, extracellular matrix, and proteinaceous extracellular matrix. The green module DEGs were mainly enriched in mitochondrial elongation, mitochondrial mutation termination, translation, mitochondrial inner membrane, mitochondrion, ATP biosynthetic process, mitochondrial large ribosomal subunit, mitochondrial intermembrane space, nucleolus, and ribosome. Visualization analysis of the bioactive components of Huangqi showed compounds such as quercetin, resveratrol, 7-O-methylisomucronulatol, and isoquercetin, which had more targets.

CONCLUSIONS: Differentially expressed genes in diabetic nephropathy were mainly enriched in immune response and inflammatory response. Various components of Huangqi have positive application value in the treatment of diabetic nephropathy and can be considered for clinical promotion.

Key Words:
Diabetic nephropathy, Weighted gene co-expression network analysis, Milkvetch root, Key target, Interventional mechanism.

Introduction

Diabetic nephropathy (DN) is a common complication among patients with diabetes that seriously affects quality of life[1]. Proteinuria, hematuria, hypertension, and renal function impairment usually occur among DN patients. The above symptoms have adverse effects on their physiological functions[2,3]. DN patients suffer from albuminuria and gradual loss of renal function in clinical treatment. Patients with advanced DN suffer from renal failure, which greatly increases the mortality[4]. At present, the prevalence of diabetes among global patients is rapidly growing, especially in developing countries. With the rise in the prevalence of diabetes, the complications of DN should be actively prevented and treated. If clinical strategies are not improved and prevention and treatment effects are poor, the prevalence of DN will still continuously increase[5,6]. DN is the severest complication caused by diabetes and poses a serious threat to patients' lives. The mortality of diabetes patients with complicated DN is approximately 30 times higher than that of patients without complicated DN[7,8]. DN is closely associated with cardiovascular diseases. DN causes hyperglycemia, further leads to vascular dysfunction and complicated cardiovascular diseases, and aggravates the disease and its severity. As a re-

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sult, mortality increases[9,10]. Besides, oxidative stress often occurs among DN patients, which results in inflammatory cell infiltration. Even worse, fibrosis comes into being[11]. Understanding the key characteristics of inflammation mechanisms related to the occurrence and development of DN has positive clinical significance in identifying new potential intervention targets and formulating and implementing more advantageous and targeted anti-inflammation strategies[12]. During the occurrence and development of DN, the molecular level changes abnormally. Therefore, it is necessary to detect the changes of abnormal molecular markers to provide a reference for the diagnosis and treatment of disease[13,14]. The combined application of multi-omics data further improves the diagnostic and prognostic indicators for DN and predicts the treatment mechanism of disease[15]. It has positive clinical values.

Deficiency of both qi and yin is the main symptom of DN. When disease is aggravated, deficiency of both yin and yang occurs and develops into deficiency of qi and blood, as well as yin and yang[16,17]. During the treatment of DN, benefiting qi and nourishing yin are frequently applied. As a traditional Chinese drug used for benefiting qi and nourishing yin, milkvetch root possesses positive application values in the prevention and treatment of DN[18,19]. During traditional biological research, a single gene or transcriptome is selected as the research object for analysis. The mechanism of life is revealed at a molecular level, and local characteristics of biological systems are analyzed and explained. However, the overall behaviors and characteristics of biological systems can hardly be comprehensively and systematically investigated and analyzed. The interaction between different biomolecules can be reflected at the system level based on the analysis of biological networks. Nonetheless, complex biological phenomena cannot be analyzed[20,21]. A biological network is a systematic and intuitive connection network that links the relationship between different body positions and physiological functions. In addition, it can perform an overall study on organisms from the macroscopic and systematic points of view[22]. The similarity of gene expressions can be overall investigated by weighted gene co-expression network analysis (WGCNA). Moreover, gene modules are collated and identified. According to the correlation among different gene expression profiles, the co-expression modules in multiple biological samples are identified, and the co-expression modules with high correlation are searched after phenotypic correlation[23]. Unlike other co-expression analysis methods, soft threshold is employed by WGCNA network to provide the sensitivity of network for module identification and it is widely applied in the analysis of biological co-expression mode. Besides, soft threshold can extract and integrate complex contents in data and compare them with sample characteristics. After that, it integrates relevant genes to form co-expression modules for corresponding investigation and analysis[24,25].

In the research, the key targets and interventional mechanism of milkvetch root in DN were investigated. WGCNA was employed to explore the gene expressions of DN samples and normal control samples to search for the key genes that caused the occurrence of DN and explain the molecular network mechanism of DN progression and the drug targets of the treatment of DN with milkvetch root. Besides, the research was implemented to provide theoretical basis for the clinical discovery of therapeutic targets for DN at molecular level.

Materials and Methods

Source of Research Data

Gene expression analysis data were obtained from the high-throughput gene expression omnibus (GEO) database. DN-related chip data were selected, and the GSE1009 dataset was finally selected as the research objects, according to research objects and sample size. There were 3 DN samples and 2 normal control human body samples in the database. The samples consisted of 3 females and 2 males with an average age of 43.67±6.54. The course of diabetes was 8.32±2.17 years. The samples in the dataset were from DN patients. After that, a high-throughput chip was employed for detection and analysis. The dataset was divided into the DN group (accession number: GSM15968, GSM15969, and GSM15970) and the control group (accession number: GSM15965 and GSM15966). Relevant gene records with $p<0.05$ were selected and included in WGCNA.

Data Pre-Processing

After gene data were obtained, they needed to be pre-processed. Besides, abnormal expression data caused by experimental techniques should be eliminated to ensure that all selected gene data had biological significance.

Data Filtering

Gene expression yielded negative data values or zero, while negative numbers and zero could not be logarithmized. Hence, these unusable data needed to be filtered out.

Data Logarithm

After the corresponding research data were selected, a logarithm was taken to analyze whether gene expressions changed.

Estimation of Missing Values

Missing values were imputed by the k-nearest neighbor value weighting method. The method was employed to process large data. Inference and analysis were carried out to impute missing data based on established data.

Selection of Differentially Expressed Genes (DEGs)

In the research, gene expression profile data at the genome level were obtained from the database for the background correction of original data. In addition, an independent sample *t*-test and multiple methods were adopted to obtain DEGs. The analysis tool of the gene database was employed to compare and analyze the DEGs of DN samples and normal control samples. The screening conditions were set as the corrected *p*-value lower than 0.05 and the absolute value of the multiple of log gene expression differentiation. To investigate the internal correlation and interaction between genes, DEGs should be screened first. In the research, DEGs of DN and control samples were selected.

Limma method was commonly used for the screening of DEGs from small samples. It possessed great advantages in data statistics and analysis and data analysis time and was not affected by genes with small variations and limited by sample size. Hence, it was widely applied and adopted in the research. "Flash Clust" in R language pack (Macromedia., San Francisco, California, USA) was utilized for the clustering analysis of included samples. The "Pick Soft Threshold" function was employed to adjust the weight of weighted coefficient β. The matrices that were correlated and adjacent to each other were calculated as topological overlap matrices (TOM) by WGCNA (Shanghai Bohao Biotechnology Co., Ltd., Shanghai, China). After that, dissimilarity – set as a distance measurement criterion for gene hierarchical clustering – was calculated. Consequently, identification modules were obtained. Highly similar modules were marked and merged by clusters. Next, "Plot Dendro and Color" function was employed to visualize gene module and the target genes in the module were selected to draw heat map. Finally, the genes in the modules closely correlated with severe burn were searched and the clustering analysis was performed on relationship heat map based on clinical characteristics.

Basis of WGCNA Algorithm

Gene co-expression network was constructed by WGCNA method, and the similarity of gene expression characteristics was used to construct Pearson's correlation coefficient matrix-based similarity matrix. After that, it was transformed into adjacency matrix to calculate soft threshold, which was more authentic and applicable. The process of WGCNA analysis is displayed in Figure 1.

At first, the similarity matrix of gene co-expression was defined. Then, the co-expression network was utilized to construct samples and matrix *Z* of relevant gene expressions (Figure 2). It was assumed that a referred to gene and b represented the detected sample size value. The matrix was expressed as equation (1) below.

$$Z = \{z_{ab}\} = \{z_1, z_2, \ldots, z_n\} \quad (1)$$

The similarity coefficient of gene co-expression was shown in equation (2) and the correlation matrix was denoted by equation (3) below.

$$S_{ab}^{\text{unsigned}} = |cor(a,b)| \quad (2)$$

$$S_{ab}^{\text{unsigned}} = (1+|cor(a,b)|)/2 \quad (3)$$

Adjacency function was defined. Power-exponential adjacency function was used in the research. β referred to soft threshold. The adjacency coefficient was K_{ab} [equation (4)].

$$K_{ab} = power(S_{ab}, \beta) \equiv |S_{ab}|^{\beta} \quad (4)$$

Correlation matrix was transformed into adjacency matrix [equation (5)].

$$W = [w_{ab}] \quad (5)$$

The dissimilarity between nodes was determined. The correlation between genes could be calculated by TOM, which had positive biological significance. The calculation method for unweighted network by TOM zab was presented in equation (6). x_{ab} referred to the sum of products of adjacency coefficients of nodes jointly connected genes a and b [equation (7)]. k_q represented the sum of adjacency coefficients of all nodes connected by single gene [equation (8)]. The equation was extended to weighted network to form topological matrix.

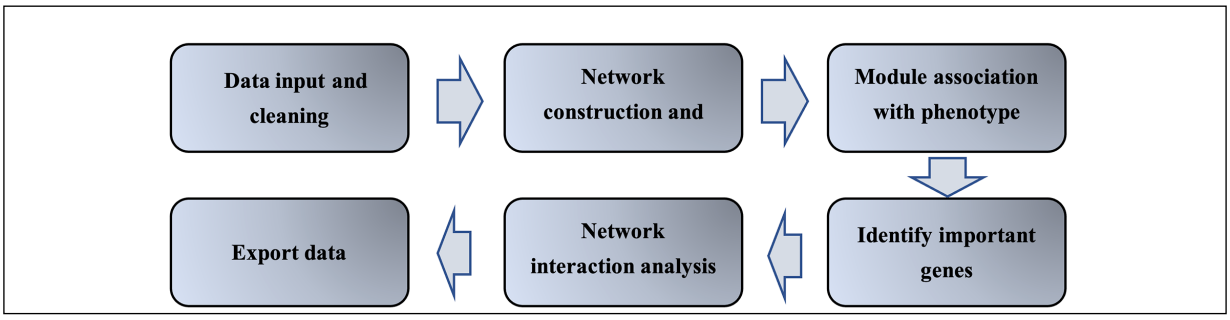


Figure 1. Process of WGCNA analysis.

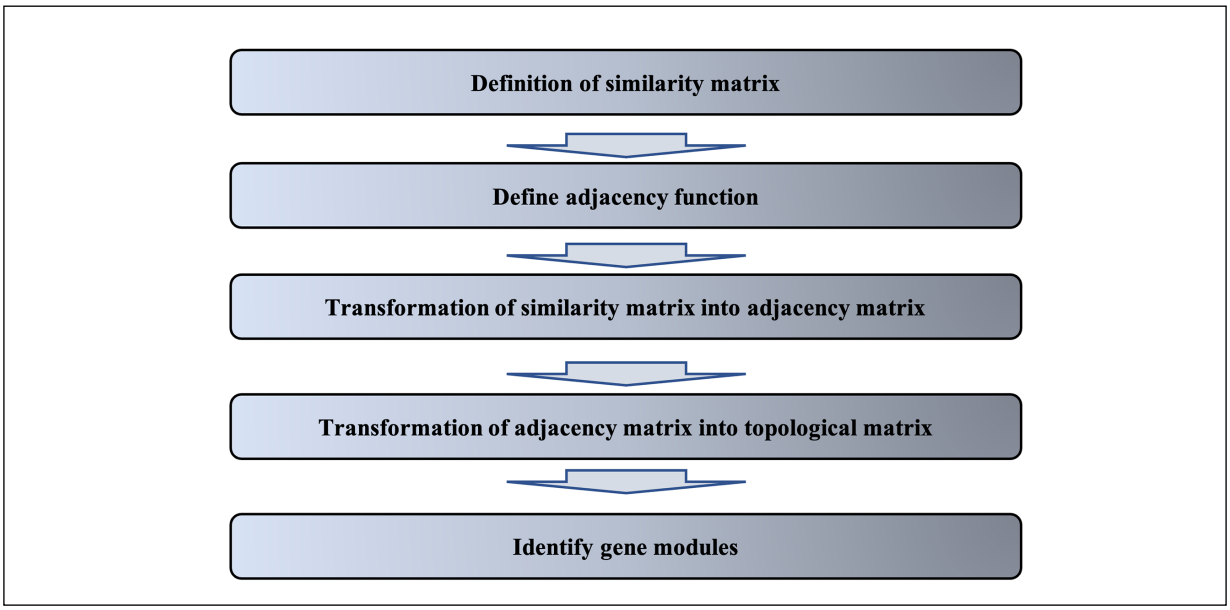


Figure 2. Basic procedures for constructing weighted gene co-expression network.

In this case, the degree of the dissimilarity between nodes was more practical.

$$z_{ab} = x_{ab+s_{ab}} / (\min\{k_a, k_b\}+1-s_{ab}) \quad (6)$$

$$x_{ab} = \sum us_{au}\, s_{ub} \quad (7)$$

$$k_a = \sum us_{au} \quad (8)$$

The similarity of co-expression of the definition of the absoluteness of correlation coefficient was shown in equation (9). The similarity values of gene a and b expression profiles ranged between 0 and 1.

$$Similarity_{ab} = |cor(n_a, n_b)| \quad (9)$$

Based on WGCNA, TOM was used to calculate the obtained dissimilarity for hierarchical clustering to obtain different gene modules of different branches. Highly correlated modules were searched by gene co-expression network based on systematic biological method. The weighted approach could be employed to construct co-expression network and search for hub genes in the modules of interest. Hub genes could be set by threshold or searched with the function called network screening.

Analysis of Gene Ontology (GO) and Pathway Enrichment of DEGs

GO could be adopted for the functional annotation of genes. The analysis of GO functional enrichment included molecular function (MF), biological process (BP), and cell component (CC). In the research, gene set enrichment analysis software (Beijing Zhongkangbo Biotechnology Co., Ltd., Beijing, China) and profiler online tool (Shenzhen Miluo Technology Co., Ltd., Shenzhen, China) were employed for the

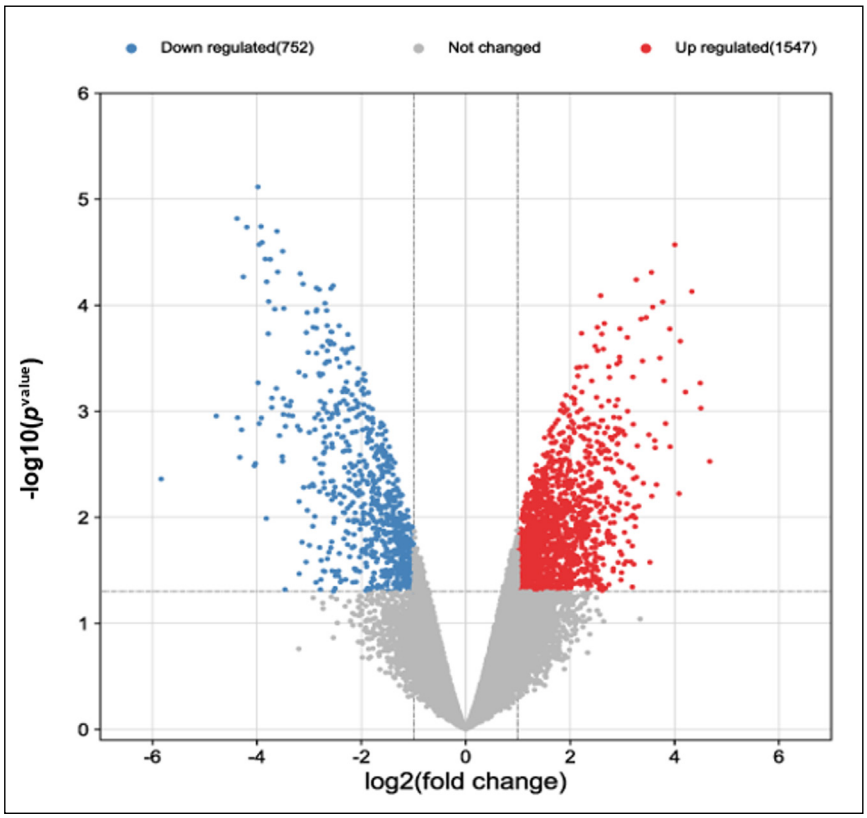


Figure 3. Volcano plot of DEGs. Gray, blue, and red areas represented the genes without statistical significance, low-expressed genes with a statistical difference, and high-expressed genes with statistical significance, respectively.

analysis and annotation of GO and Kyoto encyclopedia of genes and genomes (KEGG) enrichment of the genes in modules.

Statistical Analysis

SPSS 22.0 (IBM Corp., Armonk, NY, USA) was used for experimental data processing and statistical analysis. The differences in gene expressions between groups were compared by independent sample *t*-test and denoted by mean±standard deviation. $p<0.05$ indicated that the difference revealed statistical significance.

Results

Analysis of DEGs

12,622 DEGs were selected from the database, and the volcano plot of DEGs is shown in Figure 3. It was demonstrated there were no outlier samples in the clustering results of the genes screened from the dataset.

Hence, they could be included in the subsequent WGCAN analysis. Unlike those in control samples, there were 752 down-regulated DEGs and 1,547 up-regulated DEGs in DN samples. *PLCE1, CLIC5, PTPRO, HSPA12A, AIF1, GMDS,* and *SEMA5A* were significantly inhibited, while *CEP152, LUNAR1,* and *SLC9A1* were remarkably up-regulated. The number of up-regulated and down-regulated DEGs is displayed in Figure 4.

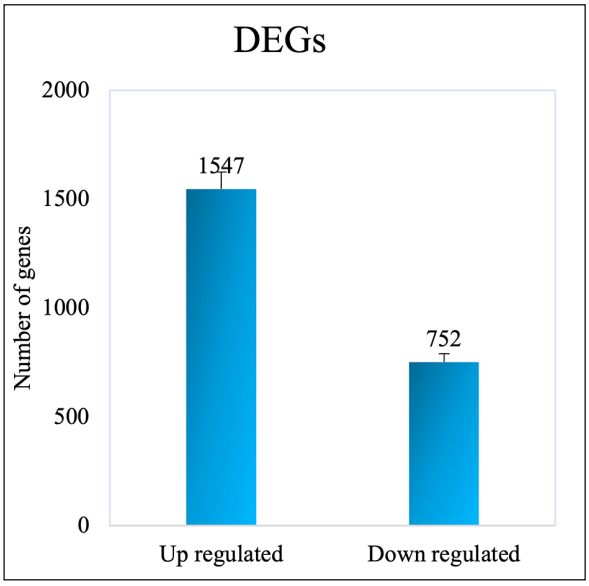


Figure 4. Statistical analysis of the number of DEGs.

WGCNA

Figures 5 and 6 represent the box plots of the analysis of the difference values of log2fc and *p*-value, respectively. It was suggested that no abnormal values existed, and all sample data could be included in the scope of the research. Besides, the determination of soft thresholds of the WGCNA network is illustrated in Figures 7 and 8.

WGCNA Network-Based Clinical Correlation Analysis

The correlation between external information and network modules was searched in the gene co-expression network. Furthermore, highly similar network modules were searched. It was assumed that the squared value of the relevant coefficient between log (k) and log [*p*(k)] was greater

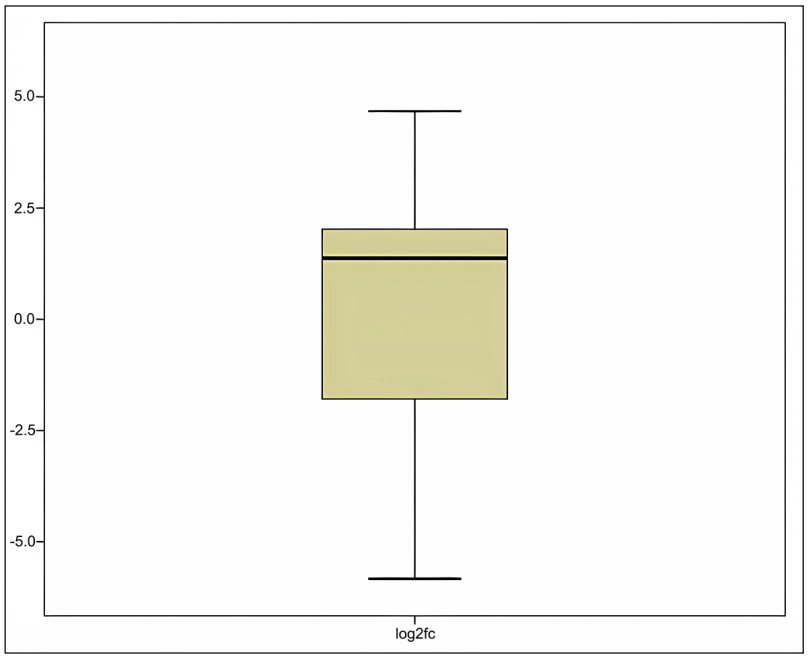


Figure 5. Box plot of the analysis of the difference values of log2fc.

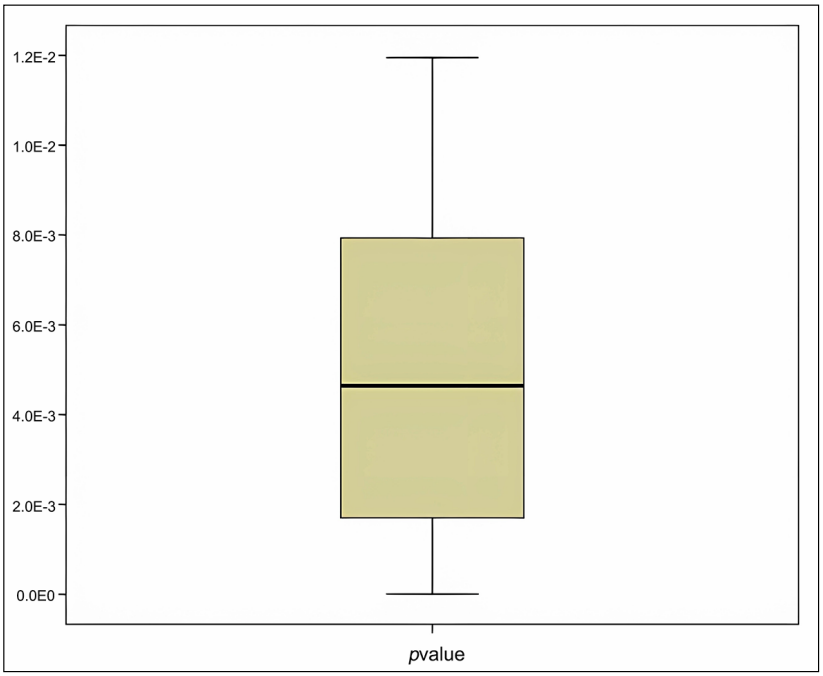


Figure 6. Box plot of the analysis of the difference values of the *p*-value.

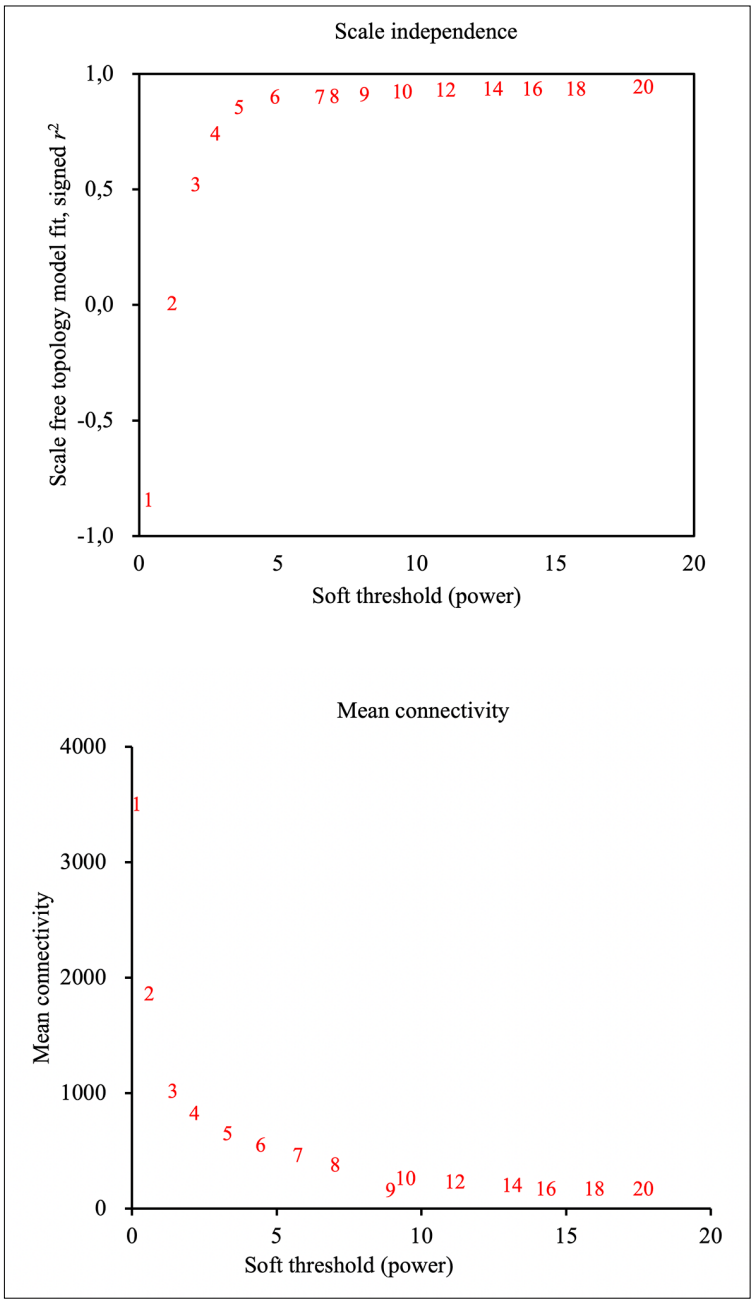


Figure 7. Determination of soft thresholds of WGCNA network.

than 0.9 when β=12 in the WGCNA network. The constructed WGCNA network is shown in Figure 9. In addition, hierarchical clustering analysis was carried out to detect the co-expression clusters with corresponding color assignments. A total of 9 modules were identified, and each of them was represented by one color.

After that, the correlation heat map and clustering analysis of WGCNA network modules were constructed (Figure 10). Apparently, 9 corresponding modules were screened and obtained, and clinical characteristics were closely associated with gray, blue, green, and brown modules in WGCNA modules.

WGCNA Network-Based Analysis of DEGs in DN

Hub DEGs were selected based on the WGCNA network. GO and KEGG were adopted to perform the functional annotation of DEGs

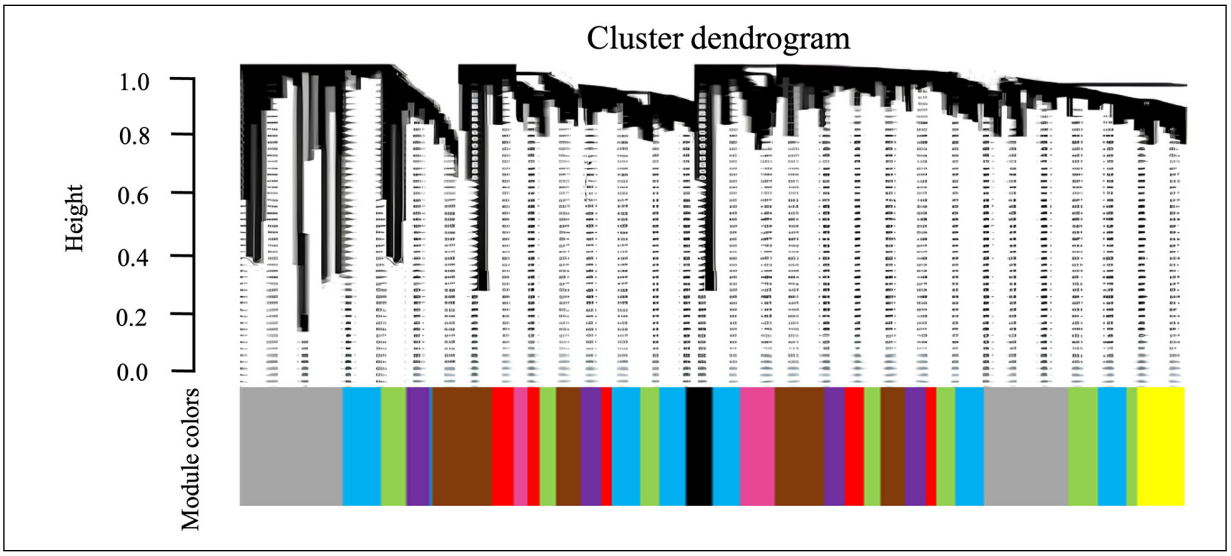


Figure 8. WGCNA network modules of DEGs in DN and normal samples.

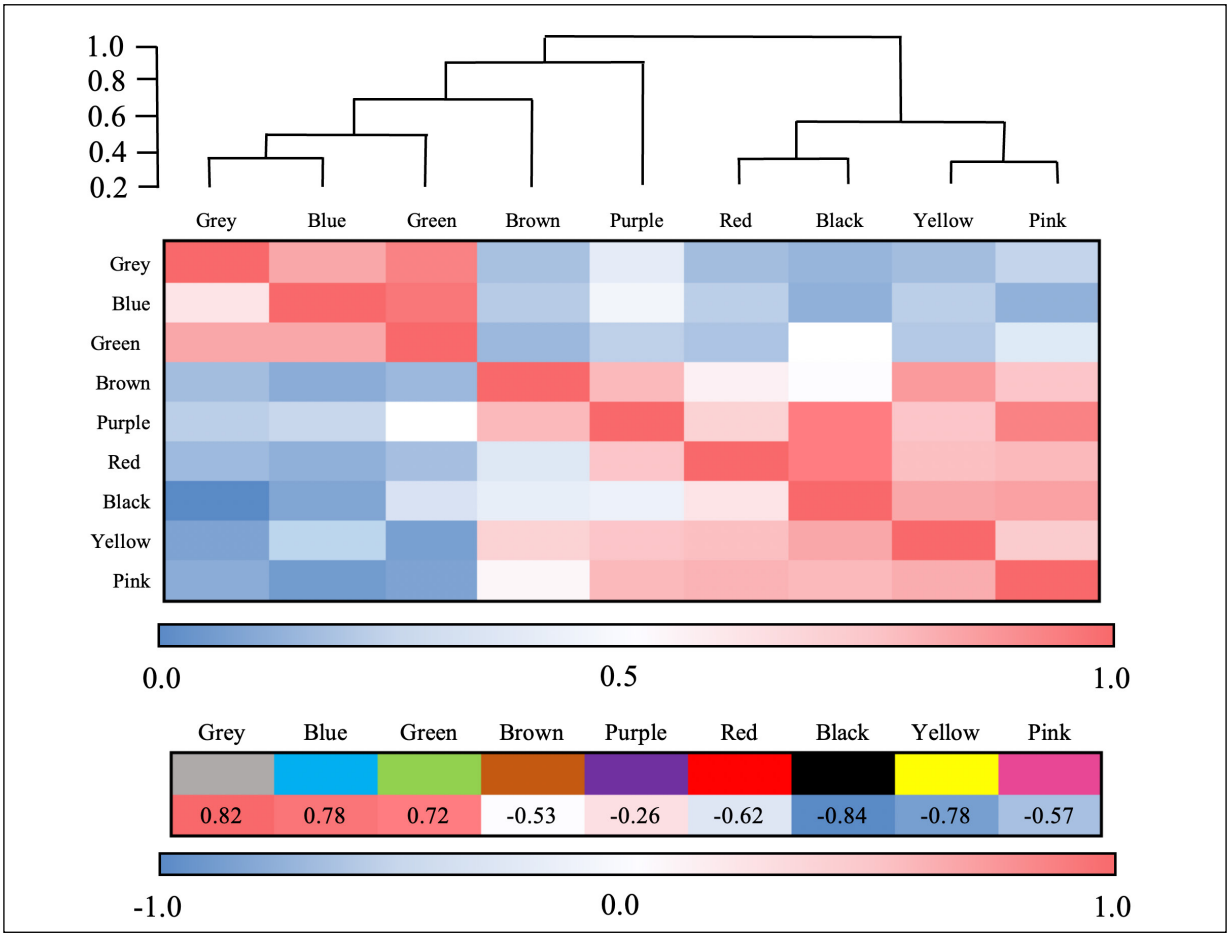


Figure 9. Analysis of WGCNA network modules and the interaction with clinical characteristics.

and enrichment analysis of signal pathways (Figures 10 and 11). As indicated in Figure 10, DEGs in blue modules were mainly enriched in immunological reaction, inflammatory reaction, signal transduction, plasmalemma, integral components of plasmalemma, extracel-

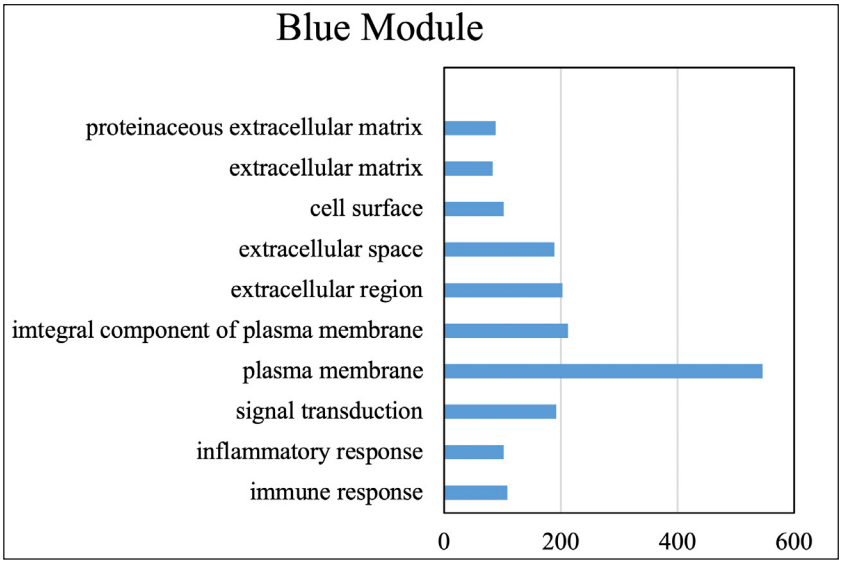
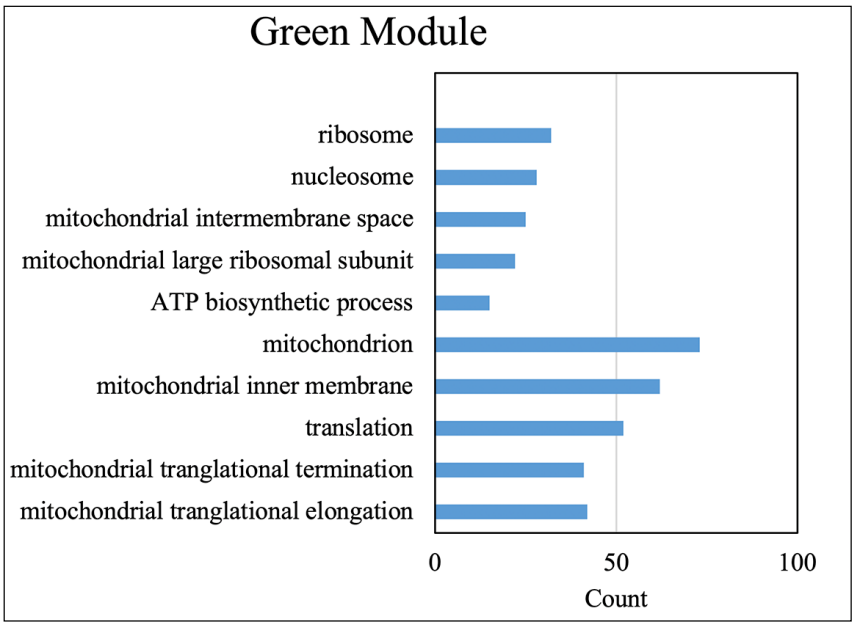


Figure 10. Main enrichment of DEGs in blue modules.

Figure 11. Main enrichment of DEGs in green modules.



lular region, extracellular space, cell surface, extracellular matrix, and protein extracellular matrix. As illustrated in Figure 11, DEGs in green modules were mainly enriched in excessive elongation of the mitochondrion, mutation termination of mitochondrion, translation, mitochondrial inner membrane, mitochondrion, adenosine triphosphate (ATP) biosynthesis, mitoribosome subunit, intermembrane space, nucleosome, and ribosome.

Analysis of Target Prediction by Milkvetch Root

According to the analysis of visualized predictive targets by main biological components of milkvetch root, quercetin, kaempferol, 7-O-methyl group-isomucronulatol, and isorhamnetin were the compounds with more targets. The above components had 133, 54, 47, 28, and 22 targets, which possessed positive significance in the treatment of DN.

Discussion

Diabetes is a very common disease with high morbidity and complexity, showing various complications at a later stage[26]. DN is one of the frequently occurring complications among patients with advanced diabetes, which seriously affects rehabilitation effect and quality of life among patients[27]. Moreover, DN exerts significant impacts on renal functions. At present, the main control and treatment methods for DN include a change in living habits, regulation of diet, strict control of blood glucose, blood pressure, and blood fat, and drug therapy for lipid abnormalities such as angiotensin-converting enzyme inhibitor (ACEI) and angiotensin receptor blocker (ARB)[28,29].

Milkvetch root is a commonly used Chinese drug for invigorating qi for consolidating superficies and is widely applied in the treatment of DN with high clinical values[30]. The investigation into the key targets and interventional mechanisms of milkvetch root in DN can more effectively exert its therapeutic effect. As a result, the treatment becomes more targeted and shows more significant efficacy[31,32]. Milkvetch root possesses remarkable clinical efficacy in DN. However, the overall molecular mechanism of the therapeutic effect has not been fully elucidated. Based on WGCNA, the correlation between genes and the relationship with disease could be analyzed.

Shen et al[33] used WGCNA to predict the central genes that influenced the relapse of lung adenocarcinoma (LUAD) and selected the modules with the highest correlation with tumor relapse for functional enrichment analysis. They found that a total of 8 hub genes (*ACTR3, ARPC5, RAB13, HNRNPK, PA2G4, WDR12, SRSF1,* and *NOP58*) were closely correlated with LUAD relapse. The research was conducive to the invention of targeted therapeutic drugs and the understanding of the mechanism of LUAD relapse. Liang and Sun[34] revealed specific central genes associated with diabetic heart failure and the significant pathways for central gene localization. Besides, they adopted WGCNA to analyze central modules for the identification of key genes. Based on the KEGG pathway and GO enrichment, they[34] analyzed the functions of genes in the modules of clinical interest. In addition, it was related to the construction of a protein-protein interaction (PPI) network in sequence.

Finally, the key genes were determined. 20 gene co-expression modules were detected by WGCNA, and the modules marked in light yellow were most significantly correlated with diabetes (p=0.08). The genes involved in the module were mainly located in immunological reaction, plasmalemma, and receptor binding and were mainly assembled in endocytosis and phagosome of KEGG pathway enrichment. Besides, three key genes (*STK39, HLA-DPB1,* and *RAB5C*) were identified and might be the key genes that caused diabetic heart failure. DN was the main complication of diabetes and the main cause of end-stage nephrosis, but its potential molecular mechanism is still unclear[35]. Gholaminejad et al[36] employed the WGCNA algorithm to analyze the microarray dataset of DN for a better understanding of the pathogenesis of DN and the exploration of key genes in disease progression. Moreover, they introduced identified DEGs in the DN dataset GSE47183 into the WGCNA for the construction of co-expression modules. After that, GO and Reactome pathway enrichment analysis were performed on each module to understand the involvement of the co-expression modules in biological processes and pathways. It was found that 2,475 important DEGs were identified through the WGCNA algorithm, and then they were clustered into 6 different co-expression modules. Metabolic process, cell cycle control, and cell apoptosis were the most abundant terms. In the genes module, 23 hub genes were identified, and 5 out of them were verified in another DN dataset, including *FN1, SLC2A2, FABP1, EHHADH,* and *PIPOX*. In the research, multiple DEGs were obtained based on WGCNA and performed with GO functional annotation and KEGG signal pathway enrichment analysis. It was revealed that there were 752 down-regulated DEGs and 1,547 up-regulated DEGs in DN samples compared to those in control samples. *PLCE1, CLIC5, PTPRO, HSPA12A, AIF1, GMDS,* and *SEMA5A* were significantly inhibited, while *CEP152, LUNAR1,* and *SLC9A1* were remarkably up-regulated in DN samples. Hierarchical clustering analysis was performed to detect the co-expression clusters with corresponding color assignments. A total of 9 modules were identified, and clinical characteristics were closely correlated with gray, blue, green, and brown modules in WGCNA modules. According to the results of GO analysis and KEGG pathway enrichment analysis, DEGs in blue modules were mainly enriched in immunological reaction, inflammatory reaction, signal transduction, plasmalemma, integral components of plasmalemma, extracellular region, extracel-

lular space, cell surface, extracellular matrix, and protein extracellular matrix. DEGs in green modules were mainly enriched in excessive elongation of mitochondrion, mutation termination of mitochondrion, translation, mitochondrial inner membrane, mitochondrion, ATP biosynthesis, mitoribosome subunit, intermembrane space, nucleosome, and ribosome.

Milkvetch root was a common traditional Chinese drug widely applied in the treatment of multiple diseases[37]. The mechanism of the treatment of laryngeal cancer with milkvetch root was investigated based on gene co-expression network and molecular docking in some research. Key modules were screened to obtain the important therapeutic targets for laryngeal cancer. Besides, external dataset was utilized for differentially expressed analysis and survival analysis. The pathways through which important targets were involved in were revealed by gene set enrichment analysis (GSEA) enrichment analysis. It was demonstrated that some components of milkvetch root could effectively bind to important targets, including quercetin, rutin, and chlorogenic acid, which might be the main mechanism of the anti-cancer effects of milkvetch root[38]. Guo et al[39] obtained the chemical components of milkvetch root from Traditional Chinese Medicine Systems Pharmacology Database (TCMSP) and determined the potential targets by therapeutic target database (TTD). DisGeNET and GeneCards databases were adopted to collect DN-related target genes. Besides, STRING database was utilized to establish DN-milkvetch root common target protein interaction network. Based on GO analysis and KEGG pathway enrichment analysis, the action mechanism and therapeutic effects of DN-milkvetch root were further explored. Eventually, a total of 16 active ingredients and 78 putative target genes were screened from milkvetch root, including 42 overlapping with DN targets. They were considered potential therapeutic targets. According to the results of network analysis, the activity of milkvetch root containing quercetin, formononetin, verbascoside, and 7-O-methyl group-isomucronulatol methyl isobutyl fenchyl alcohol was correlated with top 10 screened targets, such as vascular endothelial growth factor A (VEGFA), tumor necrosis factor (TNF), interleukin-6 (IL-6), mitogen-activated protein kinase (MAPK), chemotactic factor 3 (CCL3), nitric oxide synthase 3 (NOS3), post-transcriptional gene silencing 2 (PTGS2), IL-1 β, JUN, and epidermal growth factor receptor (EGFR). According to GO and KEGG analysis, these targets were associated with inflammatory reaction, angiogenesis, oxidative stress reaction, rheumatoid arthritis, and other biological processes. Dai et al[40] investigated the key active ingredients and potential pharmacological action mechanism of the treatment of DN with milkvetch root based on network pharmacology, which provided scientific evidence for clinical efficacy. The active ingredients of milkvetch root were obtained from TCMSP. A treatment goal database was used to determine potential targets of milkvetch root. Besides, relevant target genes of milkvetch root were acquired from the GEO microarray dataset GSE1009 and three widely used databases (DisGeNET, GeneCards, and Comparative Toxicogenomics Database). STRING database was employed to establish a DN-AM common target protein interaction network. In addition, Cytoscape was utilized to construct an active ingredient candidate target protein network for visualization. GO and KEGG gene and genome pathway analysis were performed. Eventually, 17 active ingredients and 214 target proteins were screened from milkvetch root, and 61 candidate co-expression genes that had therapeutic effects on DN were obtained and viewed as potential therapeutic targets. The analysis of GO and KEGG and genome enrichment analysis demonstrated that three genes mainly got involved in the inflammatory reaction, angiogenesis, oxidative stress reaction, hypoxia-inducible factor (HIF) signal pathway, TNF signal pathway, and VEGF signal pathway. The above research findings suggested that milkvetch root played a positive role in the treatment of DN. In the research, the bioactive ingredients of milkvetch root were performed with visualization analysis, and the prediction targets were investigated. It was found that quercetin, kaempferol, 7-O-methyl group-isomucronulatol, and isorhamnetin were the compounds with more targets. The above components had 133, 54, 47, 28, and 22 targets. To sum up, milkvetch root possessed remarkable efficacy and high application values in DN and played an important role in the clinical prevention and control of DN.

Strengths and Limitations

The limitations of this research lie in gene co-expression network analysis only by published data and the small sample size. In follow-up research, more clinical cases should be included

as the samples for transcriptome analysis. Furthermore, reconstruction was implemented, and then WGCNA analysis was conducted to provide a reference for the diagnosis and treatment for DN patients.

Conclusions

In the research, WGCNA and pharmacology networks were employed to investigate the changes of DEGs in DN samples and perform correlation analysis and drug target investigation. It was demonstrated that 9 network modules were closely associated with DN. The number of DEGs in gray, blue, green, and brown modules of WGCNA modules was the greatest. According to GO and KEGG analysis of DEGs, the main functions of these genes were annotated as immunological reaction, inflammatory reaction, and signal transduction. Quercetin, kaempferol, 7-O-methyl group-isomucronulatol, and isorhamnetin were the compounds with more targets.

Ethics Approval
Not applicable.

Informed Consent
Not applicable.

ORCID ID
Ying Li: 0009-0005-5746-3537.

Data Availability
All data are available upon request by contact with the corresponding author.

Funding
None.

Conflicts of Interest
The authors have no conflicts of interest to declare.

Authors' Contributions
Sheng Nan Zeng: Conceptualization, validation, formal analysis, investigation, resources, writing-original draft. Yang Meng, Qi Li: Validation, formal analysis, investigation, resources. Shu Rong Wang: Conceptualization, resources. Ying Li: Visualization, supervision, project administration, and funding acquisition, writting-review and editing.

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