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Bioinformatics-based analysis of *Cordyceps Sinensis* in the treatment of diabetic nephropathy-associated depression

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Abstract

Background Diabetic nephropathy (DN) and depression exhibit complex pathological association; yet western medicine shows limited efficacy with notable side effects. Cordyceps sinensis (CS), a traditional Chinese medicine, demonstrates advantages in improving renal function and regulating neuroimmunity, but its mechanism for treating DN with depression remains unclear.

Methods Active components of CS were screened via TCMSP, disease-related targets were identified from GeneCards and OMIM, and potential targets were determined by intersection analysis. PPI network, GO/KEGG enrichment analyses, and "drug-component-target-pathway" network were constructed. Molecular docking and molecular dynamics simulations were employed to verify binding affinity and stability.

Results Seven active ingredients (e.g.β-sitosterol, ergosterol) and 40 key targets were identified, with PIK3CA, AKT1, and MAPK1 as core hubs. CS primarily modulated PI3K-Akt, MAPK-ERK, and androgen receptor pathways. Molecular docking revealed binding energies < -7.0 kcal/mol, and MD simulations confirmed complex stability within 50 ns.

Conclusion CS treats DN with depression via multi-component and multi-target synergism, targeting PIK3CA/AKT1/MAPK1 to regulate signaling pathways, thereby offering mechanistic molecular bases for clinical intervention.

Key words cordyceps sinensis, diabetic nephropathy, depression, network pharmacology, molecular docking, molecular dynamics simulation

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Introduction

Diabetic nephropathy (DN) is among the most prevalent microvascular complications associated with diabetes mellitus [1]. At the clinical level, it is characterized by significant proteinuria, hypertension, and progressive renal dysfunction, making it one of the leading causes of end-stage renal disease. Hyperglycemia-induced oxidative stress, inflammation, and fibrosis represent its pathophysiological mechanisms [2]. The increasing global prevalence of DN necessitates the development of molecularly targeted therapeutics designed to rectify the core pathogenic mechanisms of the disease [3].

Depression is a chronic, relapsing mental disorder marked by persistent low mood, cognitive slowing, poor concentration, anhedonia, and loss of interest and is often accompanied by somatic symptoms including sleep disturbances and appetite loss [4-6]. The prevalence of depression is currently increasing dramatically worldwide, with a prevalence rate of 4.4% [7]. There is a complex interaction between diabetes and depression. Dysregulation of the hypothalamic–pituitary–adrenal–immune (HPAI) axis and activation of proinflammatory cytokines are associated with depression, which may lead to insulin resistance and increase the risk of diabetes mellitus [8].

Cordyceps sinensis (CS), a renowned traditional Chinese medicine, is considered a valuable natural resource and cultural heritage of China [4]. It represents a unique parasitic complex formed by the fusion of cysts of the CS and the corpse of the larvae. The Cordyceps fungus belongs to the family Clavicipitaceae [9]; it parasitizes the body of larvae of the family Batidae. Naturally, CS thrives in high-altitude regions, typically between 3,500 and 5,000 meters above sea level, such as Tibet, Qinghai, Gansu, Sichuan, and Guizhou, where the environmental conditions favor its growth and development [10]. In addition to its significant immunomodulatory [11], antioxidative stress [12], and anti-inflammatory pharmacological activities, CS can improve renal function and protect the kidneys from damage by alleviating glomerulosclerosis and fibrosis, and by inhibiting the inflammatory response and oxidative stress. Moreover, CS has also shown potential efficacy in improving depressive symptoms by regulating the central nervous system, intestinal flora [13], and immune function. Therefore, CS has unique advantages in the treatment of depression complicated by diabetic nephropathy.

Currently, for the treatment of depression complicated by DN, Western medical therapies have limited effects. Although antidepressants like selective 5-hydroxytryptamine reuptake inhibitors (SSRIs) and tricyclic antidepressants (TCAs) can alleviate depressive symptoms, they have limited therapeutic effects on DN and may aggravate the burden on renal function [14, 15]. In addition, these drugs have many side effects, and their long-term use may lead to metabolic disorders and increased cardiovascular risk. Therefore, developing a multitarget therapeutic regimen that improves renal function and relieves depressive symptoms is imperative.

However, the specific mechanism of action of CS as a treatment for depression complicated by DN has not yet been clarified. Here, we employ network pharmacology along with complementary analytical approaches to investigate the underlying mechanisms through which CS serves as a treatment of depression complicated by DN, aiming to lay a theoretical foundation for subsequent studies. **Figure 1** represents the research flowchart.

Materials and methods

Collection and screening of the components of CS

The constituents of CS were obtained from the Traditional Chinese Medicine System Pharmacology Database and Analysis Platform (TCMSP) (https://old.tcmsp-e.com/tcmsp.php) [16]. According to previous literature, compounds with oral bioavailability (OB) ≥ 30% and drug-likeness (DL) ≥ 0.18 were selected as screening criteria to identify the orally active components of CS.

Prediction of the action targets of CS active ingredients

The screened active components were retrieved from PubChem (https://pubchem.ncbi.nlm.nih.gov/) to obtain their corresponding SMILES structures [17]. These SMILES were then imported into the SwissTargetPrediction database (http://www.swisstargetprediction.ch/) database [18], with the target species restricted to humans (Homo sapiens). Targets with a predicted probability ≥ 0 were selected as the screening criterion, and the most relevant potential target information for each active compound was subsequently obtained.

Prescreening for disease target acquisition

Using "Depression" and "Diabetic Nephropathy" as keywords, we searched the GeneCards (https://www.genecards.org/) [19] and OMIM (https://www.genecards.org/) [20] databases for disease targets. The OMIM database was used to search for disease targets to screen the targets of DN and depression and removes duplicates from the two databases before organizing and merging the information to obtain the disease targets related to depression and DN. Using the jvenn (https://jvenn.toulouse.inrae.fr/app/example.html) platform, the intersection between the disease-related targets and the predicted targets of the active ingredients was identified, yielding the potential therapeutic targets of CS for depression and DN.

Prediction of protein–protein interaction (PPI) information

The intersecting targets were uploaded into the STRING (https://cn.string-db.org/) [21] database, and on the search page, "Multiple proteins" and "Homo sapines" were selected for each species. The unconnected targets were removed to obtain the PPI network diagram.

Gene Ontology (GO) analysis

The shared targets of CS for the treatment of DN complicated with depression were uploaded to the DAVID (https://davidbioinformatics.nih.gov/) [22] database for GO enrichment analysis. The results from three items, biological process (BP), cellular component (CC), and molecular function (MF), were visualized and displayed via Bioinformatics (http://www.bioinformatics.com.cn/).

Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis

KEGG pathway analysis was performed via the DAVID database for common targets, and the results were visualized via Bioinformatics. (http://www.bioinformatics.com.cn/).

Construction of a "drug–component–target–pathway" network

An Excel file of "drug-ingredient-target-pathway" was created with the active ingredients of CS, the intersecting targets of CS in the treatment of DN complicated with depression, and the pathways obtained from the enrichment analysis, which were then imported into Cytoscape 3.10 to construct a network diagram showing the interactions between the four components.

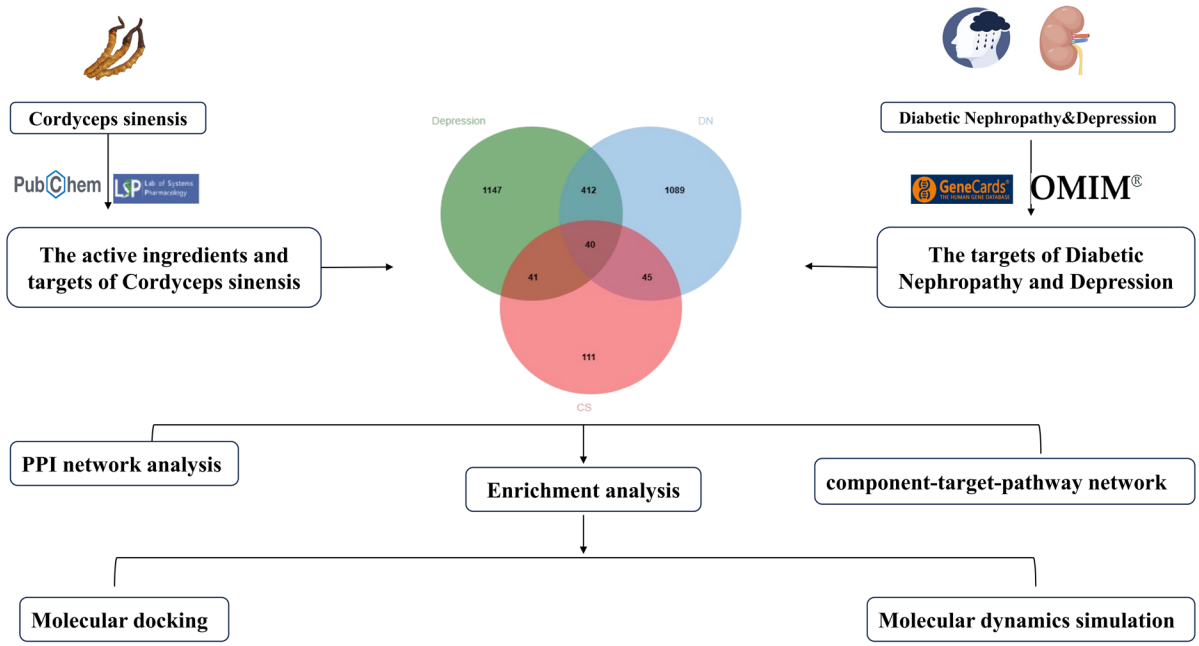

Figure 1. The flow chart of this study.

Molecular docking

The three core target proteins screened were used to perform molecular docking with the receptor proteins of their active ingredients. The 3D structures of the core target and protein receptor were obtained from the PubChem, PDB [23], and UniProt databases [24], and molecular docking was carried out via AutoDock vina1.5.7 software to determine the interaction strengths of the core target and the active ingredient. The docking interactions were visualized using PyMOL software, while Origin software was employed to generate thermograms illustrating the molecular docking binding energies.

Molecular dynamics simulation

The molecular dynamics (MD) simulations were carried out in the Yinfo Cloud Computing Platform (YCCP) via the Amber Tools 20 package with AMBER ff19SB [25] and GAFF [26] force fields for the three groups of complexes with the highest binding affinities during the molecular docking process. The system was solvated via the TIP3P water model with a truncated octahedral water box with a 10 Å edge. Periodic boundary conditions (PBC) were applied to neutralize the system's net charge using Na^+ counterions. To eliminate unfavorable atomic contacts, two consecutive minimization steps were conducted—10,000 steps of steepest descent followed by 10,000 steps of conjugate gradient minimization. After energy minimization, the system underwent equilibration under NVT and NPT ensembles for 200 ps and 500 ps, respectively. The temperature was maintained at 300 K using a Berendsen thermostat with a 1 ps coupling constant, while the pressure was controlled at 1 atm using a Monte Carlo barostat with a 1 ps relaxation time. Subsequently, a 50 ns MD production run was performed under the NVT ensemble without any positional restraints. The resulting trajectories were analyzed using the CPPTRAJ module [27]. Finally, binding free energies were computed using the Molecular Mechanics Generalized Born Surface Area (MM/GBSA) method implemented in AmberTools 20, based on 200 snapshots extracted from the MD trajectory [28]. For each snapshot, the free energy of the receptor, ligand, and complex was calculated using a "single-trajectory" approach.

Result

Prediction of active ingredients and targets of CS

Thirty-eight components of CS were included in the TCMSP, and seven active components were obtained by screening with OB ≥ 30% and DL ≥ 0.18, as shown in **Table 1**. After screening via the Swiss Target Prediction database with probability>0 as the screening criterion, 237 targets corresponding to active ingredients were finally obtained after de-weighing.

Prediction of disease targets

Depression and DN-related targets were collected from the GeneCards and OMIM disease databases. As a result, 1586 targets for depression and 1640 targets for CS were obtained. A total of 1586 targets and 1640 targets for DN and the active targets of CS intersected with the other two diseases, and 85 targets intersected between depression and CS. Eighty-one targets intersected between DN and CS, and a total of 40 targets were obtained, as demonstrated by the visualized Venn diagram shown in **Figure 2A**.

PPI network construction

Forty common targets of CS for the co-treatment of DN and depression were uploaded to the STRING database, and the target protein PPI network was obtained, which was later visualized and analyzed via Cytoscape 3.10.0 software (**Figure 2B**). Overall, the network had a 40 nodes and 320 edges in total, with the average node degree value of 16.

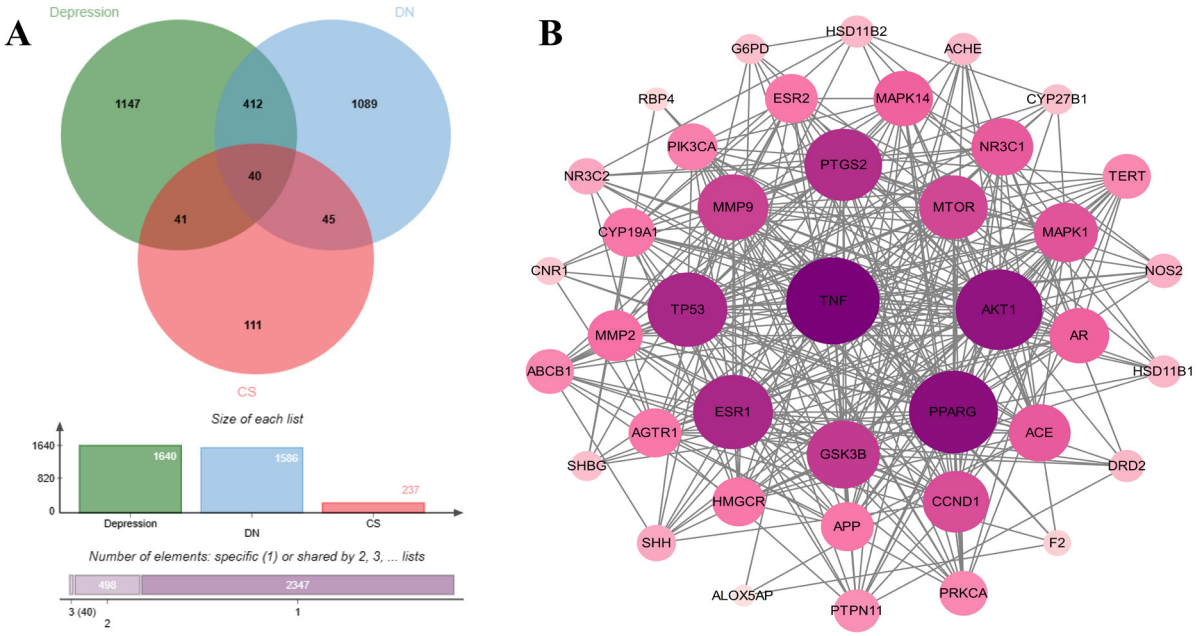

Figure 2. Analysis results of common targets and their interactions. (A) Effects of CS on the intersection of diabetic nephropathy and depression; (B) Protein–protein network interaction map.

Target enrichment analysis (GO analysis)

The 40 intersecting targets of CS for the treatment of DN and depression were subjected to GO enrichment analysis. A total of 341 GO entries were analyzed (**Figure 3A-D**), of which 263 entries were for BP, as shown in Figure 3A; 31 entries were for CC, as shown in Figure 3B; 48 entries were for MF, as shown in Figure 3C; and the top 10 entries with the smallest P values were visualized through bubble diagrams. The first 10 entries with the smallest P values among the entries were drawn into bubble diagrams for visualization, and the results suggested that the BPs were focused mainly on the positive regulation of transcription by RNA polymerase II, the positive regulation of gene expression, the negative regulation of gene expression, etc. The cellular components were mostly composed of the cytoplasm, cytoplasmic solute, nucleus, etc. The MFs were composed mainly of the functions of binding of the same proteins, binding of zinc ions, and binding of enzymes.

Target enrichment pathway analysis (KEGG analysis)

Pathway analysis of the intersection targets of disease and drug components was performed through the DIVAD database. A total of 135 pathways were obtained, and the top 20 items with the smallest P value were selected for graphical visualization, as shown in **Figure 3E-F**. The results of the analysis suggested that CS cotreats DN and depression via PI3K–Akt, MAPK–ERK, androgen receptor signaling, and other pathways.

Table 1. The main ingredients of CS.

MOL number	Compound name	OB/%	DL
MOL001439	Arachidonic acid	45.57	0.2
MOL001645	Linoleoyl acetate	42.1	0.2
MOL000358	β-sitosterol	36.91	0.75
MOL011169	Peroxyergosterol	44.39	0.82
MOL008998	Cerevisterol	39.52	0.77
MOL008999	Cholesteryl palmitate	31.05	0.45
MOL000953	Cholesterol	37.87	0.68

CS: cordyceps sinensis; DL: drug-likeness; OB: oral bioavailability.

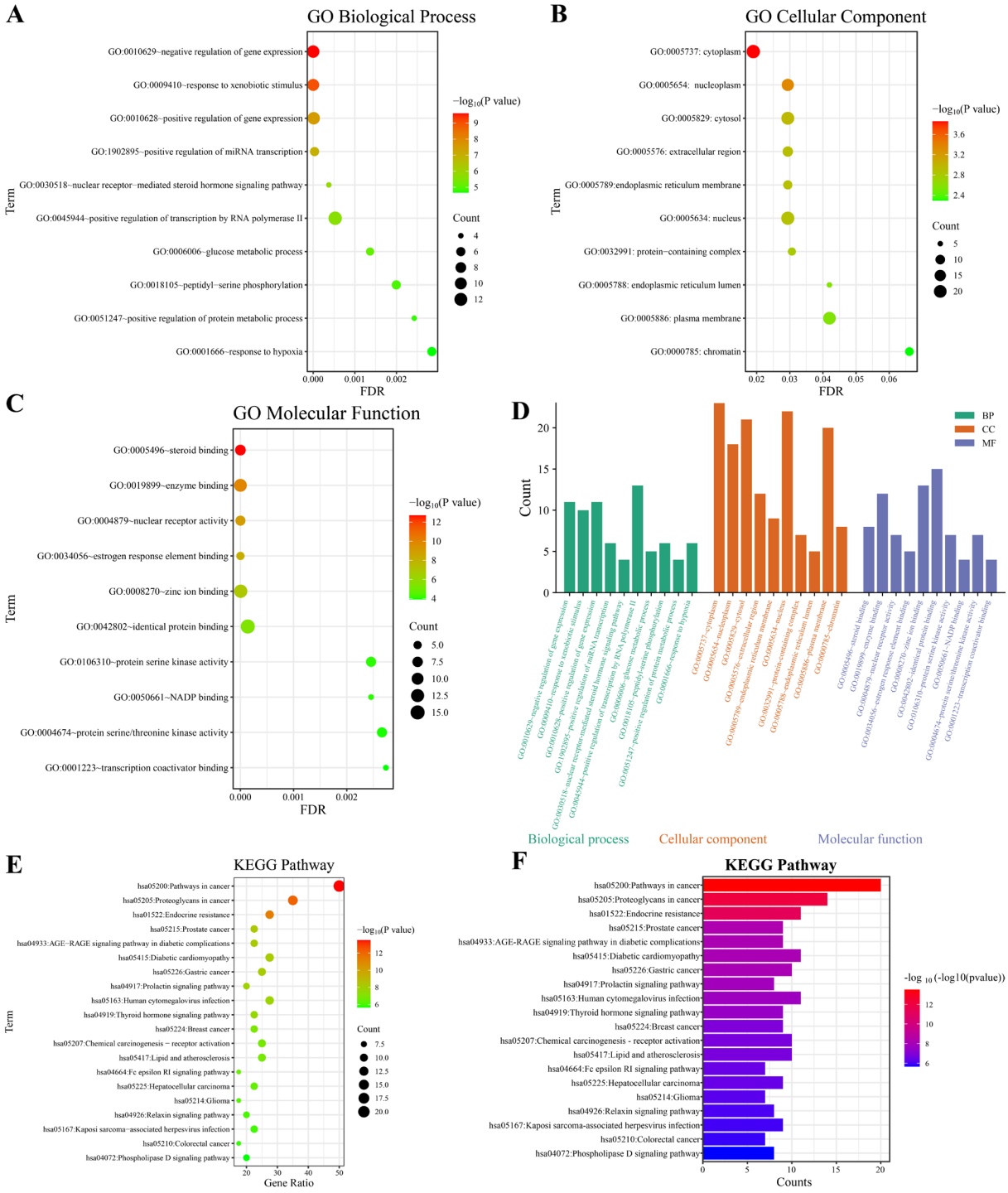

Figure 3. Enrichment analysis results. (A) GO BP enrichment analysis bubble map; (B) GO CC enrichment analysis bubble map; (C) GO MF enrichment analysis bubble map; (D) Histogram of the results of GO enrichment analysis; (E) Bubble diagram of the KEGG pathway enrichment analysis results; (F) KEGG pathway classification diagram.

Drug–component–target–pathway network

The CS-constituent-target-pathway network was constructed via Cytoscape 3.10.1 software (**Figure 4**). The red triangle represents CS, the surrounding light pink color represents the core constituents of CS, the green rectangle at the bottom represents the targets of CS in treating the disease, and the blue color at the top represents the signaling pathways. This network contains 264 nodes (drug: 1; ingredient: 6; target: 237; pathway: 20) and 439 edges (drug-ingredient: 6; ingredient-target: 273; target-pathway: 196). The results of the analysis conducted through the network indicated that the top three targets with highest degree value were PIK3CA, AKT1, and MAPK1, which were taken as the core targets in this study for subsequent research.

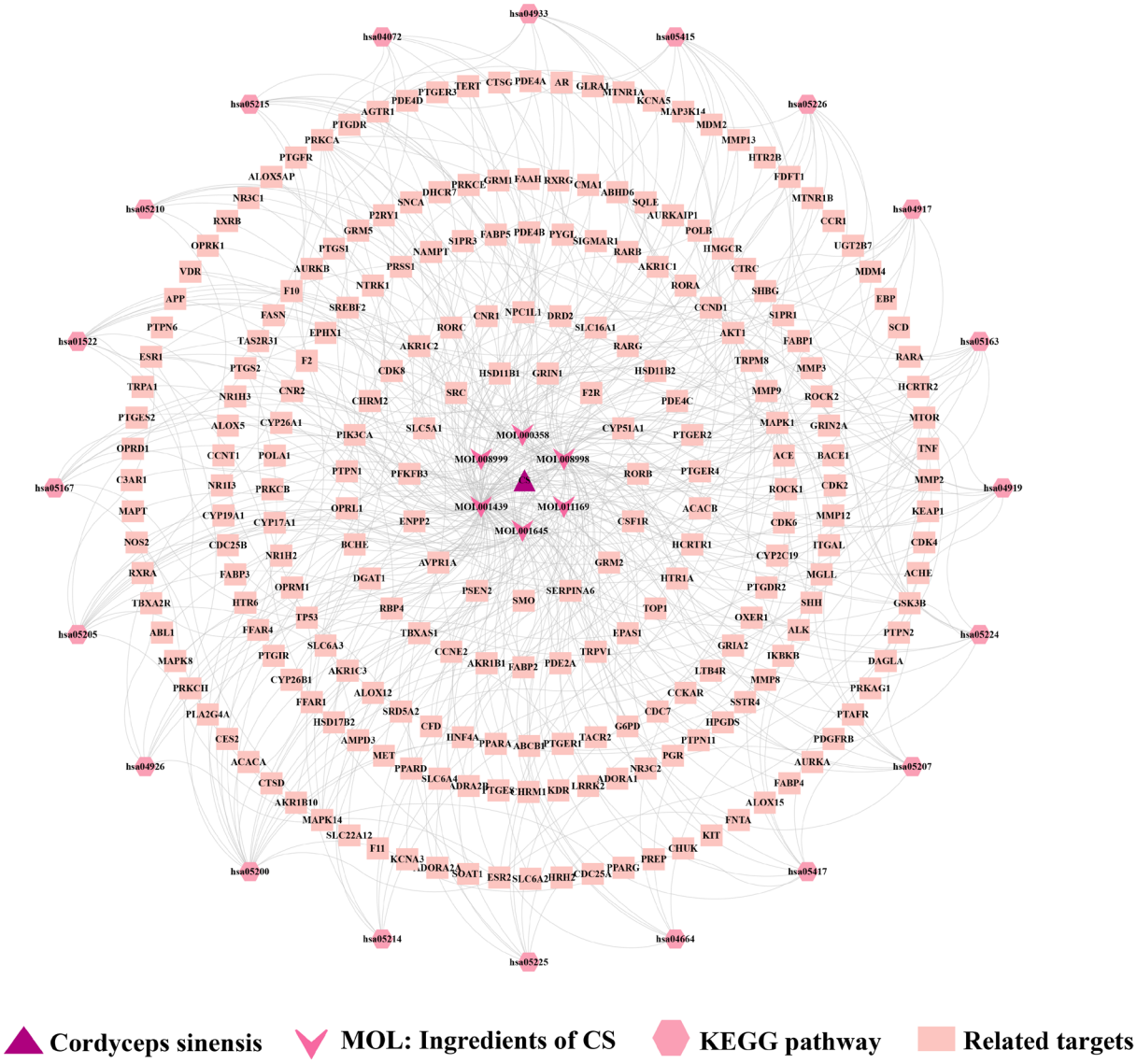

Figure 4. CS-component-target-pathway network diagram.

Molecular docking

The top three ranked targets were screened for molecular docking with the top six core components via AutoDock Vina 1.5.7. A lower affinity (kcal/mol) value indicates more stable binding between the compound and target protein, reflecting stronger binding affinity between the two molecules. It is generally believed that a binding energy value below -4.25 kcal/mol indicates a certain binding activity between the compound and target protein; a value below -5.0 kcal/mol indicates better binding activity, and a value below -7.0 kcal/mol indicates particularly strong binding activity. The results, as shown in **Table 2** and **Figure 5A** below, revealed that PIK3CA, AKT1, MAPK1 and the first six active ingredients all had good binding activities, with the highest binding activity of the PIK3CA protein to β-sitosterol reaching -9.4 kcal/mol, followed by the binding activity of the AKT1 protein to cerevisterol, with a binding activity of -9.2 kcal/mol, and the binding activity of the MAPK1 protein with cerevisterol was in third place, with a binding activity of -8.6 kcal/mol; thus, we can infer that PIK3CA may present a key target of CS for the treatment of DN and depression. As shown in **Figure 5B**, some molecular docking results are visualized, and all three groups of complexes clearly formed hydrogen bonds during the redocking process.

Molecular dynamics simulation

Three groups of complexes with the highest binding activity from the molecular docking experiments were screened for verification via MD simulations. System stability was monitored via root mean square deviation (RMSD), with lower average RMSD values reflecting greater conformational stability of the complexes throughout the simulation. According to **Figure 6A**, the RMSD values of the three sets of complexes remained low and smooth throughout the 50 ns of the simulation. These findings indicate that the ligands and receptors are more tightly bound and that the complexes are more stable. The root mean square fluctuation (RMSF) can characterize the fluctuation of small-molecule ligands on the spatial structure of proteins, and a larger value suggests that the residues of the proteins interact more heavily with small molecules. As shown in **Figure 6B**, the RMSF values of the three

Table 2. Molecular docking results.

Gene name	Degree	UniProt ID	PDB ID	Structural unit	Binding energy (kcal/mol)
PIK3CA	21	P42336	2RD0	MOL001439	-6.4
				MOL001645	-5.7
				MOL000358	-9.4
				MOL011169	-8.3
				MOL008998	-8.2
				MOL008999	-7.4
AKT1	21	P31749	1H10	MOL001439	-4.3
				MOL001645	-4.2
				MOL000358	-7.1
				MOL011169	-7.4
				MOL008998	-9.2
				MOL008999	-5.1
MAPK1	20	P28482	1PME	MOL001439	-5.4
				MOL001645	-5.3
				MOL000358	-8.1
				MOL011169	-8.2
				MOL008998	-8.6
				MOL008999	-7.1

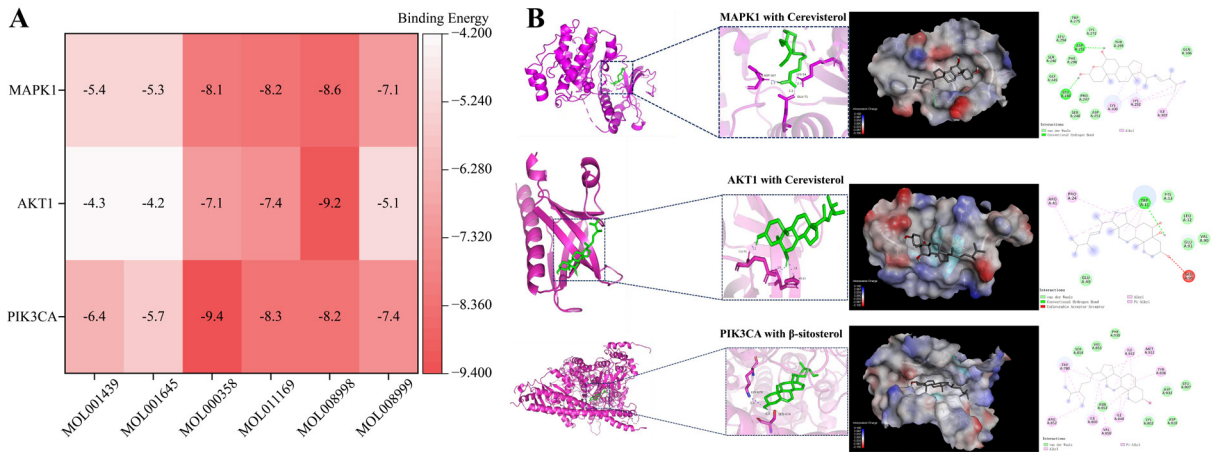

Figure 5. Results of Molecular docking. (A) Molecular docking binding energy heatmap (the color shading is inversely proportional to the magnitude of the binding energy); (B) Results of docking visualization of active ingredients with core target proteins.

groups of complexes were greater during the simulation process, and the analysis revealed that TYR, MET, and ARG may serve as key residues in the binding of the AKT1–cerevisterol complex; GLY, VAL, and ASP may play critical roles in the MAPK1–cerevisterol interaction; and ARG, MET, and LYS are likely essential residues contributing to the stability of the PIK3CA-β-sitosterol complex. The radius of gyration (ROG) can characterize the spatial compactness and changes in the morphology of a molecular system during a simulation. According to the analysis of **Figure 6C**, the AKT1-cerevisterol complex has a small ROG of approximately 1.47 nm during the simulation process, which indicates that the structure of the complex is relatively compact, the complex is relatively stable, and the possibility of structural changes during the simulation process is relatively small. The complex MAPK1-cerevisterol maintained the ROG at approximately 2.18 nm during the simulation process, indicating that there is stability in the structure of the complex and that at the same time, there is space for flexibility and dynamic changes, and different degrees of conformational changes may occur during the simulation process. The ROG of the complex PIK3CA-β-sitosterol remains at approximately 3.3 nm, indicating that the complex structure is more flexible and less stable and is prone to conformational changes during the simulation process.

The MM/GBSA method is a well-established computational approach used to calculate the binding free energies following simulations to predict the stability of a complex. Binding free energies were calculated for 200-frame snapshots of MD trajectories via the MM/GBSA method in AmberTools 20. GGAS denotes the gas-phase total free energy, which is the sum of the van der Waals force (VDW) and electrostatic potential energy (EEL), and ΔG Solvation Free Energy (GSOLV) denotes the solvation energy, representing the sum of the nonpolar solvation energy (NOPOLAR) and the polar solvation energy (POLAR). The binding free energies of the three groups of complexes shown in **Figure 6D** are -21.22 kcal/mol, -35.22 kcal/mol, and -35.44 kcal/mol, respectively, where the van der Waals forces are -27.82 kcal/mol, -48.52 kcal/mol, and -60.5 kcal/mol, the electrostatic potential energies are -12.84 kcal/mol, -0.59 kcal/mol, and -13.53 kcal/mol, the nonpolar solvation energies are -3.17 kcal/mol, -5.89 kcal/mol, and -5.37 kcal/mol, all three of which are favorable for the binding of the three groups of small-molecule compounds to proteins, and electrostatic potential and the van der Waals energies are stronger and play a dominant role, whereas the nonpolar solvation energies are weaker and play a secondary role, and the polar solvation energies are unfavorable for the interaction between the two.

Discussion

The comorbidity mechanism of DN and depression involves the interaction of oxidative stress, inflammatory networks, and

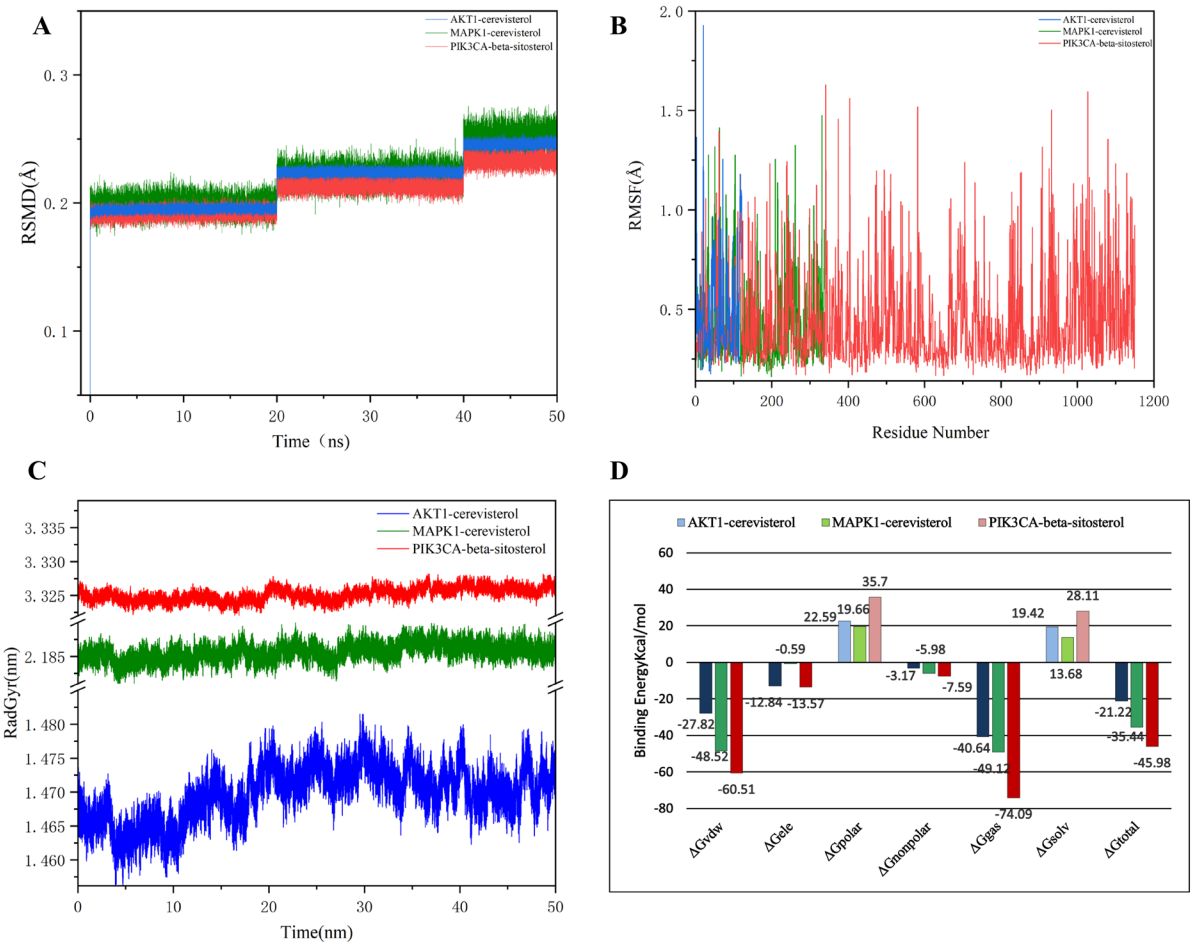

Figure 6. Results of molecular dynamics simulations. (A) RMSD curves of three complexes during molecular dynamics simulations; (B) RMSF curves of three complexes during molecular dynamics simulations; (C) ROG curves of three complexes during molecular dynamics simulations; (D) Molecular dynamics simulation energy analysis.

neuroendocrine disorders. However, current single-target Western medicine treatments face dual challenges: limited efficacy and renal toxicity. This study utilized a multi-dimensional network pharmacological approach to confirm that among the seven active components of CS, β-sitosterol and ergosterol are the core active molecules. The 40 therapeutic targets they interact with form a tightly connected network through 320 interacting edges. Notably, PIK3CA, AKT1, and MAPK1 occupy hub positions, as verified by node connection strength, highlighting their critical roles in signal transduction.

Previous studies have shown that β-sitosterol has various physiological activities, such as anti-inflammatory [29] and antioxidant [30] activities, and is pivotal in regulating body metabolism. In addition, cerevisol functions to regulate blood lipid [31] and antioxidant [32] activities. Experimental animal studies have shown that in a mouse model of DN, the administration of β-sitosterol-enriched extract resulted in a significant decrease in the levels of oxidative stress markers such as malondialdehyde (MDA) [33] and an increase in the activity of superoxide dismutase (SOD) [34] in renal tissues, as well as a downregulation of pro-inflammatory factors such as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) [35]. This downregulation tentatively suggests that β-sitosterol may delay the progression of renal lesions by attenuating oxidative stress and inhibiting inflammatory responses [33, 36]. In animal models of depression, cerevisterol supplementation has been shown to regulate the metabolism of neurotransmitters such as 5-hydroxytryptamine (5-HT) and dopamine (DA) in the brain [37, 38] and improve depressive-like behaviors, suggesting its potential value in the treatment of depression. However, there is still a lack of clinical trials on these two components in the complex condition of depression complicated by DN, and large-scale, multicenter clinical trials are warrents to further clarify their efficacy and mechanism of action, such as exploring dose-dependent effects of β-sitosterol and cerevisterol on patients' renal function indices (e.g., glomerular filtration rate, urinary protein excretion, etc.) [39, 40], depression symptom scores [41], and related biomarkers.

PIK3CA, AKT1, and MAPK1 were identified as key targets through PPI network analysis, which fits with the existing research results, but more direct evidence is still needed to confirm their roles in the triad of diabetes, depression, and nephropathy. In DN, hyperglycemia inhibits PI3K–Akt pathway activity, leading to abnormal metabolism and increased apoptosis in kidney cells [42, 43]. In cellular experiments, activation of the PI3K–Akt signaling may induce proliferative and survival benefits in renal cells and reduce the high glucose-induced cell damage [44]. The active ingredients in CS may activate the PI3K–Akt pathway through a similar mechanism to increase the resistance of renal cells to injury, reduce proteinuria production, and protect renal function [45]. For example, in a cellular model of DN, after treatment with CS extract, the level of phosphorylated Akt protein was elevated, the expression of the apoptosis-related protein Bax was reduced, and the expression of Bcl-2 was elevated, suggesting that CS may activate the PI3K–Akt pathway to induce renoprotective effects [46]. In depression research, the PI3K–Akt pathway has been shown to regulate neuroplasticity, and its activation may promote the growth, differentiation and survival of nerve cells. Likewise, in animal experiments, the upregulation of key PI3K–Akt pathway associated genes by gene editing technology has been shown to improve the behavioral performance of animal models of depression, such as increasing the ability of voluntary activity and the desire to explore and reducing the immobility time of forced swimming and tail hanging experiments [47]. The MAPK pathway is intricately associated with the cellular stress response and inflammation [48]. In patients with DN and depression, there are excessive stress responses and inflammatory states, and MAPK1 is a key member of the MAPK pathway [49]. CS ingredients may inhibit inflammatory signaling and reduce inflammatory damage by regulating MAPK1 activity [50] and, at the same time, improve the adaptability of neural cells to stress and play a therapeutic role. In inflammatory cell models, inhibition of the MAPK pathway significantly limit the secretion of pro-inflammatory factors, whereas in neuronal stress models, modulation of the MAPK pathway enhances cellular stress resistance [51]. Future studies can further construct an animal model of DN complicated by depression, and through technical means such as gene silencing or overexpression, we can investigate the specific mechanisms of PIK3CA, AKT1, MAPK1 and other key targets in the treatment of CS, as well as the interregulatory relationships among them.

GO and KEGG enrichment analyses further elucidated the underlying potential molecular mechanisms of CS in treating these two diseases. BP primarily involve the positive regulation of transcription mediated by RNA polymerase II and the enhancement of overall gene expression, which suggests that CS may influence the synthesis of multiple proteins in cells by regulating gene transcription and expression and thus participate in the regulation of the pathophysiological processes of diseases. In terms of cellular composition, it is related to the cytoplasm, cytoplasmic lysate, nucleus, etc., indicating that its action may be closely related to intracellular environmental stability, material transportation and signaling. Regarding molecular function, enrichment of the same protein binding, zinc ion binding, enzyme binding, etc., suggested that the active ingredients of CS may regulate the physiological functions of cells by interacting with specific proteins, ions, or enzymes. KEGG pathway analysis revealed that a number of pathways, such as the PI3K-Akt, MAPK-ERK, and androgen receptor signaling pathways, were significantly enriched. In addition to the previously mentioned PI3K-Akt and MAPK pathways, the androgen receptor signaling pathway also contributes to the development of both DN and depression [51, 52]. In DN, androgens exert anti-inflammatory [53] and antifibrotic [54] effects, and a decrease in androgen levels may accelerate the process of renal fibrosis; however, in depression, androgens are associated with the regulation of neurotransmitters, which may affect mood and cognitive function [55, 56]. CS may improve the pathology of both diseases by modulating the androgen receptor signaling pathway. Notably, androgen supplementation can reduce the degree of renal fibrosis in an animal model of renal fibrosis, whereas regulating androgen levels can affect neurotransmitter synthesis and release in an animal model of depression. Future studies may explore in depth the specific molecular mechanisms by which CS regulates these pathways, as well as the interactions between the pathways, to provide a more detailed theoretical basis for a comprehensive understanding of its therapeutic effects.

Molecular docking and MD simulations verified the binding activity and stability of the key components to the relevant core targets from multiple molecular perspectives. These results demonstrated that all three groups of complexes had good binding activities in both static and dynamic binding processes. These results provide direct molecular evidence that the active components of CS act on key targets, but further validation of their actual roles in physio-pathological environments through in vitro and ex vivo experiments is still necessary. Future research could build on this study and conduct in vitro and in vivo experiments. By establishing an animal model of DN complicated by depression, CS extract or active ingredients can be used to intervene, and their effects on disease-related indices, such as renal function indices, neurotransmitter levels [57], and inflammatory factor expression [56], can be evaluated. Moreover, combined with metabolomics, proteomics and other multiomics techniques, the molecular mechanisms underlying the therapeutic effects of CS in treating DN complicated by depression can be comprehensively

revealed, which will provide a more solid theoretical foundation for the development of innovative medicines and therapeutic programs based on CS.

In conclusion, this study provides new insights into the treatment of depression complicated by DN with CS, but further experimental studies are still needed to elucidate its mechanism of action, with the goal of providing more powerful support for clinical application.The translational medicine gap in this study includes: (1) the lack of pharmacokinetic data for CS components in the DN-depression model (e.g., plasma half-life and renal tissue distribution of β-sitosterol); (2) verification of causal relationships for core targets (e.g., observing linkage changes between renal function and depressive behavior through PIK3CA siRNA knockdown). Future research could integrate single-cell sequencing to analyze the transcriptional regulatory map of CS on key kidney-brain axis cells (e.g., podocytes and hippocampal CA3 region neurons), providing precise targets for innovative combination therapies based on dual-pathway activation of PI3K-Akt/MAPK-ERK.

Conclusions

This study systemically investigated the potential underlying mechanisms of CS in the treatment of depression complicated by DN, via network pharmacology, molecular docking and MD simulations. The results indicate that β-sitosterol and cerevisterol in CS may regulate the activation and signal transduction of PI3K-Akt, MAPK-ERK, and sex hormone receptor signaling pathways by binding with the core targets PIK3CA, AKT1, and MAPK1 and then intervening in the resistance of kidney cells to injury and the level of inflammatory factors, thus acting synergistically in a multi-target, multi0constituent way in the treatment of depression complicated by DN. The above comprehensive research and discussion provide valuable theoretical support and new insights for subsequent in-depth research and clinical treatment of DN and depression.

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Ethics approval

No applicable.

Data availability

The data will be available upon request.

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Authors' contribution

All authors have materially participated in the research and article preparation. The roles for all authors are as follows: Xiaohui Li: Designed the study, Data curation, Formal analysis, Writing—original draft; Yajun Qiao: Formal analysis, Investigation; Xingfang Zhang: Formal analysis, Investigation; Ruiying Cheng: Formal analysis; Yi Liu: Formal analysis; Huimin Zheng: Formal analysis; Lixin Wei: Formal analysis; Yi Ding: Formal analysis, Writing—review & editing; Hongtao Bi: Formal analysis, Writing—review & editing; Tingting Gao: Conceptualization, Writing—review & editing; All authors have approved the final version of the manuscript.

Competing interests

The authors declare that they have no competing interests.

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