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Investigating the Mechanism of Chufan Yishen Formula in Treating Depression through Network Pharmacology and Experimental Verification

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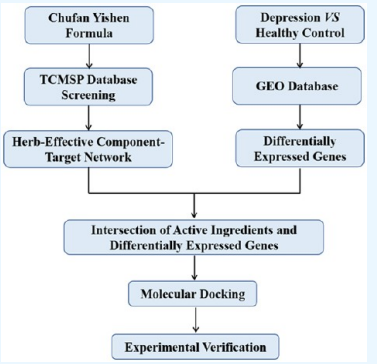
ABSTRACT: *Objective*: To investigate the antidepressant effect and potential mechanism of the Chufan Yishen Formula (CFYS) through network pharmacology, molecular docking, and experimental verification. *Methods*: The active ingredients and their target genes of CFYS were identified through Traditional Chinese Medicine Systems Pharmacology (TCMSP) and TCM-ID. We obtained the differentially expressed genes in patients with depression from the GEO database and screened out the genes intersecting with the target genes of CFYS to construct the PPI network. The key pathways were selected through STRING and KEGG. Then, molecular docking and experimental verification were performed. *Results*: A total of 113 effective components and 195 target genes were obtained. After intersecting the target genes with the differentially expressed genes in patients with depression, we obtained 37 differential target genes, among which HMOX1, VEGFA, etc., were the key genes. After enriching the differential target genes by KEGG, we found that the "chemical carcinogenesis-reactive oxygen species" pathway was the key pathway for the CFYS antidepressant effect. Besides, VEGFA might be a key marker for depression. Experimental verification found that CFYS could significantly improve the behavioral indicators of rats with depression models, including improving the antioxidant enzyme activity and increasing VEGFA levels. The results are consistent with the network pharmacology analysis. *Conclusions*: CFYS treatment for depression is a multicomponent, multitarget, and multipathway complex process, which may mainly exert an antidepressant effect by improving the neuron antioxidant stress response and regulating VEGFA levels.



1. INTRODUCTION

Depression is a mental illness characterized by persistent low mood and cognitive dysfunction, including symptoms such as anhedonia, inattention, sleep disorders, reduced appetite, and recurring suicidal thoughts.[1] In China, the number of people with depression exceeds 95 million, making depression the second most significant national disease burden. It has a high disability and mortality rate, severely affecting the health of the population.[2,3]

The etiology and pathogenesis of this disease are still unclear. Based on extensive clinical research, it is believed that genetic factors, neurobiochemical factors, psychological factors, and social factors all have a significant impact on its onset.[4−6] The neurobiochemical factors mainly include a deficiency in 5-hydroxytryptamine (5-HT) synthesis, changes in the hypothalamic−pituitary−adrenal (HPA) axis, neuroplasticity and neurogenesis, and alterations in the brain structure and function.[6] Western medicine treatment consists of nonpharmacological and pharmacological approaches. Typical nonpharmacological treatments include psychotherapy and physical exercise. While these treatments do not have drug-related side effects, they act slowly and can be easily influenced by patients' subjective feelings.[7] Pharmacological treatments mainly consist of antidepressants and other drugs with antidepressant effects. The most commonly used in clinical practice are the newer antidepressants, which regulate the functions of monoamine neurotransmitters in the brain, such as 5-HT, dopamine (DA), and noradrenaline (NA). In China, the most frequently used antidepressants belong to this category.[8−10] Though Western medications act quickly, they often lead to side effects, such as gastrointestinal reactions, dizziness, and blurred vision. Additionally, they can lead to drug resistance, and their long-term efficacy is questionable. Clinical studies show that even among patients who respond to treatment, the recurrence rate of depression can be as high as 70%.[11] Therefore, there is an urgent need to understand the mechanisms of depression and find more effective treatments to improve clinical outcomes.

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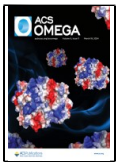

Traditional Chinese medicine (TCM) has shown notable clinical efficacy in treating depression, with certain advantages over Western medicine in terms of side effects and long-term efficacy.[2,12,] When combined with Western medication, TCM not only enhances the antidepressant effect but also mitigates some side effects. Furthermore, TCM offers a holistic treatment approach, with multicomponent and multitarget action, which is a key feature of its clinical application.[12,14] The Chufan Yishen Formula (CFYS) is an in-hospital formulation of our institution (approval number: Su Medicine System Z04001408), with effects such as resolving both the exterior and interior and calming the mind. It has proven to be effective in clinical practice for treating depression. However, due to the complexity of the active components in TCM, understanding their exact mechanisms remains a challenge.

The rapid development of network pharmacology in recent years has provided a novel approach to studying TCM. This interdisciplinary field leverages databases, high-throughput omics technologies, bioinformatics, and network visualization tools to build multidimensional biological network models. By analyzing these networks, researchers can identify key nodes and understand how drugs intervene with disease-causing networks, paving the way for predicting active components, targets, and potential mechanisms of action.[15−17] Given its compatibility with the multicomponent nature of TCM, network pharmacology can offer a new perspective on understanding and validating efficacy and safety. Therefore, this research intends to explore the mechanisms of CFYS in treating depression from a modern medical perspective using network pharmacology and experimental verification. This will provide a foundation for further promotion of the clinical application of CFYS.

2. MATERIALS AND METHODS

2.1. Data Collection. The GSE76826 transcriptome data set of blood samples from depression patients was downloaded from the GEO database (https://www.ncbi.nlm.nih.gov/geo/) for analysis. The GSE54570 depression data set was also downloaded for diagnosis efficiency validation. The GEO data set was processed according to the following standards: probes were converted into symbols based on the corresponding relationship on each platform. Probes that corresponded to multiple genes were removed, and if multiple probes corresponded to the same symbol, the average was taken. The GSE76826 data set contains transcriptome data from 20 patients with depression and 12 healthy individuals. The GSE54570 data set includes transcriptome data from 13 patients with depression and 13 healthy individuals.

2.2. Identification of Active Ingredients. The chemical components and targets of CFYS, which contains nine types of Chinese herbs (Bupleurum, Paeonia lactiflora, Rehmannia, Chuanxiong, Angelica, Dragon bone, Oyster, Alisma, and Licorice), were obtained using the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP, https://tcmsp-e.com/tcmsp.php). No information about dragon bone and oyster was found in the TCMSP database. Five components of oyster were obtained from the TCM-ID database (https://www.bidd.group/TCMID/), four of which had OB values below 30% in the TCMSP; one component (TCM-ID: TCMC2018) was predicted by SwissADME (http://www.swissadme.ch/), only one of the five drug properties (Lipinski, Ghose, Veber, Egan, and Muegge) was "yes", and the GI absorption was low, indicating that it is not an active substance. One component of dragon bone was obtained from the TCM-ID database, which is the same as one of the components in oyster (TCM-ID: TCMC2018), indicating that it is not an active substance. Therefore, we analyzed only the seven Chinese herbs (Bupleurum, Paeonia lactiflora, Rehmannia, Chuanxiong, Angelica, Alisma, and Licorice) in CFYS. The target genes were converted from targets using the protein gene correspondence in the Uniprot database.

2.3. Construction of Network of Active Components and Corresponding Target Genes. The association network was visualized using Cytoscape v3.9.1 software using the effective components and corresponding target gene data from the TCMSP database.

2.4. Disease-Related Differentially Expressed Genes and Enrichment Analysis. The limma package in R was used to determine differentially expressed genes in depression patients. Genes that were differentially expressed between the disease group and the normal group were screened based on a significance threshold p-value <0.05. GO analysis is a primary bioinformatics tool for gene annotation and its products, including three categories: cellular components (CCs), molecular functions (MFs), and biological pathways (BPs). KEGG is a collection of databases, which contains information about genomes, biological pathways, diseases, and chemicals. The clusterProfiler package was used to perform GO functional enrichment and KEGG pathway analysis on the differentially expressed genes to predict their potential molecular functions. A p-value <0.05 was considered statistically significant.

2.5. Screening for Potential Key Pathways. KEGG is a collection of databases, including information on genomes, biological pathways, diseases, and chemicals. The clusterProfiler package was used to perform KEGG pathway analysis on the differential target genes of CFYS, screening for potential key pathways of drug action. A p-value <0.05 was considered statistically significant.

2.6. Molecular Docking. The structures of the active components were obtained from the TCMSP database. Charges were adjusted, and torsion keys were detected using AutoDocktools, and they were saved as pdbqt files. The three-dimensional structure of the protein was obtained from the RCSB (https://www.rcsb.org/), prioritizing structures obtained via X-ray method, high-resolution, and having a ligand. The original ligand small molecule was removed by using PyMol to facilitate docking with other molecules. AutoDocktools was then used on this protein to dehydrate and hydrogenate, calculate charge, add atomic types, and save as a pdbqt file. Molecular docking was carried out using AutoDock vina, and the binding action diagram of the active components and proteins was displayed using PyMol.

2.7. Diagnostic Efficiency of Key Markers. The diagnostic efficiency of target genes of the potential pathway of drug action on disease was analyzed. The pROC package in R was used to analyze the diagnostic efficiency of target genes on depression. The same method was used for validation in the GSE54570 data set.

2.8. Experimental Verification. *2.8.1. Animal Grouping, Drug Administration, and CUMS Model Establishment.* After 1 week of adaptive feeding, rats were divided into control group (Control), model group (DP), and high-dose (DP + CFYS-5 g/kg), medium-dose (DP + CFYS-2.5 g/kg), and low-dose groups (DP + CFYS-1.25 g/kg) with six rats per group based on a random number table method. CUMS modeling was carried out in reference to the literature.[18,19] Stimuli included: 12 h of food

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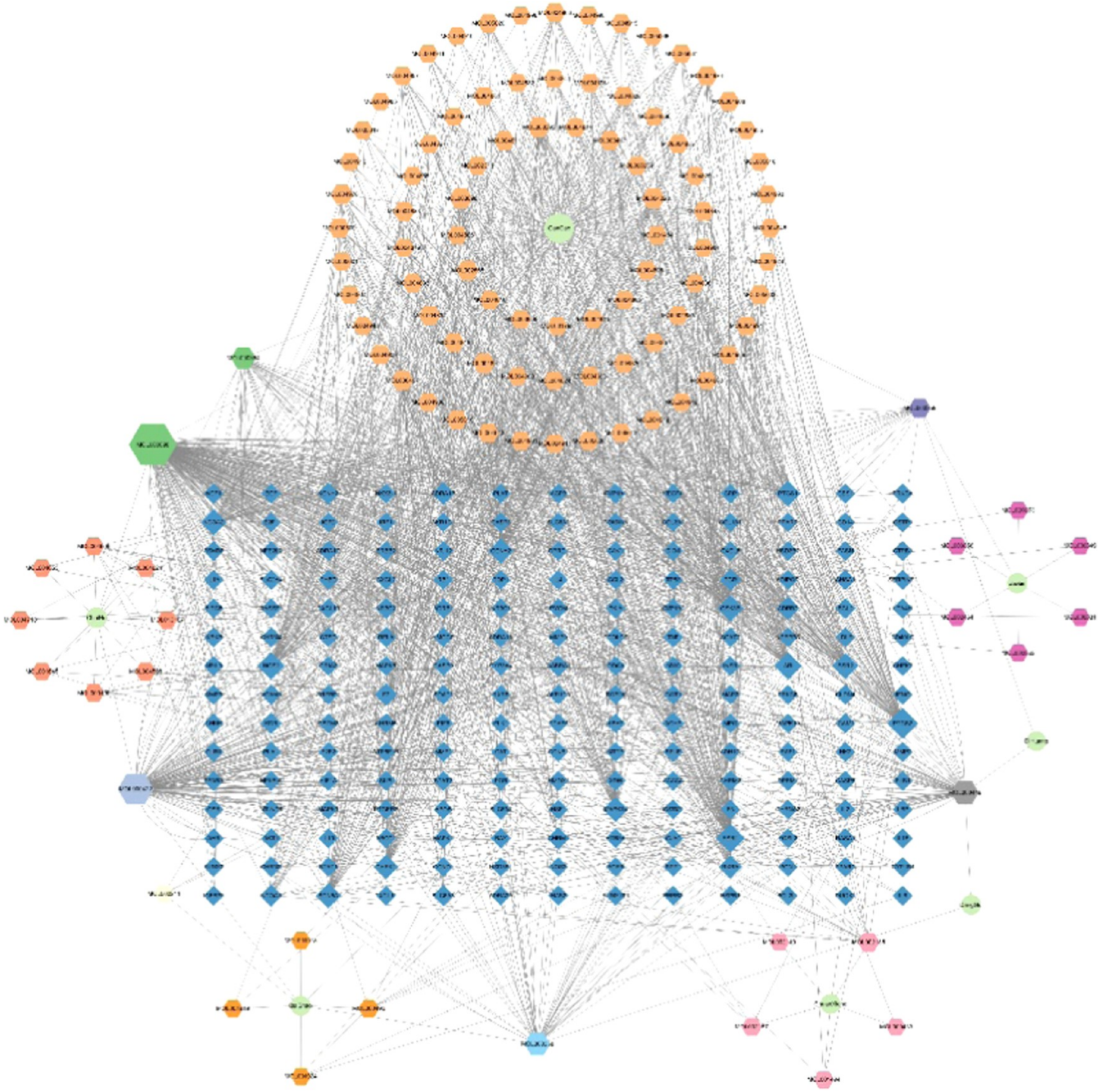

Figure 1. Herb-effective component-target network. The circles represent the herbal materials, the hexagons around the herbal materials represent the unique effective components of this kind of herbal material, and the independent hexagons (A1, A2, B1, C1, D1, E1, and F1) represent the effective components shared by two or more kinds of herbal materials. The diamonds in the middle represent the target genes, and the size of the figures represents the degree value.

deprivation, 12 h of water deprivation, reverse day and night, 8 h of wet padding, 1 h of 60 Hz noise stimulation, 2 min of tail clamping, 15 min of cage shaking, 5−10 min of swimming in 4 °C ice water, and 2 h of restraint. Two of the nine types of stimuli were randomly chosen each day, without repetition within 3 days, and the stimulus lasted 11 weeks. Drug intervention began in the seventh week of CUMS intervention, and the drug was continuously administered for 5 weeks. After 5 weeks of administration, behavioral tests were performed. After the behavioral tests, all rats were fasted overnight, euthanized with inhaled CO_2, and the entire brain tissue was removed. The right brain tissue was quickly frozen in liquid nitrogen and stored at −80 °C for later use, while the left-brain tissue was fixed in 4% polyformaldehyde and embedded in paraffin.

The CFYS ingredients were obtained from the Preparation Room for TCM in our hospital after quality control. The main procedure was the following: Bupleurum 100 g, Paeonia lactiflora 100 g, Rehmannia 100 g, Ligusticum striatum 100 g, Angelica sinensis 100 g, Dragon bone (Fossilia Ossis Mastodi) 300 g, Oyster shell (Concha Ostreae) 300 g, Alisma plantago-aquatica 100 g, and Licorice (Glycyrrhiza) 100 g. Soak in 5000 mL of water and simmer for 1 h, then retrieve the medicinal solution and add another 3000 mL of water, simmer for another hour, combine the medicinal solutions, filter, and let stand

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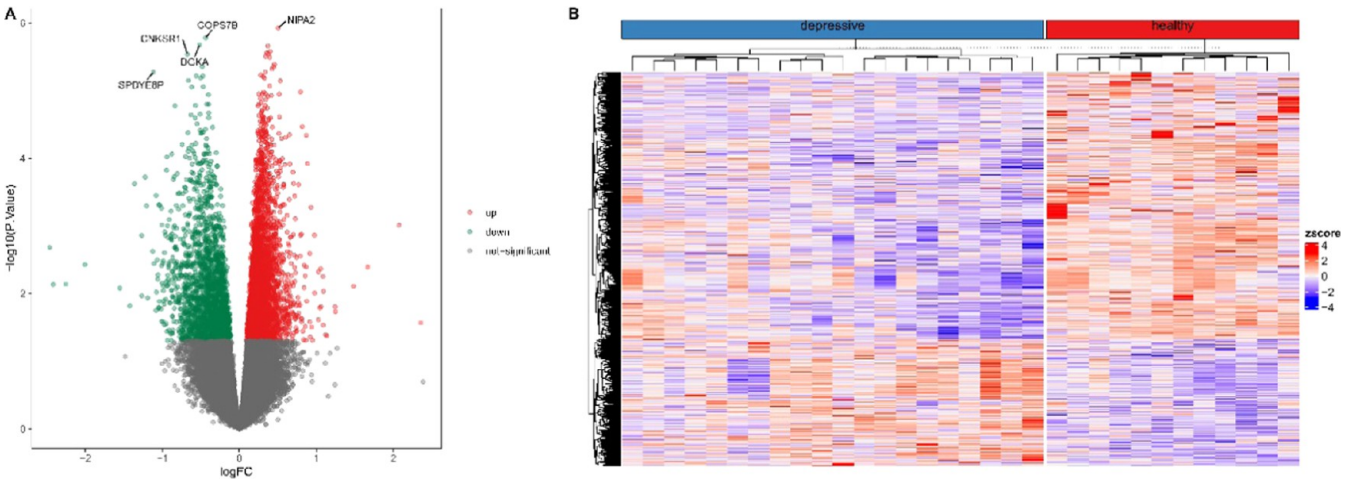

Figure 2. Differential expression analysis. (A) Volcano plot of the differential expression analysis; green represents downregulated genes, red represents upregulated genes, and gray represents genes with nonsignificant expression; (B) heatmap of differential gene clustering.

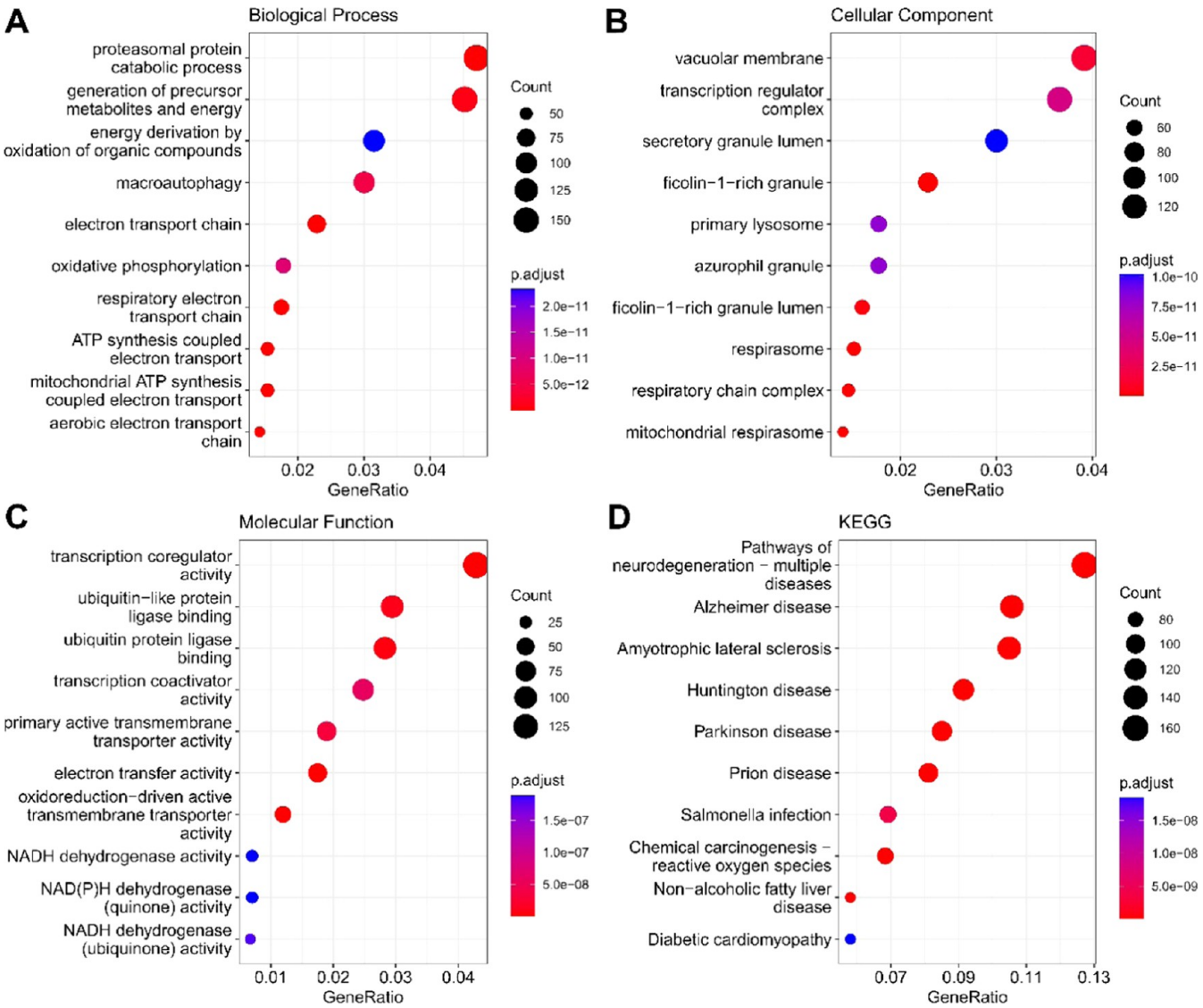

Figure 3. Enrichment analysis. (A) Biological process (BP) enrichment, (B) cellular component (CC) enrichment, (C) molecular function (MF) enrichment, and (D) KEGG pathway analysis.

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overnight, and then take the supernatant and concentrate to 950 mL. Stir while it is hot; add 4.0 g of sodium benzoate, let it settle for 48 h, and then add the solution of hydroxybenzoate dissolved in ethanol, add water to reach 1000 mL, stir, divide, and sterilize to obtain CFYS.

2.8.2. Behavioral Evaluation. Open field experiment: after the drug intervention, the rat's activity during a 5 min open field experiment was recorded, and the rat's immobility time, total movement distance, and number of grid crossings in the central area within the last 3 min were analyzed.

Forced swim test: food was restricted 12 h before the experiment without restricting water, and rats were placed in a swimming tank. The time rats remained immobile in the water for 5 min was recorded, i.e., the time when the rats stopped struggling and floated or only made minor limb movements to keep their heads above the water.

2.8.3. Detection of Oxidative Stress Indicators. Appropriate brain tissue was taken and washed with cold saline. A tissue homogenate (10% (w/v)) was prepared by homogenizing the tissue in cold saline (pH 7.0). The contents of Cu/Zn-superoxide dismutase (SOD), Mn-SOD, SOD, glutathione peroxidase (GSH-PX), catalase (CAT), glutathione (GSH), and malondialdehyde (MDA) were detected using test kits and measured under a fluorescence spectrophotometer.

2.8.4. Detection of VEGFA Content by ELISA. Appropriate brain tissue was taken, and phosphate-buffered saline (PBS) was added at a mass/volume ratio of 1:9. After grinding on ice with a hand-held homogenizer, it was centrifuged at a low temperature at 14,000 rpm for 15 min, and the supernatant was taken. The total protein in the supernatant was quantified using a BCA kit, and then the standard dilution, sample addition, and processes of incubation, washing, enzyme addition, incubation, washing, color development, and reaction termination were completed in strict accordance with the ELISA kit instructions. The optical density (OD) values of VEGFA in the rat brain tissue of each group were measured in order at a wavelength of 450 nm using an enzyme marker.

2.8.5. Statistical Analysis. Data were analyzed using SPSS 24.0 and presented as mean ± standard deviation ($x \pm s$). One-way analysis of variance was used for intergroup comparison, and LSD-t test was used for multiple comparisons between groups. $P < 0.05$ was considered statistically significant.

3. RESULTS

3.1. Compound-Target Network and Analysis. Effective components of the seven herbs in CFYS, Bupleurum, Paeonia lactiflora, Rehmannia, Ligusticum wallichii, Angelica, Alisma, and Licorice were obtained from the TCMSP database. Effective components were screened through OB ≥ 30% and DL ≥ 0.18, and a total of 113 effective components and 195 target genes were obtained. Based on the relationship among the 7 herbs in CFYS, 113 effective components, and 195 potential target genes, a herb-effective component-target network (Figure 1) was constructed. The results showed that the effective components quercetin (MOL000098) and kaempferol (MOL000422) had the most target genes and may play a more important role.

3.2. Differential Expression Analysis and Function Enrichment. In order to study the differences in gene expression between patients with depression and normal people, we used the "limma" package to perform differential expression analysis between different samples, with a differential gene screening condition of p-value <0.05. We obtained 5031 genes that are differentially expressed in patients with depression, of which 3039 are upregulated genes and 2042 are downregulated genes (Figure 2). We then used clusterProfiler for enrichment analysis. The results showed that these differentially expressed genes were mainly enriched in vesicle membrane, transcriptional regulatory complexes, interacting with transcription coregulatory factor activity, ubiquitin-like protein ligase binding, participating in the proteasome protein degradation metabolic process, precursor metabolites, energy production, etc. (Figure 3A−C). KEGG analysis results showed that differentially expressed genes are mainly enriched in various neurologically relevant diseases, such as neurodegenerative diseases, Alzheimer's disease, etc. (Figure 3D).

3.3. Differential Target Genes. The intersection of differential genes (DEGs) and CFYS target genes (X-TGs) resulted in 37 CFYS acting as differential target genes (Figure 4).

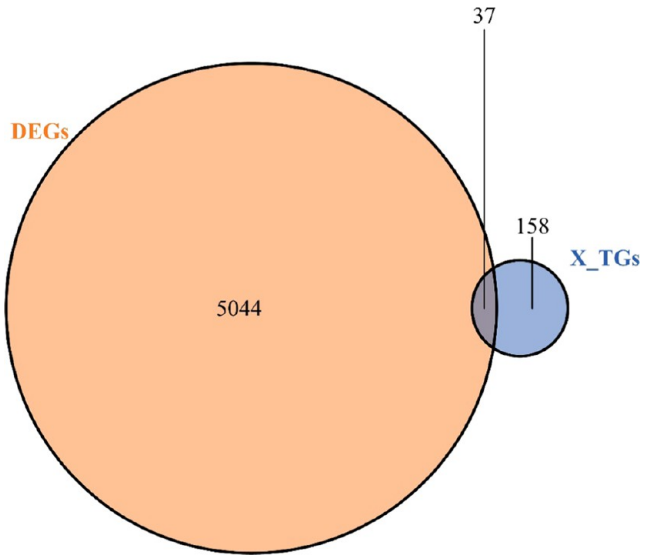

Figure 4. Differential target genes of CFYS.

To explore the interaction of differential target genes, we used the String database to construct a PPI network based on differential target genes (Figure 5A). We used Cytoscape v3.9.1 software to visualize the PPI network. Since genes GABRA1 and ABAT have no interaction with other genes, the PPI network only has interaction relations with 35 genes. The MCODE was used to identify key modules, and based on the submodules (Figure 5B,C), it can be known that genes such as HMOX1, VEGFA, etc., are key genes among the differential target genes.

3.4. Key Pathway of CFYS. To explore the key pathway of CFYS, we used clusterProfiler to conduct KEGG pathway analysis on the 37 differential target genes. A total of 120 pathways were enriched ($p < 0.05$). We sorted by the size of the P-value and selected the top 15 pathways for display (Figure 6). In addition, the pathway related to depression, "chemical carcinogenesis-reactive oxygen species", is one of the top 15 pathways.[12]

3.5. Active Ingredient and Target Gene Affinity. Through Figures 3D and 6, we can confirm that the "chemical carcinogenesis-reactive oxygen species" pathway is a key pathway in depression. Therefore, we further studied the combination activity of 10 "chemical carcinogenesis-reactive oxygen species" pathway-enriched genes and their corresponding 52 CFYS active ingredients, including GSTM2, CHUK, GSTM1, HMOX1, NFKBIA, VEGFA, MAPK1, CYP1B1,

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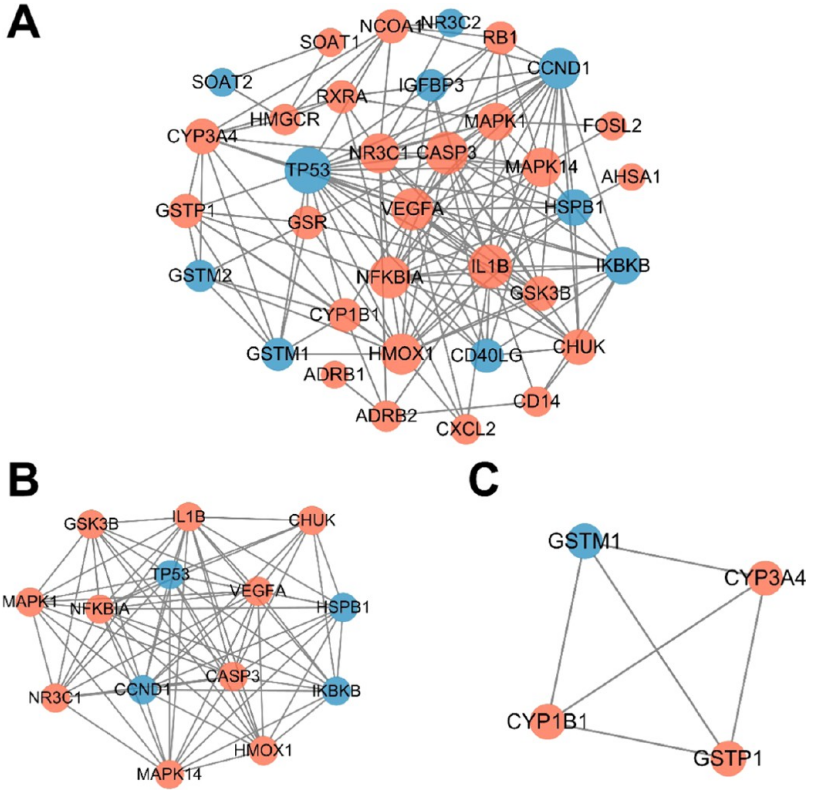

Figure 5. PPI network. (A) CFYS acting differential target gene interaction network and (B,C) two key submodules identified by MCODE in Cytoscape software; red represents differential upregulated genes, blue represents differential downregulated genes, and the area of the node represents the degree value.

MAPK14, and IKBKB. We carried out 64 docking combinations. In the AutoDock vina results, the smallest score indicates the best binding. The conformation with the smallest binding energy in each gene was selected for display (Figure 7).

3.6. Diagnostic Efficacy of Key Markers. Based on the 37 differential target genes acted on by CFYS, we used the pROC package to calculate the marker's diagnostic effect on depression and plotted the ROC curve. The same method was used in the validation set GSE54570 to validate the marker's diagnosis. The ROC is a curve composed of the true positive rate and false positive rate, and the AUC value represents the probability that the true positive is greater than the false positive. The results showed that VEGFA has a good predictive ability for depression (Figure 8).

3.7. Effect of CFYS on CUMS Rats. The results are listed in Table 1. It can be seen that the immobility time of the depressed model rats significantly increased in the open field test, while the total movement distance shortened, and the number of grid crossings in the central area decreased, indicating that the model was successfully constructed. After intervention with CFYS, the aforementioned behavioral indicators of depressed rats significantly improved.

As shown in Figure 9, the results show that in the model group, the activity of antioxidant enzymes in rats significantly decreased, including the levels of SOD, Mn-SOD, Cu/Zn-SOD, GSH, GSH-PX, and CAT decreased (Figure 9A−F), while the MDA level increased (Figure 9G). Intervention with CFYS could significantly reverse these changes and show a dose-dependent relationship. Also, the study found that iron ion levels were significantly increased in the model group (Figure 9H), and CFYS could improve the VEGFA level in a dose-dependent manner. These results suggest that CFYS may treat depression by antagonizing oxidative stress.

4. DISCUSSION

In this study, we explored the mechanism of CFYS in the treatment of depression through network pharmacology and experimental verification. First, we used the method of network pharmacology to obtain the effective components and target genes of CFYS from the TCMSP database. The analysis results show that two effective components, quercetin (MOL000098) and kaempferol (MOL000422), have the most target genes. Existing research confirms that quercetin can play a neuroprotective role through its antioxidant, anti-inflammatory, antiapoptotic properties, and anticalcium overload.[21−23] It can exert antidepressant effects through various mechanisms, including regulating the imbalance of triggering receptors expressed on myeloid cells 1/2 (TREM1/2)[24] and nuclear factor E2-related factor 2 (Nrf2) signaling pathways.[25] Kaempferol can exert a neuroprotective effect by regulating various proinflammatory signaling pathways, such as nuclear factor κB (NF-kB) and p38 mitogen-activated protein kinase (p38MAPK).[26] Kaempferol can exert an antidepressant effect by mediating neuroinflammation and oxidative stress responses through the AKT/β-catenin pathway.[27] Therefore, the effective components in CFYS, including quercetin and kaempferol, may be the main material basis for its antidepressant effects.

We further analyzed the expression differences of these target genes in depression and the biological processes and pathways in which they participated. Enrichment analysis revealed that the biological processes participated by differential genes, such as vesicle membrane, transcriptome regulation complex, protea-

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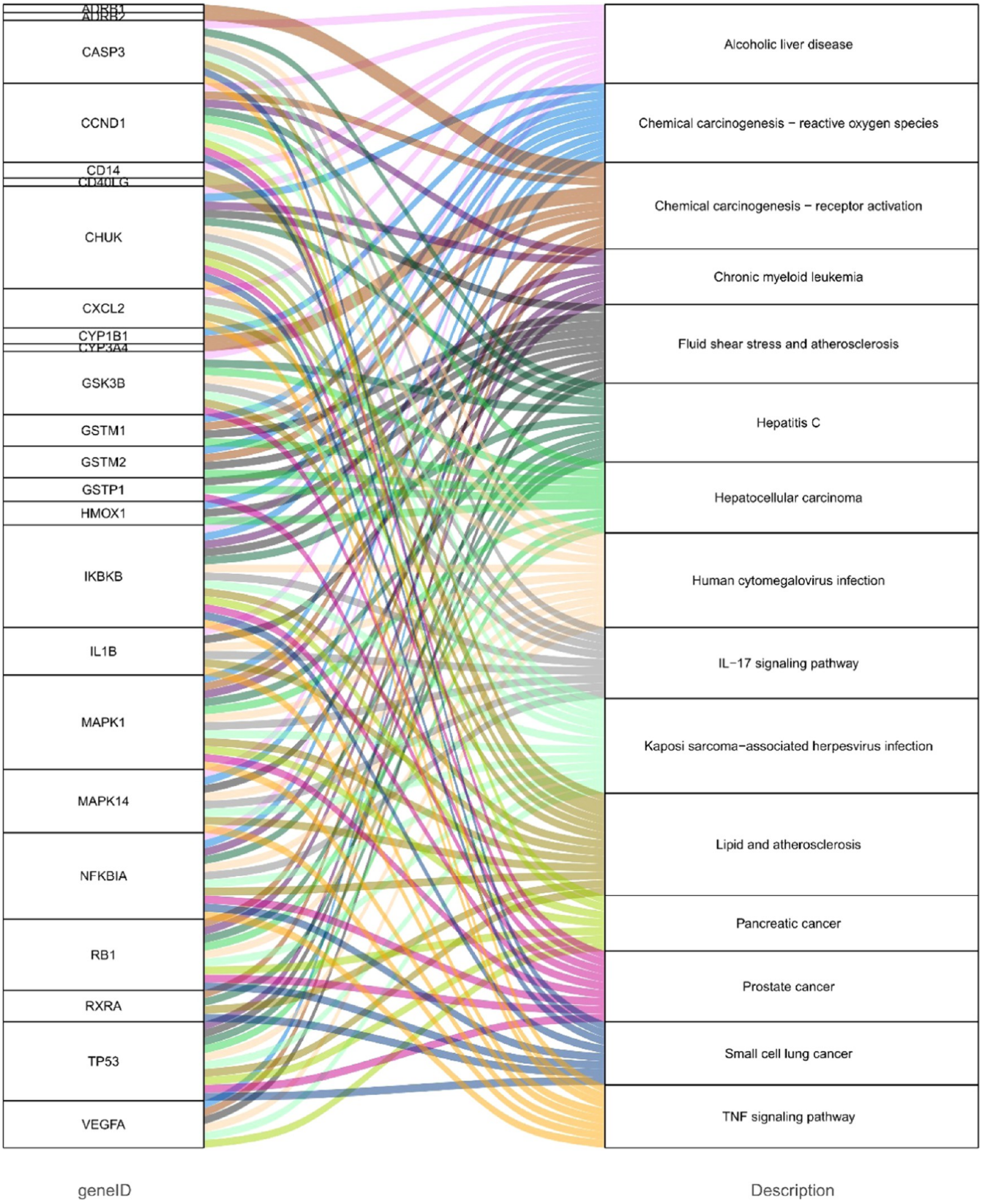

Figure 6. KEGG pathway analysis.

some protein degradation metabolic process, etc. These pathways all play a certain role in the progression of depression.[28−30] This may be one of the mechanisms by which CFYS treats depression. In addition, the study successfully constructed a PPI network, among which HMOX1 and VEGFA showed higher degrees of association and were key nodes in this network, indicating that the antidepressant effect of CFYS active ingredients is related to

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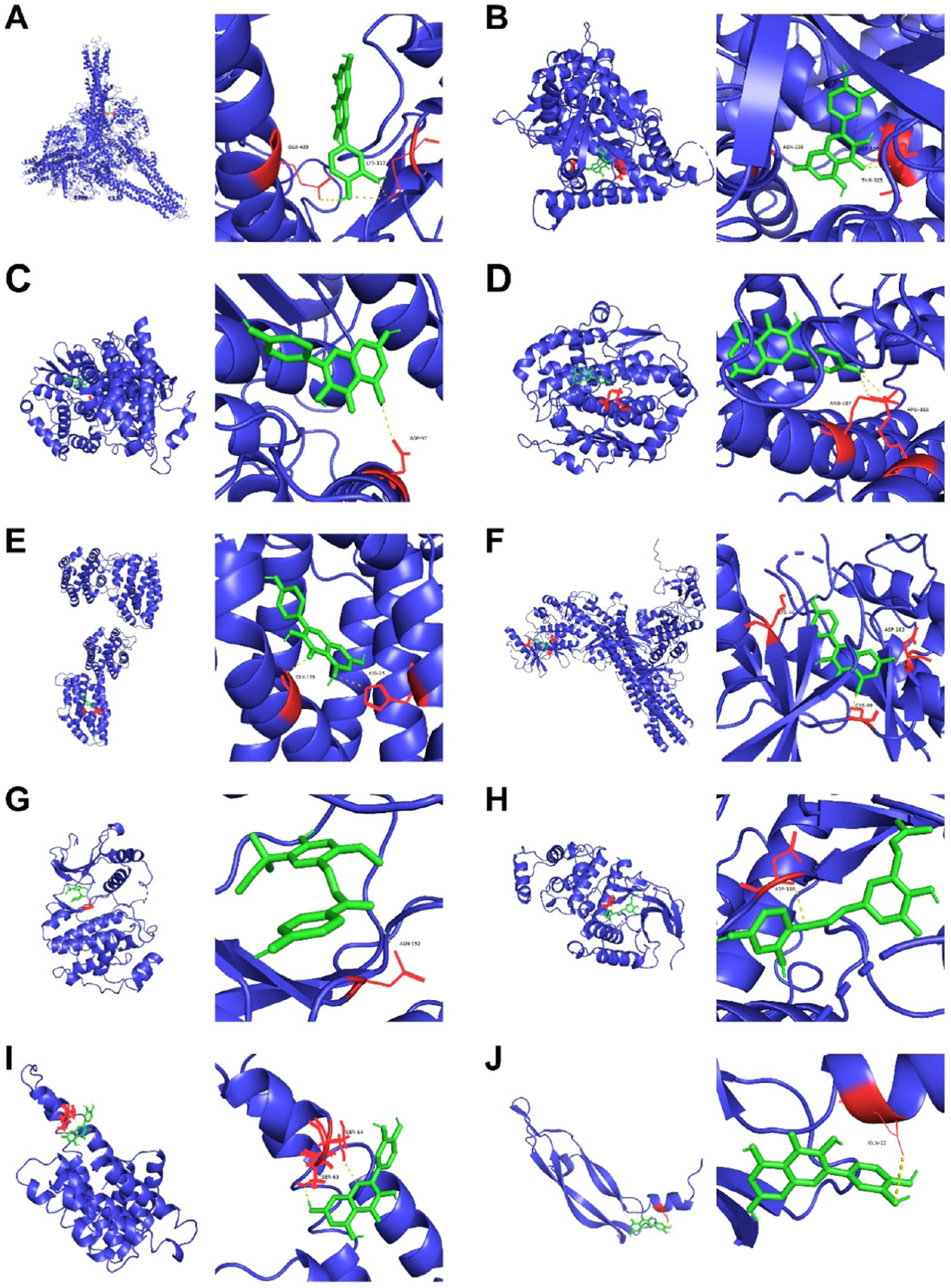

Figure 7. Typical molecular docking diagram. (A) The conformation has the smallest binding energy of CHUK and quercetin (−7.6), (B) the conformation with the smallest binding energy of CYP1B1 and quercetin (−10.0), (C) the conformation with the smallest binding energy of GSTM1 and kaempferol (−6.0), (D) the conformation with the smallest binding energy of GSTM2 and kaempferol (−6.9), (E) the conformation with the smallest binding energy of HMOX1 and kaempferol (−7.2), (F) the conformation with the smallest binding energy of IKBKB and kaempferol (−9.4), (G) the conformation with the smallest binding energy of MAPK1 and licochalcone a (−8.4), (H) the conformation with the smallest binding energy of MAPK14 and (*E*)-3-[3,4-dihydroxy-5-(3-methylbut-2-enyl)phenyl]-1-(2,4-dihydroxyphenyl)prop-2-en-1-one (−9.2), (I) the conformation with the smallest binding energy of NFKBIA and quercetin (−6.6), and (J) the conformation with the smallest binding energy of VEGFA and quercetin (−5.0).

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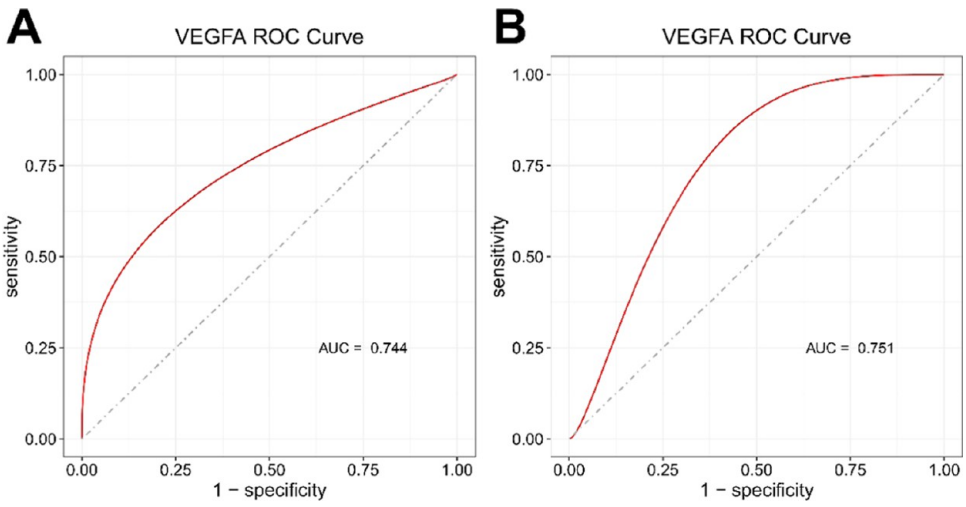

Figure 8. Diagnostic efficacy of VEGFA. (A) ROC curve of the VEGFA gene diagnosing depression in the training set GSE76826, (B) ROC curve of the VEGFA gene diagnosing depression in the validation set GSE54570.

Table 1. Effect of CFYS on the Behavior of Depression Model Rats ($\bar{x} \pm s$)[a]

groups	dose (g/kg)	immobility time	total moving distance	central area grid crossings	nonmovement time
control		56.28 ± 8.23	802.33 ± 37.21	21.42 ± 4.36	26.06 ± 9.55
DP		132.33 ± 11.47[a]	416.91 ± 24.07[a]	11.82 ± 3.85[a]	71.12 ± 6.73[a]
DP-1.25	1.25	117.23 ± 9.38[ab]	519.92 ± 22.25[ab]	14.83 ± 3.44[ab]	53.92 ± 6.37[ab]
DP-2.5	2.5	93.06 ± 8.30[ab]	565.84 ± 24.12[ab]	17.39 ± 4.06[ab]	43.72 ± 7.46[ab]
DP-5	5	82.58 ± 7.26[ab]	662.40 ± 25.38	18.93 ± 4.17[ab]	35.13 ± 5.057[ab]
DP + FXT		87.74 ± 7.54[ab]	573.59 ± 28.56[ab]	16.92 ± 3.63[ab]	42.94 ± 7.83[ab]

[a]Compared with the control group, [a]$P < 0.05$; compared with the model group, [b]$P < 0.05$.

these targets. HMOX1 is an important metabolic enzyme for iron metabolism and antioxidative stress.[31,32] HMOX1 plays an important role in the antidepressant effect of traditional Chinese medicine under the assistance of NADPH and cytochrome P450 reductase, consuming O_2 to catalyze the degradation reaction of heme.[33,34] According to the neurotrophic depression hypothesis, VEGF may participate in the progression of depression.[35] VEGFA is one of the most effective angiogenic growth factors in the human body, whose basic mechanism is to promote angiogenesis and increase blood supply.[36] Increasing numbers of studies have shown that VEGFA can affect neurogenesis in multiple ways. VEGFA can bind to its receptor VEGFR2, mediate the expression of downstream effector genes, and promote the survival, migration, and proliferation of hippocampal neurons.[37,38] VEGFA also extensively participates in the signal transduction of hippocampal nerve cells and plays an important role in the proliferation, survival, and functional maintenance of hippocampal nerve cells.[39] The diagnosis of depression by differential target genes through the ROC curve analysis also found that VEGFA has a good predictive power for depression. This suggests that the study of the mechanism of the CFYS antidepressant effect may be related to oxidative stress and neuronal function regulation mechanisms.

Using clusterProfiler to perform KEGG pathway analysis on the 37 differential target genes, we found that the "chemical carcinogenesis-reactive oxygen species" pathway might be the key route in the CFYS treatment of depression. ROS and its mediated neuronal function regulation play significant roles in the progression of depression.[40−42] This result aligns with the results of the previous target gene analysis. Further molecular docking study of 10 genes enriched in the "chemical carcinogenesis-reactive oxygen species" pathway and their corresponding 52 effective CFYS components showed that these CFYS effective components could interact favorably with key regulatory genes in the "chemical carcinogenesis-reactive oxygen species" pathway. Research has shown that oxidative stress levels in the brains and peripheral fluids of patients with depression are elevated.[43] An increase in the ROS levels can lead to neuroinflammation, further exacerbating depressive symptoms. Chronic oxidative stress can damage cellular DNA, lipids, and proteins, subsequently affecting the plasticity of neural cells. This damage can result in impaired synaptic function and hindered neural regeneration, both of which are associated with the development of depression.[44] ROS can interfere with the metabolism of neurotransmitters, such as dopamine, cholecystokinin, and 5-hydroxytryptamine (serotonin), which might lead to the onset or exacerbation of depressive symptoms. ROS can also affect various cellular signaling pathways, such as NF-κB and MAPK pathways. These pathways play a crucial role in cellular survival, proliferation, differentiation, and apoptosis, and their abnormalities may be linked to the onset and progression of depression.[45]

Upon experimental verification, it was discerned that CFYS markedly ameliorated the behavioral indicators observed in the depressive model rats. A pivotal aspect of this improvement was the elevation in the activity of the antioxidant enzymes. Elevated oxidative stress, often noted by increased levels of ROS as discussed earlier, has consistently been linked to depression. An abundance of studies delineates the adverse effects of unchecked oxidative stress, including cellular damage and impaired neuronal plasticity, which, in turn, can aggravate depressive symptoms.[20,46] Thus, the enhanced activity of antioxidant

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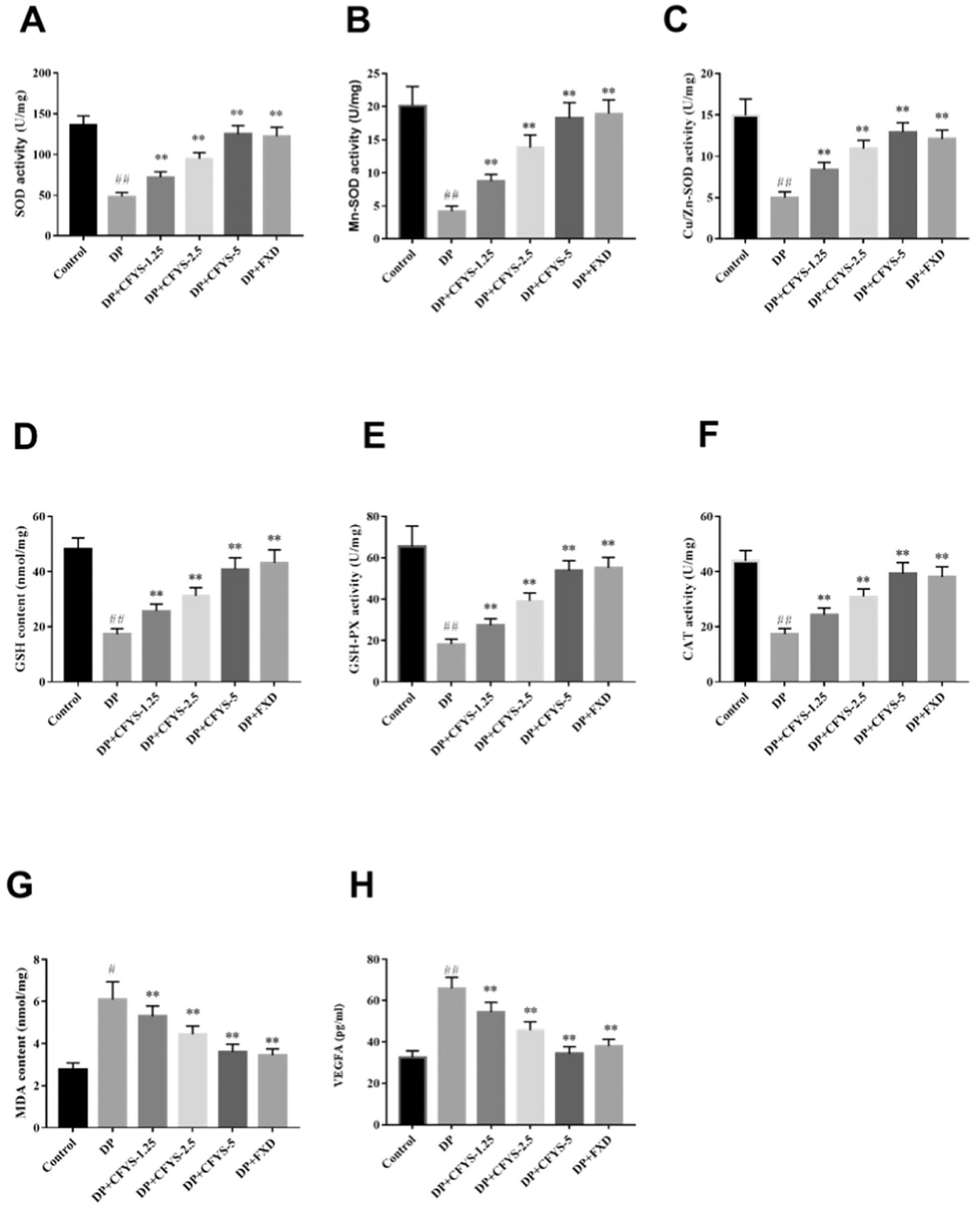

Figure 9. Effect of CFYS on oxidative stress indicators and iron ion levels in the hippocampus of depressed rats. (A) SOD, (B) Mn-SOD, (C) Cu/Zn-SOD, (D) GSH, (E) GSH-PX, (F) CAT, (G) MDA, and (H) VEGFA. **P vs DP < 0.001, #P < 0.05, ##P < 0.001.

enzymes, attributable to CFYS, is likely a protective mechanism, counteracting the detrimental impacts of oxidative stress. This

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observation aligns seamlessly with prior literature that emphasizes the role of antioxidants in mitigating depressive pathophysiology.

Moreover, our experimental results noted a reduction in the MDA levels in the treated rats. MDA, a byproduct of lipid peroxidation, serves as a tangible marker of oxidative stress and cellular damage. Elevated MDA levels have been previously linked with various neuropsychiatric disorders, including depression.[47] Therefore, the ability of CFYS to reduce MDA levels underpins its potential in curtailing lipid peroxidation and the associated neurotoxicity, reinforcing its therapeutic prospects. The observed surge in VEGFA levels in CFYS-treated rats has profound implications. VEGFA, beyond its angiogenic capabilities, has gained attention for its roles in neurogenesis and synaptic plasticity. As highlighted in prior sections, VEGFA's ability to promote the survival, migration, and proliferation of hippocampal neurons is of paramount significance in the context of depression.[37,38] By augmenting VEGFA levels, CFYS might foster an environment conducive to neuronal regeneration and synaptic strengthening. This is particularly relevant given the well-established theories positing impaired neurogenesis as a contributing factor to depression.

These results are consistent with the network pharmacology analysis and further support our hypothesis that CFYS might treat depression by improving the neuronal antioxidant stress response and regulating VEGFA levels. Unlike conventional antidepressants, which primarily target neurotransmitter regulation, CFYS offers a broader, more holistic approach. By potentially modulating oxidative stress responses and targeting key genes related to neurogenesis and angiogenesis, CFYS offers a treatment avenue that addresses both the symptoms and the potential underlying causes of depression. This nuanced mechanism distinguishes CFYS from other treatments and might explain its potential efficacy, where other treatments falter.

Despite combining network pharmacology and experimental verification methods, revealing the possible mechanism of CFYS in treating depression from multiple angles and providing important clues for further understanding the scientific basis of traditional Chinese medicine in treating depression and developing new treatment strategies for depression, our study has some limitations. For instance, mechanism research still needs to be further deepened. Additionally, our study mainly focuses on the association between the effective components of traditional Chinese medicine and their potential targets and depression, but how to effectively use these effective components and their potential targets in clinical treatment still needs to be explored more deeply.

5. CONCLUSIONS

In summary, this study retrieved the components and targets of CFYS from the TCMSP database and, after screening, obtained 113 effective components and 195 target genes. After intersecting the target genes with the differential genes of depression patients, we obtained 37 differential target genes. After KEGG enrichment of differential target genes and animal experiment verification, we found that CFYS might treat depression by improving the neuronal antioxidant stress response and regulating VEGFA levels. Moreover, VEGFA has the potential for early prediction of depression.

■ ASSOCIATED CONTENT

Data Availability Statement

The data set generated during and analyzed during the current study is available from the corresponding author on reasonable request.

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Author Contributions

Y.J. and H.Z. conceived the study; Z.D., Y.J., Q.Z., and R.L. collected the report; and Y.S. and H.Z. wrote and edited the manuscript. All authors have approved the publishment of the manuscript.

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Notes

The authors declare no competing financial interest.

The study involved human data of publicly available data from the databases, which was approved by the Ethics Committee of Wuxi Mental Health Center, with the approval number WXMHCIRB2022LLky008. This animal study was approved by the Ethics Committee of Jiangnan University, with the approval number JNERB2022019. All methods were performed in accordance with relevant guidelines and regulations.

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