Original Article

Mechanism of Chaihu Longgu oyster adjusted decoction for the treatment of depression based on network pharmacology and molecular docking technology

Chunlin Li[1], Bowen Li[2], Hui Liu[3], Linglong Qu[2], Hui Wang[4]

[1]Department of Neurology, Affiliated Hospital of Shandong University of Traditional Chinese Medicine, Jinan, China; [2]College of Traditional Chinese Medicine, Shandong University of Traditional Chinese Medicine, Jinan, China; [3]First Clinical Medical College, Shandong University of Traditional Chinese Medicine, Jinan, China; [4]Department of Acupuncture, Zaozhuang Traditional Chinese Medicine Hospital, Zaozhuang, China

Contributions: (I) Conception and design: C Li; (II) Administrative support: C Li; (III) Provision of study materials or patients: C Li; (IV) Collection and assembly of data: B Li; (V) Data analysis and interpretation: B Li; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Chunlin Li. Department of Neurology, Affiliated Hospital of Shandong University of Traditional Chinese Medicine, Jinan 250014, China. Email: 71000070@sdutcm.edu.cn.

Background: Depression is a common clinical psychiatric disorder that is responsible for health-related disease burdens globally. According to traditional Chinese medicine (TCM), mental disorders and qi stagnation are important pathogenic mechanisms of depression. The Chaihu Longgu Oyster Decoction, which has been documented in the Shanghanlun (Treatise on Typhoid), is widely used to treat various affective disorders.

Methods: Network pharmacology and molecular docking technology were used to investigate the material basis and mechanism of action of the Chaihu Longgu oyster adjusted decoction in treating depression. The main pharmacological substance bases, possible targets, and pathways of Chaihu Longgu oyster adjusted decoction in treating depression were visualized by constructing a "component-pathway-target" network.

Results: Quercetin, 7-methoxy-2-methylisoflavone, baicalein, kaempferol, and lignan are the main practical chemical components in Chaihu Longgu oyster adjusted decoction. The Chaihu Longgu oyster adjusted decoction regulates 74 protein targets and 142 pathways associated with depression. Its molecular mechanism involves inhibiting neuroinflammation and improving neurotransmitter function, neuroplasticity, etc.

Conclusions: The underlying mechanism of the anti-depressive effect of the Chaihu Longgu oyster adjusted decoction may involve neuroinflammatory response reduction and improvement of neurotransmitter function and neuroplasticity. This study revealed the mechanism of action of the Chaihu Longgu oyster adjusted decoction in the treatment of depression through network pharmacology, which provides a scientific basis for clinical application.

Keywords: Network pharmacology; Chaihu Longgu oyster adjusted decoction; molecular docking; depression; mechanism; components

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Introduction

Depression is a common clinical psychiatric disorder that is characterized by a persistently sad mood, lack of pleasure, reduced volitional activity, sleep disturbance, and even suicidal tendencies. In 2008, the World Health Organization (WHO) ranked major depression as the third largest leading cause of the global disease burden, and relevant epidemiological data indicate that the global prevalence of depression reached 4.4% in 2019, and it is expected to

become the leading disease burden by 2030 (1,2). The onset of depression is influenced by multiple factors such as genetic, psychological, biochemical, and social environment (3); however, the underlying mechanisms of its occurrence and development remain largely unclear. At present, the treatment of depression in Western medicine is mainly based on oral antidepressants such as promethazine, haloperidol tablets, and paroxetine hydrochloride. Yet, many problems with this treatment approach have been documented, such as the slow onset of action, numerous side effects, withdrawal symptoms, and poor compliance of patients.

Depression belongs to the "Yu Zheng" category in Chinese medicine (4). The Internal Medicine of Traditional Chinese Medicine (TCM) defines "Yu Zheng" as a group of diseases with clinical manifestations such as depression, emotional restlessness, fullness in the chest, swelling and rib pain, irritability and crying, and obstruction or foreign body sensation in the throat. The etiology of the disease is emotional injury, resulting in qi stagnation and dysfunction of the internal organs (5). The Chaihu Longgu Oyster Decoction, first documented in the Treatise on Typhoid, comprises Chai Hu, Huang Qin, Ban Xia, Long Gu, Oyster, Fu Ling, Ren Shen, Gui Zhi, Qian Dan, and Da Huang. It has the effect of reconciling Shaoyang and calming the nerves and is used to treat cold and fever, bitterness and fullness in the chest, as well as restlessness and agitation in China. Moreover, given its efficacy, it is also commonly used for the treatment of depression. Previous studies have established that the efficacy of the Chaihu Longgu Oyster Decoction is statistically significant (6-9). Adjustments have been made to the Chaihu Longgu Oyster Decoction, with the removal of Qian Dan and replacing Ren Shen with Dang Shen, yielding a remarkable clinical effect.

Network pharmacology is widely acknowledged to analyze the mechanism of action of active ingredients of drugs by constructing a complex "component-target-pathway-disease" network, which reflects the comprehensive network analysis thinking of pharmacological research (10,11). Furthermore, it can analyze the mechanisms of action of TCM prescription as a whole and independently describe the "active ingredient-target-metabolic pathway" related to specific diseases. The multi-target and multi-pathway pharmacological characteristics of TCM compound prescriptions for disease treatment are naturally compatible with network pharmacology (12-15).

Herein, network pharmacology was applied to analyze the active components and related pathway mechanisms of the Chaihu Longgu oyster adjusted decoction in treating depression from a molecular perspective. This study provides a theoretical basis for further biological mechanism research and clinical application.

Highlight box

Key findings

- The mechanism of the anti-depressive effect of the Chaihu Longgu oyster adjusted decoction is related to neuroinflammatory response reduction and the improvement of neurotransmitter function and neuroplasticity.

What is known and what is new?

- The Chaihu Longgu oyster adjusted decoction is often used to treat depression with remarkable effects.
- It exerts antidepressant-like effects dependent on the activation of downstream targets and pathways by active ingredients such as 7-methoxy-2-methyl isoflavone, wogonin, kaempferol, and luteolin.

What is the implication, and what should change now?

- The treatment of depression using Chaihu Longgu oyster decoction has been supported by theoretical and clinical effects, and thus, it can be applied as a treatment strategy for depression.

Methods

Building a database of the main active components in the Chaihu Longgu oyster adjusted decoction

The active chemical constituents and corresponding targets of eight Chinese phytomedicines (Chai Hu, Huang Qin, Dang Shen, Fu ling, Gui Zhi, Ban Zia, Da Huang, and Da Zao) in the Chaihu Longgu oyster adjusted decoction were retrieved from the Traditional Chinese Medicine System Pharmacology database (TCMSP, https://old.tcmsp-e.com/tcmsp.php) based on the following criteria: oral bioavailability (OB) ≥30% and drug sample (DL) ≥0.18. The Bioinformatics Analysis Tool for Molecular mechANism of the Traditional Chinese Medicine database (BATMAN-TCM, http://bionet.ncpsb.org.cn/batman-tcm/) was used to search for the chemical constituents of two mineral medicines (Long Gu, Oyster) in the Chaihu Longgu oyster adjusted decoction. The candidate compounds were comprehensively screened for further analysis using "Score cutoff >20" and "P>0.05" as the screening criteria.

Prediction of the component targets

The corresponding action targets of the effective chemical components were converted and corrected using the Uniprot database (http: //www.uniprot.org/). Then, the repeated

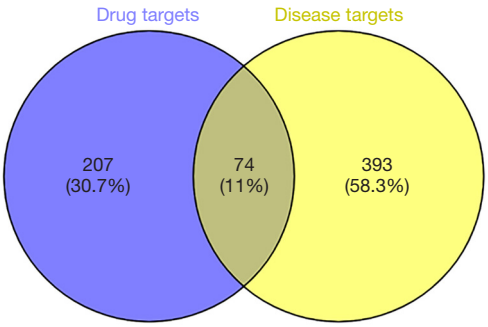

Figure 1 The intersecting targets of drugs and diseases.

targets of the active components in the Chaihu Longgu oyster adjusted decoction were screened out and eliminated.

Prediction of depression targets

The Disgenet (http://www.disgenet.org/search) and Genecards (http://www.genecards.org) databases were searched using the keywords "Depressive Disorder" and "mental depression" to predict the depression-related targets. Venny 2.1.0 (https://bioinfogp.cnb.csic.es/tools/venny/) was employed to map the targets of the active ingredients in the Chaihu Longgu oyster adjusted decoction and the depression-related targets, and the intersecting targets were obtained. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Construction and analysis of the protein-protein interaction (PPI) network

The intersecting targets of the Chaihu Longgu oyster adjusted decoction and depression were imported into STRING 11.0 software (http://string-db.org), and the species was limited to "Homo sapiens". The PPI network of the Chaihu Longgu oyster adjusted decoction was constructed by selecting data with confidence scores higher than 0.7. The core genes of the network were identified according to the number of genes and adjacent genes in the network.

Enrichment analysis of Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways

The data of core targets were imported into the Database for Annotation, Visualization and Integrated Discovery (DAVID) bioinformatics resource (https://david.ncifcrf.gov/tools.jsp), and the species was limited to "Homo sapiens" to analyze the annotated GO biological process (BP) and enriched KEGG pathways of the core targets.

Construction of the component-target-pathway network

The active ingredient-target-pathway co-expression network was constructed using Cytoscape 3.9 software (https://cytoscape.org/). The network's topological properties were analyzed using the Network Analyzer function to investigate the importance of nodes in the network based on parameters such as degrees of freedom, closeness, and betweenness.

Molecular docking

The top three target proteins in the PPI network underwent molecular docking simulation with the main active ingredients of the Chaihu Longgu oyster adjusted decoction using Autodock software (Scripps Research).

Statistical analysis

Protein-protein interaction analysis was performed using STRING 11.0, and pathway enrichment analysis was performed by Cytoscape 3.9. Molecular docking analysis of proteins and active ingredients was performed using Autodock Vina. The results of all relevant data analysis are presented in the Result section below.

Results

The active ingredients and targets of the Chaihu Longgu oyster adjusted decoction

Analysis on the TCMSP platform (using the search criteria OB ≥30%, DL ≥0.18) and the BATMAN database yielded a total of 162 active ingredients for Chai Hu (n=17), Huang Qin (n=37), Fu Ling (n=15), Dang Shen (n=21), Gui Zhi (n=7), Da Huang (n=16), Ban Xia (n=13), Da Zao (n=29), Long Gu (n=2), and Oyster (n=5). After removing the duplicate targets predicted by the active components, 281 targets were finally obtained.

The targets of depression

A score greater than one-third of the maximum score was used as the screening criterion, yielding 467 disease targets. The intersection of the targets of the Chaihu Longgu oyster adjusted decoction with depression-related targets yielded 74 common targets (*Figure 1*). After matching the

active components with the common targets, 88 active components were found to regulate the 74 targets, among which beta-sitosterol was the common compound among Huang Qin, Gui Zhi, Ban Xia, and Da Huang; stigmasterol was the common compound among Huang Qin, Dang Shen, and Ban Xia; sitosterol was the common compound among Huang Qin and Gui Zhi; etc. It is suggested that the same compound in the Chaihu Longgu oyster adjusted decoction corresponds to many different Chinese medicinal materials, and multiple compounds can correspond to the same target. The 88 active ingredients are shown in *Table 1* (16).

Construction of the PPI network and analysis of the core targets

The 74 intersecting target proteins were uploaded onto the String database to obtain the PPI network, which consisted of 74 nodes and 266 edges (*Figure 2*). The "degree" is an important topological characteristic, with higher values indicating more edges connected to the point, highlighting a relationship with more targets and hence its importance in the network. The results were imported into Cytoscape 3.9 software for visualization and network topology analysis (*Figure 3*). The targets were further screened according to the degree cutoff ≥12 (greater than their median degree). A total of 18 key protein nodes were obtained, and the eligible targets with scores are shown in *Figure 4*. The top five key targets included AKT serine/threonine kinase 1 (AKT1, degree =24), tumor necrosis factor (TNF, degree =24), interleukin-6 (IL-6, degree =22), vascular endothelial growth factor (VEGFA, degree =21), and epidermal growth factor receptor (EGFR, degree =20).

GO annotation and KEGG pathway enrichment analysis

GO annotation was used to identify the significantly enriched GO terms for the 74 potential therapeutic targets. A total of 584 GO items were obtained, including 439 BPs, 57 cellular components (CCs), and 88 molecular functions (MFs). The top 20, 10, and 10 items with $P<0.05$, respectively, were selected for visualization and analysis by sorting the P values from smallest to largest (*Figure 5*). The significantly enriched BPs of the Chaihu Longgu oyster adjusted decoction included the positive regulation of gene expression, response to the drug, response to xenobiotic stimulus, signal transduction, regulation of synaptic vesicle exocytosis, etc. The significantly enriched GO terms in the CC category included components of the presynaptic membrane, plasma membrane, postsynaptic membrane, etc. Finally, significantly enriched GO terms in the MF category mainly included neurotransmitter receptor activity, enzyme binding, serotonin binding, etc.

KEGG pathway enrichment analysis of the 74 potential therapeutic targets yielded 142 signaling pathways. The top 20 items were selected for analysis ($P<0.01$ and the number of genes ≥7) (*Figure 6*). We found that the advanced glycation end products (AGE)-receptor of AGE (RAGE) signaling pathway, IL-17 signaling pathway, dopaminergic synapse, serotonergic synapse, the hypoxia inducible factor (HIF)-1 signaling pathway, the calcium signaling pathway, and the cyclic adenosine monophosphate (cAMP) signaling pathway played important roles in the treatment of depression using the Chaihu Longgu oyster adjusted decoction.

Compound-target-pathway network construction

Cytoscape 3.9 software was used to construct the compound-target-pathway network (*Figure 7*), including 182 nodes and 675 edges, with blue nodes representing the active ingredients, pink nodes representing the targets, and purple nodes representing the pathways. The network analysis results showed that the degree value of quercetin was 36, the betweenness was 4,095.326, and the closeness was 0.1913, suggesting that quercetin is the main active ingredient of the Chaihu Longgu oyster adjusted decoction in the treatment of depression, followed by 7-methoxy-2-methyl isoflavone (degree value =16, betweenness =890.7535, closeness =0.18357) and wogonin (degree value =15, betweenness =627.0686, closeness =0.1839), as shown in *Table 2*.

Molecular docking

Molecular docking is the process of mutual recognition between molecules and targets through geometric and energy matching. The binding strength and activity of compounds and target proteins can be evaluated based on the binding energy value. A negative binding energy value suggests that the ligand and receptor can bind spontaneously. A binding energy value <−5 kcal/mol indicates good binding activity, and the lower the binding energy value, the better the docking effect. The top three target proteins in the PPI network underwent molecular docking simulation with the main active ingredients of the Chaihu Longgu oyster adjusted decoction (*Figure 8*). The

Table 1 The active ingredients in the Chaihu Longgu oyster adjusted decoction (16)

Symbol	MOL	Name	OB (%)	DL	Source
CH1	MOL001645	Linoleyl acetate	42.1	0.2	Chai Hu
CH2	MOL000354	isorhamnetin	49.6	0.31	Chai Hu
CH3	MOL000422	kaempferol	41.88	0.24	Chai Hu
CH4	MOL004598	3,5,6,7-tetramethoxy-2-(3,4,5-trimethoxyphenyl)chromone	31.97	0.59	Chai Hu
CH5	MOL004609	Areapillin	48.96	0.41	Chai Hu
CH6	MOL013187	Cubebin	57.13	0.64	Chai Hu
CH7	MOL004624	Longikaurin A	47.72	0.53	Chai Hu
CH8	MOL004653	(+)-Anomalin	46.06	0.66	Chai Hu
CH9	MOL004718	α-spinasterol	42.98	0.76	Chai Hu
CH10	MOL000490	Petunidin	30.05	0.31	Chai Hu
A3	MOL000098	Quercetin	46.43	0.28	Chai Hu, Da Zao
HQ1	MOL001689	Acacetin	34.97	0.24	Huang Qin
HQ2	MOL000173	Wogonin	30.68	0.23	Huang Qin
HQ3	MOL000228	(2R)-7-hydroxy-5-methoxy-2-phenylchroman-4-one	55.23	0.2	Huang Qin
A4	MOL002714	Baicalein	33.52	0.21	Huang Qin, Ban Xia
HQ4	MOL002909	5,7,2,5-tetrahydroxy-8,6-dimethoxyflavone	33.82	0.45	Huang Qin
HQ5	MOL002910	Carthamidin	41.15	0.24	Huang Qin
HQ6	MOL002913	Dihydrobaicalin_qt	40.04	0.21	Huang Qin
HQ7	MOL002914	Eriodyctiol (flavanone)	41.35	0.24	Huang Qin
HQ8	MOL002915	Salvigenin	49.07	0.33	Huang Qin
HQ9	MOL002917	5,2',6'-Trihydroxy-7,8-dimethoxyflavone	45.05	0.33	Huang Qin
HQ10	MOL002925	5,7,2',6'-Tetrahydroxyflavone	37.01	0.24	Huang Qin
HQ11	MOL002927	Skullcapflavone II	69.51	0.44	Huang Qin
HQ12	MOL002928	oroxylin a	41.37	0.23	Huang Qin
HQ13	MOL002932	Panicolin	76.26	0.29	Huang Qin
HQ14	MOL002933	5,7,4'-Trihydroxy-8-methoxyflavone	36.56	0.27	Huang Qin
HQ15	MOL002934	NEOBAICALEIN	104.34	0.44	Huang Qin
HQ16	MOL002937	DIHYDROOROXYLIN	66.06	0.23	Huang Qin
A5	MOL000358	Beta-sitosterol	36.91	0.75	Huang Qin, Gui Zhi, Ban Xia, Da Huang
A6	MOL000359	Sitosterol	36.91	0.75	Huang Qin, Gui Zhi
HQ17	MOL000525	Norwogonin	39.4	0.21	Huang Qin
HQ18	MOL000552	5,2'-Dihydroxy-6,7,8-trimethoxyflavone	31.71	0.35	Huang Qin
A7	MOL000073	ent-Epicatechin	48.96	0.24	Huang Qin, Gui Zhi
A2	MOL000449	Stigmasterol	43.83	0.76	Huang Qin, Dang Shen, Ban Xia

Table 1 (*continued*)

Table 1 (*continued*)

Symbol	MOL	Name	OB (%)	DL	Source
HQ19	MOL001458	Coptisine	30.67	0.86	Huang Qin
A8	MOL002879	Diop	43.59	0.39	Huang Qin, Dang Shen
HQ20	MOL002897	epiberberine	43.09	0.78	Huang Qin
HQ21	MOL008206	Moslosooflavone	44.09	0.25	Huang Qin
HQ22	MOL012245	5,7,4'-trihydroxy-6-methoxyflavanone	36.63	0.27	Huang Qin
HQ23	MOL012246	5,7,4'-trihydroxy-8-methoxyflavanone	74.24	0.26	Huang Qin
HQ24	MOL012266	Rivularin	37.94	0.37	Huang Qin
DS1	MOL001006	poriferasta-7,22E-dien-3beta-ol	42.98	0.76	Dang Shen
DS2	MOL002140	Perlolyrine	65.95	0.27	Dang Shen
DS3	MOL003036	ZINC03978781	43.83	0.76	Dang Shen
DS4	MOL003896	7-Methoxy-2-methyl isoflavone	42.56	0.2	Dang Shen
DS5	MOL004355	Spinasterol	42.98	0.76	Dang Shen
DS6	MOL005321	Frutinone A	65.9	0.34	Dang Shen
DS7	MOL000006	Luteolin	36.16	0.25	Dang Shen
DS8	MOL007059	3-beta-Hydroxymethyllenetanshiquinone	32.16	0.41	Dang Shen
DS9	MOL008400	glycitein	50.48	0.24	Dang Shen
DS10	MOL008407	(8S,9S,10R,13R,14S,17R)-17-[(E,2R,5S)-5-ethyl-6-methylhept-3-en-2-yl]-10,13-dimethyl-1,2,4,7,8,9,11,12,14,15,16,17-dodecahydrocyclopenta[a]phenanthren-3-one	45.4	0.76	Dang Shen
DS11	MOL008411	11-Hydroxyrankinidine	40	0.66	Dang Shen
GZ1	MOL001736	(-)-taxifolin	60.51	0.27	Gui Zhi
B1	MOL000492	(+)-catechin	54.83	0.24	Gui Zhi, Da Zao
GZ2	MOL004576	taxifolin	57.84	0.27	Gui Zhi
FL1	MOL000273	(2R)-2-[(3S,5R,10S,13R,14R,16R,17R)-3,16-dihydroxy-4,4,10,13,14-pentamethyl-2,3,5,6,12,15,16,17-octahydro-1H-cyclopenta[a]phenanthren-17-yl]-6-methylhept-5-enoic acid	30.93	0.81	Fu Ling
FL2	MOL000275	Trametenolic acid	38.71	0.8	Fu Ling
FL3	MOL000279	Cerevisterol	37.96	0.77	Fu Ling
FL4	MOL000296	Hederagenin	36.91	0.75	Fu Ling
BX1	MOL001755	24-Ethylcholest-4-en-3-one	36.08	0.76	Ban Xia
BX2	MOL002670	Cavidine	35.64	0.81	Ban Xia
BX3	MOL000519	Coniferin	31.11	0.32	Ban Xia
BX4	MOL006957	(3S,6S)-3-(benzyl)-6-(4-hydroxybenzyl)piperazine-2,5-quinone	46.89	0.27	Ban Xia
BX5	MOL003578	Cycloartenol	38.69	0.78	Ban Xia
BX6	MOL006967	Beta-D-Ribofuranoside, xanthine-9	44.72	0.21	Ban Xia

Table 1 (*continued*)

Table 1 (*continued*)

Symbol	MOL	Name	OB (%)	DL	Source
DH1	MOL002235	EUPATIN	50.8	0.41	Da Huang
DH2	MOL002259	Physciondiglucoside	41.65	0.63	Da Huang
DH3	MOL002268	Rhein	47.07	0.28	Da Huang
DH4	MOL002280	Torachrysone-8-O-beta-D-(6'-oxayl)-glucoside	43.02	0.74	Da Huang
DH5	MOL002281	Toralactone	46.46	0.24	Da Huang
DH6	MOL002288	Emodin-1-O-beta-D-glucopyranoside	44.81	0.8	Da Huang
DH7	MOL000471	Aloe-emodin	83.38	0.24	Da Huang
B2	MOL000096	(-)-catechin	49.68	0.24	Da Huang, Da Zao
DZ1	MOL012921	Stepharine	31.55	0.33	Da Zao
DZ2	MOL012946	Zizyphus saponin I_qt	32.69	0.62	Da Zao
DZ3	MOL012976	Coumestrol	32.49	0.34	Da Zao
DZ4	MOL012986	Jujubasaponin V_qt	36.99	0.63	Da Zao
DZ5	MOL012992	Mauritine D	89.13	0.45	Da Zao
DZ6	MOL001454	Berberine	36.86	0.78	Da Zao
DZ7	MOL001522	(S)-Coclaurine	42.35	0.24	Da Zao
DZ8	MOL004350	Ruvoside_qt	36.12	0.76	Da Zao
DZ9	MOL000627	Stepholidine	33.11	0.54	Da Zao
DZ10	MOL007213	Nuciferin	34.43	0.4	Da Zao
DZ11	MOL000787	Fumarine	59.26	0.83	Da Zao
DZ12	MOL002773	Beta-carotene	37.18	0.58	Da Zao
LG1	Kwy0001112	Calcium Phosphate			Long Gu
ML1	Kwy0001114	Calcium Sulphate			Mu Li
ML2	Kwy0001115	Silicon			Mu Li

Data are from TCMSP, https://old.tcmsp-e.com/tcmsp.php. TCMSP, Traditional Chinese Medicine System Pharmacology database; MOL, molecular; OB, oral bioavailability; DL, drug sample.

results of molecular docking showed that the binding value of quercetin, 7-methoxy-2-methyl isoflavone, and wogonin with three target proteins AKT1, TNF, and IL-6 was less than-5 kcal/mol, indicating good binding affinity between the selected active components and the target proteins. Among these, quercetin exhibited the strongest binding activity to the AKT1 receptor (*Table 3*).

Discussion

It is well-established that the Chaihu Longgu oyster adjusted decoction is composed of Chai Hu, Huang Qin, Fu Ling, Gui Zhi, Dang Shen, Ban Xia, Da Huang, Da Zao, Long Gu, and Oyster. Chai Hu tastes slightly cold and bitter and has the effect of clearing heat, soothing the liver, and regulating qi. Huang Qin tastes cold and bitter and has the effect of clearing heat and eliminating annoyance. Fu Ling has the effect of tranquilizing the mind and tonifying the heart. Gui Zhi tastes pungent, sweet, and warm and has the effect of warming meridians. Dang Shen tastes sweet and has the effect of invigorating qi and the spleen and benefiting the lung. Ban Xia tastes pungent, sweet, and warm and has the effect of drying dampness and resolving phlegm. Da Huang tastes cold and bitter and has the effect of dissipating

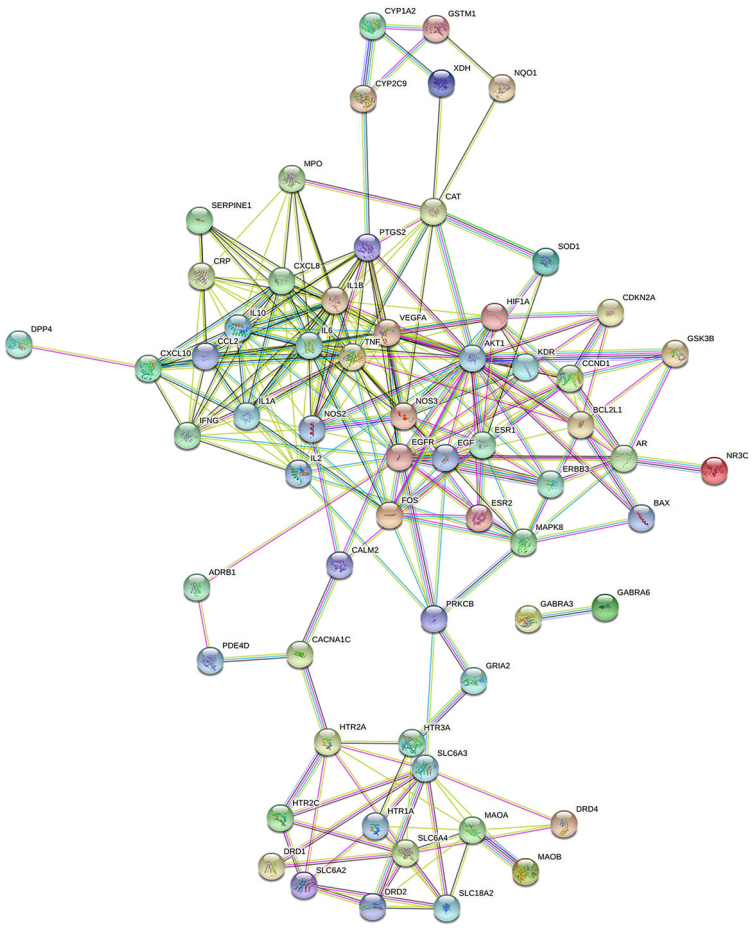

Figure 2 Target-protein interaction network.

heat. Da Zao tastes warm and sweet and has the effect of invigorating qi and the spleen. Finally, Long Gu and Oyster have the effect of tranquilizing and calming nerves. Taken together, these TCM have the effect of soothing the liver, invigorating the spleen, and calming the mind. Therefore, the Chaihu Longgu oyster adjusted decoction is good at treating the symptoms of evil qi invading Shaoyang, poor running of meridian qi, and mental disorders. The clinical treatment of depression using the Chaihu Longgu oyster adjusted decoction is effective. In addition, other traditional Chinese medicines, such as Xiaoyaosan and Banxia-Houpu decoction, have more advantages in terms of effectiveness and acceptability in the treatment of depression. And they also deserve further study.

In this study, we applied network pharmacology and molecular docking technology to explore the mechanism of action of the Chaihu Longgu oyster adjusted decoction in treating depression. According to the "compound-target-pathway" network, the main active ingredients in the formula were quercetin, 7-methoxy-2-methyl isoflavone, baicalein, kaempferol, lignocaine, etc. Quercetin is a flavonoid and a secondary plant metabolite that exists widely in nature. It has numerous pharmacological effects, such as antioxidation, anti-inflammation, anti-tumor, antibacterial, immunosuppression, neuroprotection, etc. (17), and exerts well-established pharmacological activity against psychiatric disorders (18). An increasing body of evidence suggests that the inflammatory response is strongly associated with the onset of depression (18,19). In this respect, prolonged chronic stress and inflammatory responses activate microglia and astrocytes in the brain, triggering a neuroinflammatory response that leads to the release of the inflammatory cytokines IL-1β, IL-6, and TNF-α. Clinical studies have demonstrated that

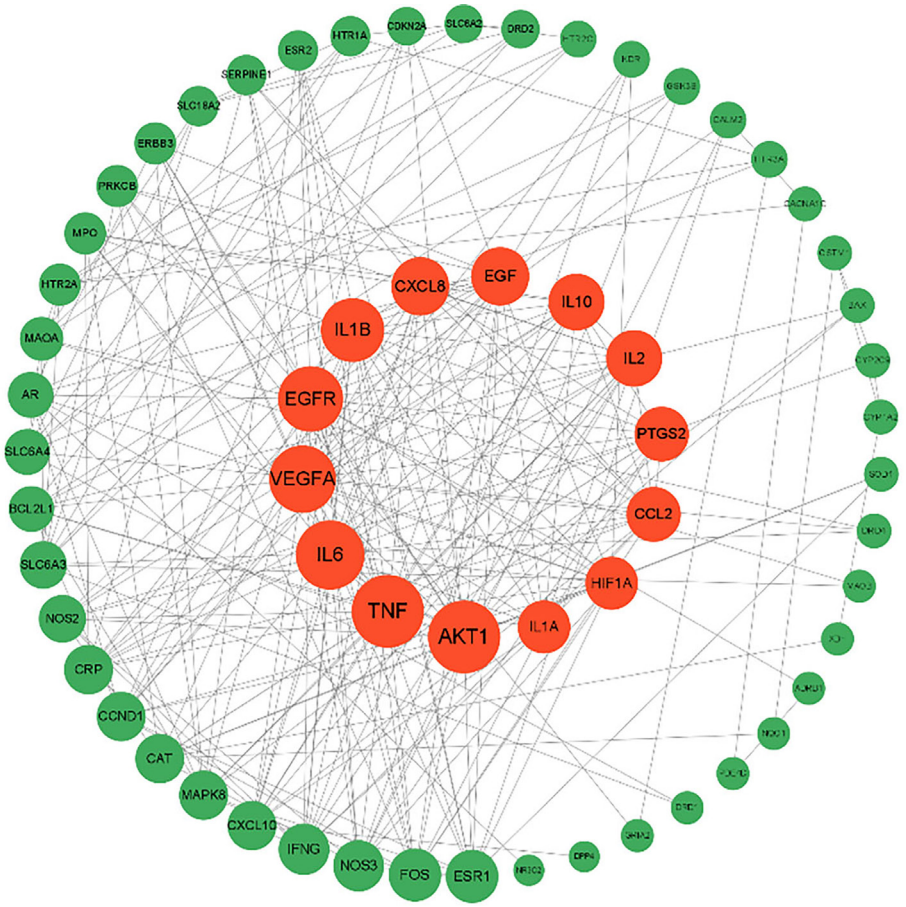

Figure 3 PPI association network. PPI, protein-protein interaction.

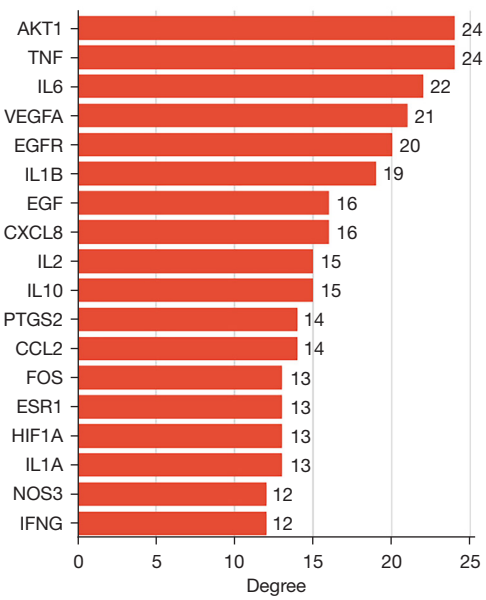

Figure 4 Histogram of the core targets.

IL-6 and TNF-α levels are significantly elevated in the blood and cerebrospinal fluid of major depressive disorder patients (20,21), and the increase of central and peripheral inflammatory cytokines induces depressive symptoms such as loss of pleasure and motor retardation (22). Quercetin can also inhibit the nuclear factor kappa-B (NF-κB) and Phosphatidylinositide 3-kinases (PI3K) signaling pathways; decrease TNF-α, IL-1β, and IL-6 levels; and inhibit COX-2 secretion and Caspase-3 activation, thereby exerting antidepressant effects by suppressing inflammatory cytokine expression and inflammatory enzyme activity (23,24). It is widely acknowledged that depressed patients exhibit hypothalamic-pituitary-adrenal (HPA) axis hyperactivity. Importantly, quercetin can inhibit the expression of chronic renal failure (CRF) mRNA to normalize the hyperactive HPA axis and significantly reduce serum cortisol levels (25-27). Quercetin also regulates neurotransmitters and synaptic plasticity (28,29).

The 7-methoxy-2-methyl isoflavone is an isoflavone

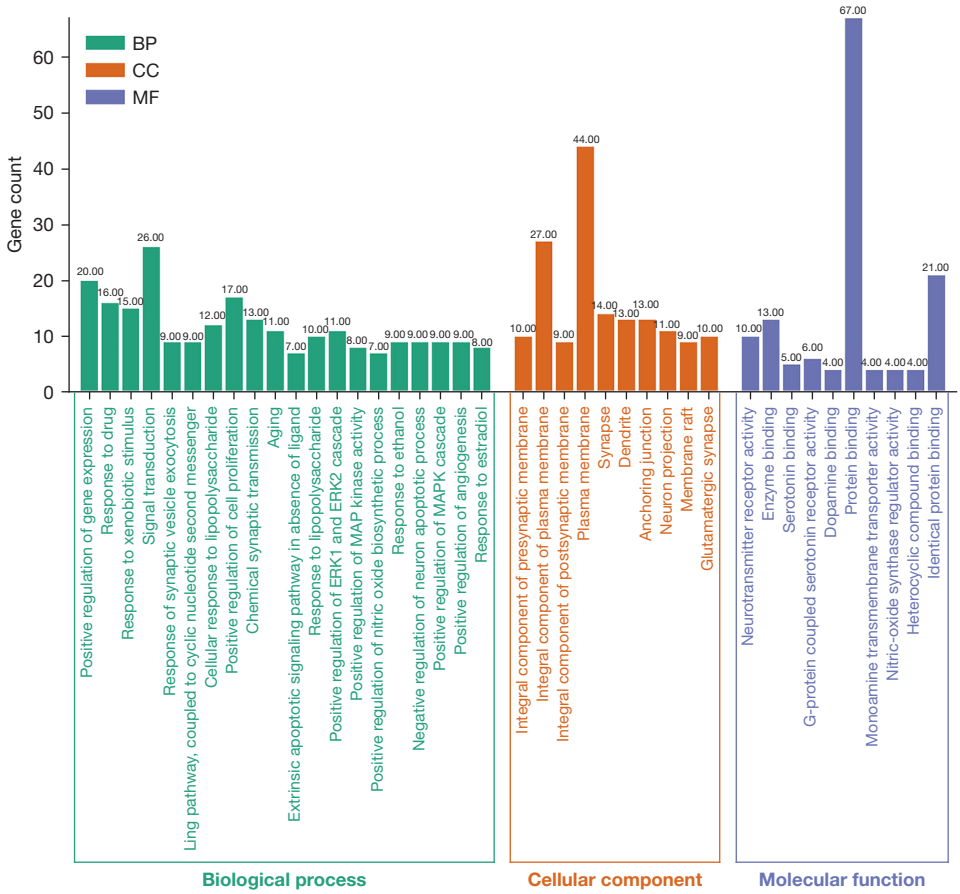

Figure 5 GO enrichment analysis. BP, biological process; CC, cellular component; MF, molecular function; ERK, extracellular regulated protein kinases; MAPK, mitogen-activated protein kinase; GO, Gene Ontology.

derivative and an activator of Amp-activated protein kinase (AMPK). Activated AMPK can catalyze UNC-51-Like Kinase (ULK1) phosphorylation to promote neuronal autophagy and reduce depression-like behavior (30,31). It is widely thought that Wogonin has significant anti-inflammatory, anti-oxidative stress, and neuroprotective effects, and its mechanism is related to the downregulation of phosphoinositide-dependent protein kinase 1 (PDK1), PDK4, and IL-6/signal transducer and activator of transcription 3 (STAT3), PI3K/Akt pathway-related protein expression (32). Kaempferol exerts anti-inflammatory, antioxidant, neuroprotective, and other biological activities. Kaempferol can reduce the secretion of inflammatory factors nitric oxide (NO), IL-1β, and TNF-α; inhibit the expression of Bax and Caspase-3; and reduce the apoptosis rate of hippocampal neurons (33). Also, luteolin has strong anti-inflammatory and antioxidant effects (34), and its antidepressant effect may be mediated by inhibition of the Janus Kinase 1 (JAK1)/STAT3 signaling pathway, which in turn inhibits the polarization of microglia to the M1 type (35,36).

It is widely thought that the greater the degree value of the target, the greater the possibility that the target participates in the regulation and control of the PPI network. In the present study, PPI network analysis showed that the key targets of the Chaihu Longgu oyster adjusted decoction in treating depression were AKT1, TNF, IL-6, etc., mirroring the multi-target characteristics of TCM. Akt belongs to serine/threonine protein kinase, an important target downstream of PI3K. As its most important subtype, Akt1 participates in numerous BP, including cell survival, proliferation and apoptosis, neovascularization, and cell cycle regulation, and has been explored in studies on depression since its polymorphism was found to be is related to the severity of depression, anxiety symptoms, suicidal tendencies, and so on (37-39). TNF is a cytokine

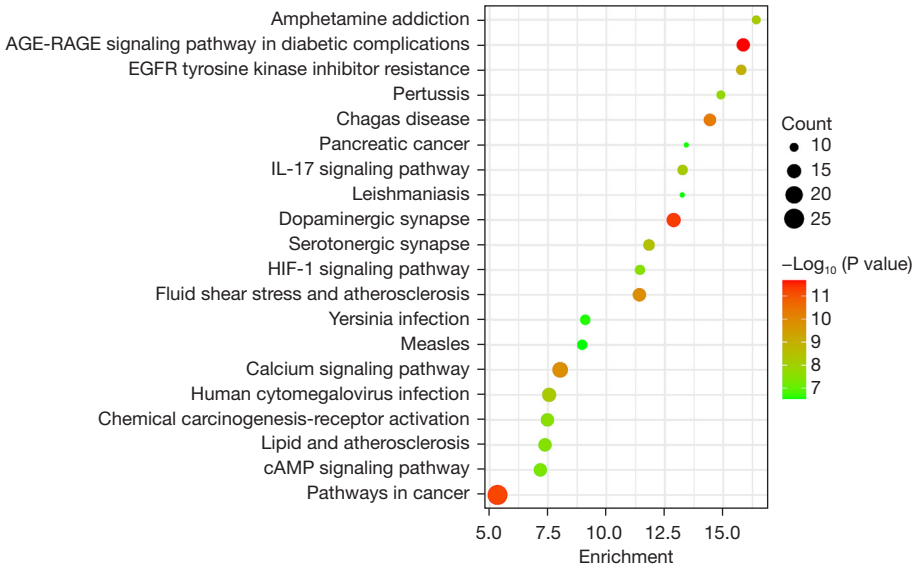

Figure 6 KEGG pathway enrichment analysis. AGE-RAGE, advanced glycation end products-receptor of AGE; EGFR, epidermal growth factor receptor; IL, interleukin; HIF, hypoxia inducible factor; cAMP, cyclic adenosine monophosphate; KEGG, Kyoto Encyclopedia of Genes and Genomes.

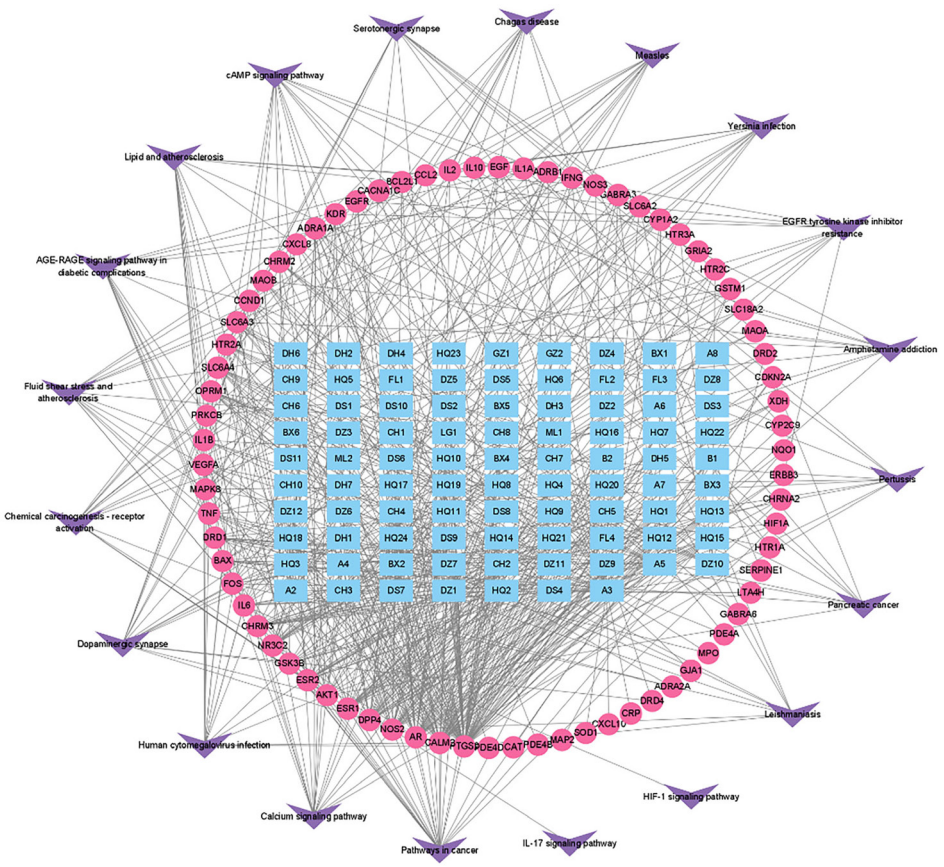

Figure 7 Compound-target-pathway network.

Table 2 Topological attribute parameters of the main active ingredients

Symbol	MOL	Active ingredients	Degree	Betweenness	Closeness
A3	MOL000098	Quercetin	36	4095.325797	0.191331924
DS4	MOL003896	7-methoxy-2-methyl isoflavone	16	890.7534956	0.18356998
HQ2	MOL000173	Wogonin	15	627.0686026	0.183943089
CH3	MOL000422	Kaempferol	14	795.9774427	0.182828283
DS7	MOL000006	Luteolin	14	561.8771078	0.182459677
DZ1	MOL012921	Stepharine	14	607.43105	0.180638723
A2	MOL000449	Stigmasterol	14	5569.933817	0.185831622
DZ10	MOL007213	Nuciferin	13	612.6255617	0.180278884
A5	MOL000358	Beta-sitosterol	12	895.203479	0.181726908
CH2	MOL000354	Isorhamnetin	11	361.4164746	0.181362725
DZ9	MOL000627	Stepholidine	11	481.6842091	0.180278884
DZ11	MOL000787	Fumarine	11	509.2990757	0.180278884

MOL, molecular.

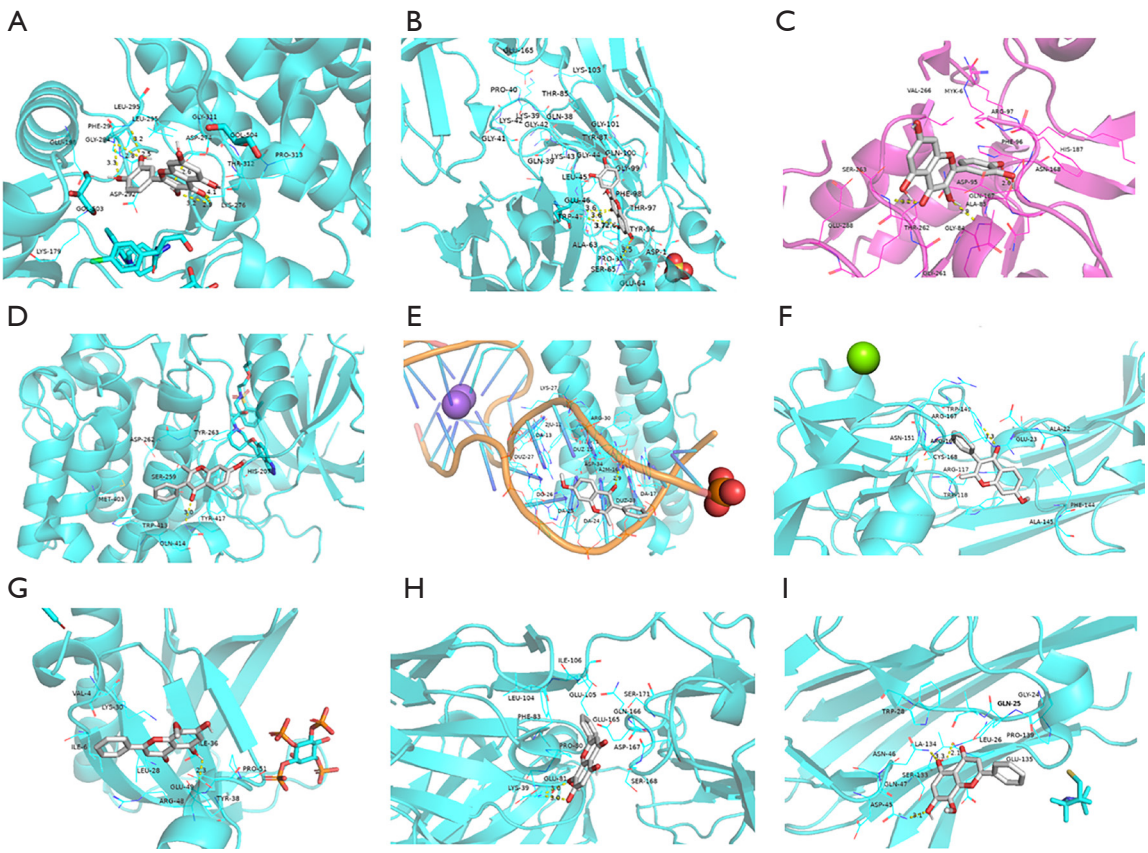

Figure 8 Molecular docking results. (A) Quercetin and AKT1; (B) Quercetin and IL-6; (C) Quercetin and TNF; (D) 7-methoxy-2-methyl isoflavone and AKT1; (E) 7-methoxy-2-methyl isoflavone and IL-6; (F) 7-methoxy-2-methyl isoflavone and TNF; (G) Wogonin and AKT1; (H) Wogonin and IL-6; (I) Wogonin and TNF. AKT1, AKT serine/threonine kinase 1; IL, interleukin; TNF, tumor necrosis factor.

Table 3 Binding energy values of the main active ingredients to the core targets

Active ingredient	Target	PDB ID	Energy (kcal/mol)
Quercetin	AKT1	4gv1	–8.8
Quercetin	TNF	4cni	–8.2
Quercetin	IL-6	4y6o	–7.9
7-methoxy-2-methyl isoflavone	AKT1	3qkl	–7.1
7-methoxy-2-methyl isoflavone	TNF	3l9j	–7.5
7-methoxy-2-methyl isoflavone	IL-6	4ni7	–7.2
Wogonin	AKT1	1UNQ	–6.2
Wogonin	TNF	5uui	–6.5
Wogonin	IL-6	4cni	–8.0

PDB, Protein Data Bank; AKT1, AKT serine/threonine kinase 1; IL, interleukin; TNF, tumor necrosis factor.

that constitutes the acute phase response and is involved in systemic inflammation. It is mainly produced by activated macrophages and plays a role in regulating immune cells. Abnormal TNF expression is linked with various human diseases, including Alzheimer's disease, cancer, and major depression (40-42). Many major neuropathologic features of TNF or IL-6 in the central nervous system are similar to those found in cytokine-related immune inflammatory neurological diseases in humans (42). TNF, as an essential pro-inflammatory factor, participates in immunopathological reactions, regulates the immune-inflammation signal network, and influences depression development. Growing evidence suggests that IL-1β, IL-6 (a proinflammatory cytokine), and TNF-α are positively correlated with depression in clinical and community samples and are maintained at high levels in depressive patients (43,44).

GO annotation showed that the related targets were mainly involved in BPs such as the positive regulation of gene expression, response to heterogeneous stimuli, signal transduction, regulation of synaptic vesicle exocytosis, and so on. KEGG enrichment analysis showed that the AGE-RAGE, IL-17, HIF-1, and cAMP signal pathways were the key therapeutic pathways, and dopaminergic synapses and serotonergic synapses also played an important role in treating depression with the Chaihu Longgu oyster adjusted decoction. The AGE-RAGE signaling pathway, which consists of advanced glycosylation end products (AGEs) and their receptors (RAGE), leads to oxidative stress, activates downstream signaling pathways such as mitogen-activated protein kinase (MAPK), NF-κB, and PI3K, promotes the formation of reactive oxygen species, and activates caspase-3, resulting in neuronal cell damage and even apoptosis (45,46). IL-17 is a proinflammatory factor induced by T cells. By binding to surface receptors and activating related signaling pathways, IL-17 induces proinflammatory cytokines such as IL-1, IL-6, and TNF-α to bind to their corresponding receptors and induce an inflammatory response (47). HF1 and its mediated VEGF signaling pathway play important roles in maintaining the integrity of the vascular wall and the normal development of nerves (48). According to the monoamine hypothesis, the deficiency of monoamine neurotransmitters such as dopamine and 5-hydroxytryptamine (5-HT) or the disorder of binding to receptors is closely related to depression (49).

In the present study, the molecular docking results showed that the binding energies of the main active components to the core protein were less than –5 kcal/mol, indicating that the active components could stably bind to the core receptor protein and play a role in the treatment of depression.

In recent years, network pharmacology has been widely used in the field of traditional Chinese medicine. Researchers used network pharmacology to predict the mechanism of various diseases, providing a novel solution to TCM's ambiguous effective ingredients and mechanism (50-52). Zheng proposed an innovative high-throughput research strategy, combining computational and experimental network pharmacology methods, and selected 22 health-strengthening herbs (53). Zhang *et al.* studied the anti-depression mechanisms of single medicinal herbs and the compatibility of different medicinal herbs (14,54,55).

As can be seen, network pharmacology plays an important role in discovering and investigating TCM properties, compatibility rules, mechanisms of TCM efficacy, and new indications for TCM compounds.

Conclusions

In this study, network pharmacology was used to explore the mechanism of the Chaihu Longgu oyster adjusted decoction in the treatment of depression, which reflects the characteristics of multi-target, multi-pathway, and coordinated therapy in the treatment of diseases with TCM compound prescriptions. The underlying mechanism of the anti-depressive effect of the Chaihu Longgu oyster adjusted decoction may involve neuroinflammatory response reduction and improvement of neurotransmitter function and neuroplasticity. This phenomenon may be related to the synergistic activity of the main active components (including quercetin, 7-methoxy-2-methyl isoflavone, wogonin, kaempferol, and luteolin) with AKT1, IL-6, TNF, and other proteins, which further regulate signaling pathways such as AGE-RAGE, IL-17, HIF-1, cAMP, dopaminergic synapses, and 5-hydroxytryptamine synapses. It is worth noting that quercetin, kaempferol, and other active ingredients sifted in this study have a wide range of activities and are highly similar in sifting different drugs or prescriptions. This phenomenon needs to be further explored in future studies. In addition, since the drug has to reach a particular dose or concentration to be effective, subsequent studies will confirm the efficacy of this study with animal tests or cell tests.

In summary, based on network analysis and molecular docking technology, this study took the Chaihu Longgu oyster adjusted decoction as the research object to analyze its chemical constituents, targets, and binding ability of key pharmacodynamic components. It initially explored the possible material basis and mechanism of the Chaihu Longgu oyster-adjusted decoction in the treatment of depression. This study can provide data support and a theoretical basis for further screening and evaluating antidepressant drugs based on active ingredients in traditional Chinese medicine.

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Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-23-236/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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