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Research Article
Pharmacological Targets and Active Components of *Gastrodiae rhizoma* Against Depression: Findings of Network Pharmacology

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Abstract

Background and Objective: Depression is one of the most frequent mental illnesses all over the world. *Gastrodiae rhizoma* (GR) has been used as both herbal medicine and functional food in China. Herein, we aim to decipher the pharmacological targets and active components of GR against depression by using network pharmacology, bioinformatic analysis and molecular docking. **Materials and Methods:** GR active components were screened based on *in silico* prediction models of pharmacological properties. The potential targets of GR active components were predicted. Protein-protein interaction networks were constructed using the STRING database. Hub genes were identified by the cytoHubba plugin in Cytoscape. Functional enrichment analysis were carried out using the "clusterProfiler" package in R software. Molecular docking simulation was conducted to evaluate the binding affinity between the active components and hub targets. **Results:** A total of 24 active components and 38 targets were identified to interpret the anti-depressive effect GR. Functional enrichment analysis showed that the anti-depressive activity of GR may be associated with various biological processes such as regulation of neurotransmitter levels and monoamine transport, as well as multiple pathways such as neuroactive ligand-receptor interaction and dopaminergic synapse. SLC6A3, SLC6A4, CNR1 and MAOA were identified as hub targets and they had a good binding ability with the GR active components. (-)-Variabilin, bis-(4-hydroxybenzyl) sulfide and 6-ethoxysanguinarine may be promising anti-depressive leading compounds. **Conclusion:** This study uncovers the synergistic anti-depressive effect of multiple active components in GR and provides a scientific basis for developing GR as complementary medicine or functional food in depression prevention and treatment.

Key words: *Gastrodiae rhizoma*, depression, network pharmacology, molecular docking, bioinformatic analysis, mechanism of action, active components

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Depression, which accounts for 1.7% of disability-adjusted life-years worldwide, is one of the most common and debilitating mental disorders[1]. The estimated lifetime prevalence of depression was 11.1 and 14.6% in the low-middle income and high-income countries, respectively[2]. Additionally, depression is associated with an increased risk of chronic conditions such as coronary heart disease, diabetes and cancer[3-5]. Even worsen, depression accounts for at least half of suicide deaths, thereby further increasing its harmfulness[6].

Despite great advances that have been achieved in the pharmacotherapy of depression over the past decades, many depressed patients could not obtain satisfactory effects after anti-depressant treatment. According to the Sequenced Treatment Alternatives to Relieve Depression trial report, only 36.8% of depressed patients were remitted with 12-14 weeks of treatment of citalopram[7]. Furthermore, the rates of tachyphylaxis ranged from 9-57% in patients during long-term anti-depressants therapy[8]. Additionally, the currently available antidepressants were involved in considerable adverse drug reactions, such as gastrointestinal symptoms, headaches, dizziness, weight gain and sexual dysfunction[9]. It is, therefore, of great significance to discover a new therapeutic regimen with a safety profile for the treatment of depression.

Gastrodiae rhizoma (GR), the dried tuber of *Gastrodia elata* Blume, has been used as herbal medicine in many eastern Asian countries, including China, Japan and Korea[10]. GR has effectively been used for treating many neurological conditions, including epilepsy, headache, dizziness, stroke, convulsions and so forth[11]. Additionally, GR is commonly used to make medicinal food in Chinese folk, such as "Stewed chicken with GR", a dish that is supposed to have the effect of treating dizziness and headache.

Many studies in recent years have revealed the anti-depressive effect of GR. Zhou *et al.*[12] had, firstly, demonstrated that the anti-depressant-like effect of 75% ethanol extract of GR was not inferior to fluoxetine in the mouse depression model. Later, other studies have documented that the GR water extract can improve the depressant-like symptoms in rats exposed to unpredictable chronic mild stress or forced-swimming[13,14]. A recent study has indicated that GR water extract improves depressive-like behaviors in the chronic stress model of rats by modulation of inflammatory response[15]. Although the above-mentioned studies have revealed the anti-depressive effect of GR, the active components and anti-depressive targets of GR remain poorly understood.

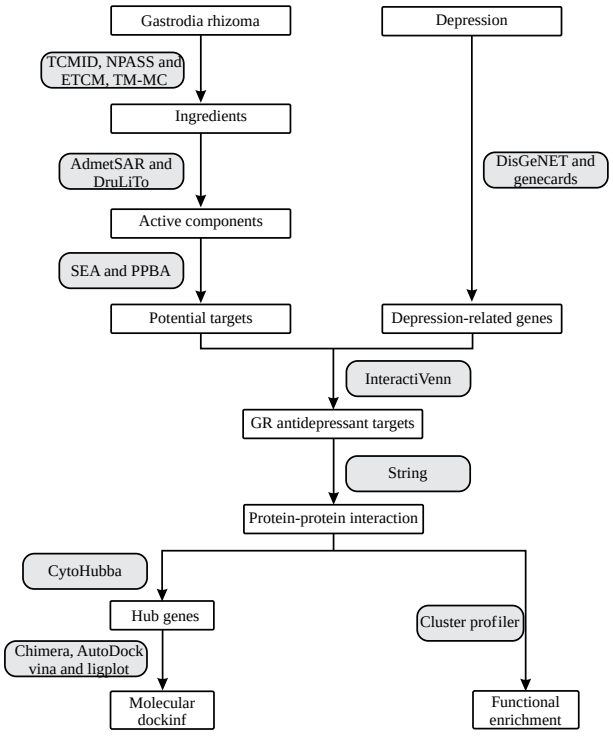

Fig. 1: Flowchart of this study

Network pharmacology was proposed as a new paradigm in drug discovery by Hopkins A.L.[16]. It offers a brand-new perspective to understand the multi-targets and multi-pathways mode of action of drugs. By adopting the network pharmacology approach, we have successfully revealed the active components and anti-depressive mechanisms of *Gardeniae* fructus[17]. Herein, we utilized the network pharmacology method to uncover the candidate active components of GR and its anti-depressive targets and conducted molecular docking simulation to verify the interaction between GR active components and it's targets. The flowchart of this study was illustrated in Fig. 1.

MATERIALS AND METHODS

Study area: The present study was carried out at the Department of Pharmacy, Shanghai University of Medicine and Health Sciences Affiliated Sixth People's Hospital South Campus, Shanghai, People's Republic of China, between January-September, 2020.

Screen GR active components: The GR chemical ingredients were comprehensively collected from Traditional Chinese Medicines Integrated Database (TCMID)[18], Natural Product Activity and Species Source Database (NPASS)[19], the

Encyclopedia of Traditional Chinese Medicine Database (ETCM)[20] and Database of Medicinal Materials and Chemical Compounds in Northeast Asian Traditional Medicine (TM-MC)[21].

Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) are important properties for evaluating the potential efficacy and safety of a certain chemical compound. In this study, four ADMET parameters, namely Human Intestinal Absorption (HIA), Caco-2 permeability (Caco-2), Blood-brain Barrier Permeability (BBB) and Oral Bioavailability (OB) were used to screen the GR active components. The ADMET Structure-Activity Relationship Database (admetSAR[22]), a free web service for the prediction of chemical ADMET profiles, was used to calculate the ADMET properties of GR chemical ingredients herein. Prediction results of the above-mentioned four parameters were classified as positive (+) or negative (-) by the admetSAR.

Drug-likeness (DL), which is considered as to whether a chemical compound has the physicochemical properties similar to known drugs, was also adopted for screen the GR active components. A metric called the Quantitative Estimate of Drug-likeness (QED) was used to calculate the DL of GR chemical ingredients[23]. Drugs with high QED scores are more likely to exhibit a higher fraction absorbed, resulting in higher oral bioavailability, decreased dose size and food effect[24]. Molecules with a score of less than 0.600 are often considered "bad" in the literature[25]. The drug-likeness tool (DruLiTo, software, an open-source virtual screening tool, was used to calculate the QED scores.

Additionally, we found that 27 of 29 available antidepressants were HIA^+, $Caco\text{-}2^+$ and BBB^+ properties according to admetSAR calculation. Thus, those chemical ingredients with HIA^+, Caco-2 permeability$^+$, BBB^+ and QED score$\geq$0.600 properties were considered as GR active components with anti-depressive potential.

Target fishing and diseases enrichment analysis: Since the polypharmacology browser 2 (PPB2)[26] and the Similarity Ensemble Approach (SEA)[27] showed the best quality and quantity for the accurate predictions among nine target prediction tools[28], the potential targets of GR active components were predicted by using PPB2 and SEA. All the protein names of predicted targets were converted to their unique gene names in the UniProt database. Subsequently, diseases enrichment analysis of predicted targets was conducted by using WebGestalt, a web tool of functional enrichment analysis[29]. In the WebGestalt, the "DisGeNET" and the "affy hugene 2.0 st v1" were selected as disease database and reference gene set, respectively.

Collection of genes associated with depression: The term "depressive disorder" was used as a keyword to retrieve the DisGeNET database[30] and Genecards[31] to gather genes associated with depression. The inclusion criteria for depression-related genes screening through DisGeNET and GeneCards database were a gene-disease association score greater than 0.1 and a gene score greater than 10, respectively.

Protein-protein Interaction (PPI) network construction and hub genes identification: The overlap of GR active components targeted genes and depression-related genes, which were regarded as anti-depressive targets of GR, were obtained by using InteractiVenn[32]. To reveal the interaction among GR anti-depressive targets, the PPI network was constructed by using the STRING database[33], in which the species was limited to "Homo sapiens" and the confidence score>0.4. Furthermore, hub genes in the PPI network were identified by the overlap of the top 10 genes according to six different ranking methods, including Maximum Neighborhood Component (MNC), degree, Edge Percolated Component (EPC), bottleneck, closeness and radiality in cytoHubba plugin[34].

Gene Ontology (GO) Enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis: GO enrichment and KEGG pathway analyses were conducted by using the "clusterProfiler" package in R software[35]. The p-value was adjusted by using the Benjamini and Hochberg method and adjusts p-value (p.adjust)<0.05 was considered as statistical significance. The analysis results were visualized by using the "GOplot" package in R[36].

Molecular docking simulation: The molecular docking simulation of hub genes and their corresponding GR active components were performed by using UCSF Chimera software[37], a program for the interactive visualization and analysis of molecular structures and related data. Firstly, the 3D structures of compounds and proteins were downloaded from PubChem[38] and The Protein Data Bank[39], respectively. The homologous modeling structure of human SLC6A3 was obtained from SWISS-MODEL[40]. Secondly, the structures of proteins and compounds were prepared for docking by using the Dock Prep plugin. The preparation process includes replacing incomplete side chains, adding hydrogens and charges[41,42]. Thirdly, molecular docking was conducted by using the AutoDock Vina[43]. Finally, 3D and 2D molecular docking models were visualized by Chimera and LigPlot$^+$[44], respectively.

RESULTS

GR active components: After deleting duplicates, a total of 109 chemical ingredients derived from GR were collected from TCMID, NPASS, ETCM and TM-MC. All these ingredients were subjected to ADMET and DL screening and 23 of the 109 with HIA^+, Caco-2^+, BBB^+ and QED score≥0.600 properties. Besides, despite not meeting the inclusion criteria, gastrodin was also considered as active components because it is a major GR chemical ingredient. Thus, a total of 24 chemical ingredients were considered as GR active components for further analysis. The detailed information is listed in Table 1.

Targets of GR active components and diseases enrichment analysis: There were 147 and 123 targets of GR active components predicted by SEA and PPB2, respectively. After removing the overlapped targets, a total of 171 targets were obtained. As shown in Fig. 2, the GR active components target network consists of 195 nodes and 496 interactions. Each of the GR active components is associated with multiple targets. The diseases enrichment analysis by using the WebGestalt showed that the top 10 enriched diseases of the 171 targets were mood disorders, mental depression, depressive disorder and so on (Fig. 3).

PPI network and enrichment analysis: There were 614 genes with a gene-disease association score greater than 0.1 in the DisGeNET database and 123 genes with a gene score greater than 10 in the Genecards database. After removing duplicates, 650 depression-related genes were obtained. A total of 38 intersection genes, which were regarded as GR anti-depressive targets, were obtained by using a Venn diagram (Fig. 4). The PPI analysis of the 38 targets was then conducted by using STRING database. As shown in Fig. 4, the PPI network consisted of 38 nodes and 156 edges.

GO enrichment analysis consisted of three major categories, namely, Biological Process (BP), Cellular Component (CC) and Molecular Function (MF). A total of 712 GO entries with p.adjust<0.05 were identified by GO enrichment analysis. The bubble diagram of top 10 entries of each GO category was illustrated in Fig. 5a. For the BP, it can be found that the 38 targets were mainly enriched in the regulation of neurotransmitter levels (GO: 0001505) and monoamine transport (GO: 0015844). Additionally, synaptic membrane (GO: 0097060) and neuronal cell body (GO: 0043025) ranked the highest in the CC category, while G protein-coupled amine receptor activity (GO: 0008227), ammonium ion binding (GO: 0070405), steroid binding (GO: 0005496) and neurotransmitter receptor activity (GO: 0030594) were the major MF category involved. There were 23, 16 and 18 targets involved in top 10 BP, CC and MF entries, respectively (Fig. 5b-d).

There were 11 significant pathways identified by KEGG pathway enrichment analysis. As shown in Fig. 6a, the

Table 1: Information of GR active components

ID	Chemical name	Molecular formula	HIA	Caco2	BBB	QED	Information source
GR01	(-)-Variabilin	$C_{17}H_{16}O_5$	+	+	+	0.931	TC
GR02	4-(Methoxymenthyl) benzene-1,2-diol	$C_8H_{10}O_3$	+	+	+	0.656	TM
GR03	4-(4'-Hydroxybenzyloxy)benzyl methyl ether	$C_{15}H_{16}O_3$	+	+	+	0.895	TC/E/TM
GR04	4-(Ethoxymethyl)-2-methoxyphenol	$C_{10}H_{14}O_3$	+	+	+	0.779	TM
GR05	4,4'-Dihydroxybenzyl sulfone	$C_{14}H_{14}O_4S$	+	+	+	0.929	TM
GR06	4,4'-Dihydroxybenzyl sulfoxide	$C_{14}H_{14}O_3S$	+	+	+	0.910	TM
GR07	4-Ethoxybenzyl alcohol	$C_9H_{12}O_2$	+	+	+	0.715	TM
GR08	4-Ethoxymethylphenyl-4'-Hydroxybenzylether	$C_{16}H_{18}O_3$	+	+	+	0.887	TC/E/TM
GR09	4-Hydroxybenzyl alcohol	$C_7H_8O_2$	+	+	+	0.600	TC/E/TM/N
GR10	4-Hydroxybenzyl methyl ether	$C_8H_{10}O_2$	+	+	+	0.673	TC/E/TM
GR11	4-Hydroxylbenzyl ethyl ether	$C_8H_{10}O_2$	+	+	+	0.716	TC/E/TM
GR12	4-Methoxybenzyl alcohol	$C_8H_{10}O_2$	+	+	+	0.671	TM/N
GR13	5-Hydroxymethylfuraldehyde	$C_6H_6O_3$	+	+	+	0.615	TM
GR14	6-Ethoxysanguinarine	$C_{22}H_{19}NO_5$	+	+	+	0.715	TC/N
GR15	Beta-sitosterol	$C_{29}H_{50}O$	+	+	+	0.742	TC/TM/N
GR16	Bis-(4-hydroxybenzyl)ether	$C_{14}H_{14}O_3$	+	+	+	0.858	TC/E/TM
GR17	Bis-(4-hydroxybenzyl)sulfide	$C_{14}H_{14}O_2S$	+	+	+	0.870	TM/N
GR18	Disogenin	$C_{27}H_{42}O_3$	+	+	+	0.691	TM/N
GR19	Gastrodamine	$C_{14}H_{15}NO_3$	+	+	+	0.757	TC/E
GR20	Gastrodin	$C_{13}H_{18}O_7$	-	-	+	0.521	TC/E/TM/N
GR21	Suchilactone	$C_{21}H_{20}O_6$	+	+	+	0.605	TC
GR22	Vanillic acid	$C_8H_8O_4$	+	+	+	0.704	TM/N
GR23	Vanillin	$C_8H_8O_3$	+	+	+	0.673	TC/E/TM/N
GR24	Vanillyl alcohol	$C_8H_{10}O_3$	+	+	+	0.676	TC/E/TM

TC: TCMID, E: ETCM, N: NPASS, TM: TM-MC

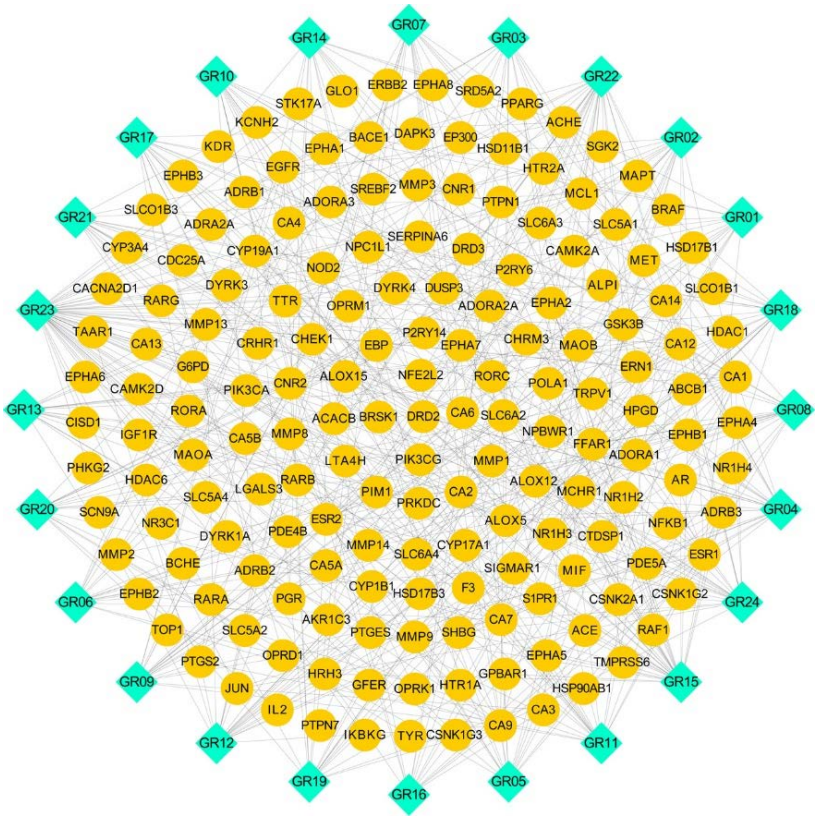

Fig. 2: GR active components-targets network

Green diamond represents GR active components and the orange round represents predicted targets

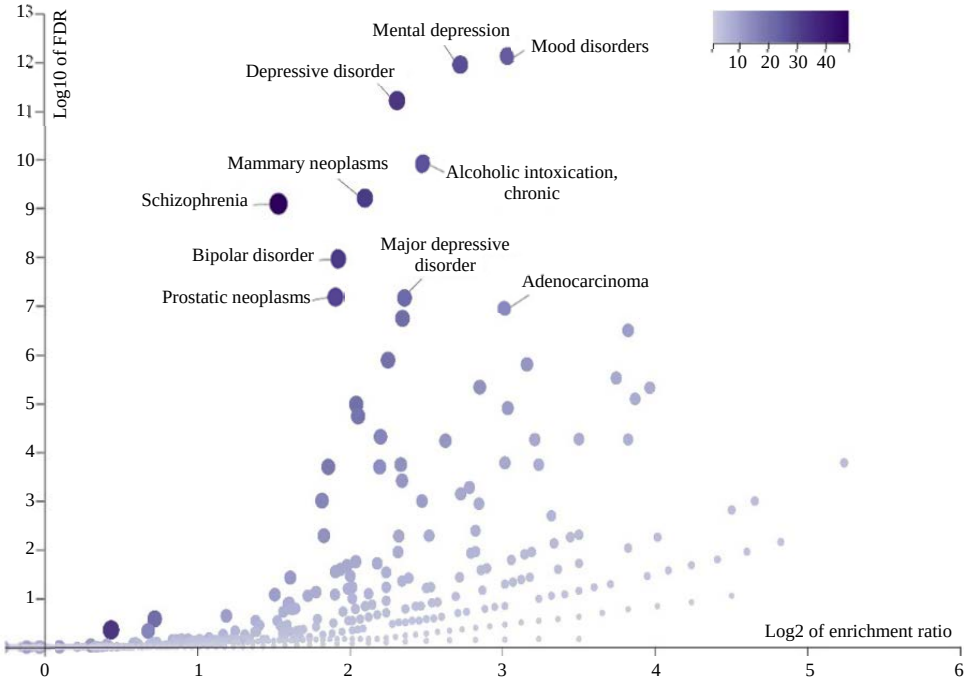

Fig. 3: GR active components targets-diseases enrichment analysis

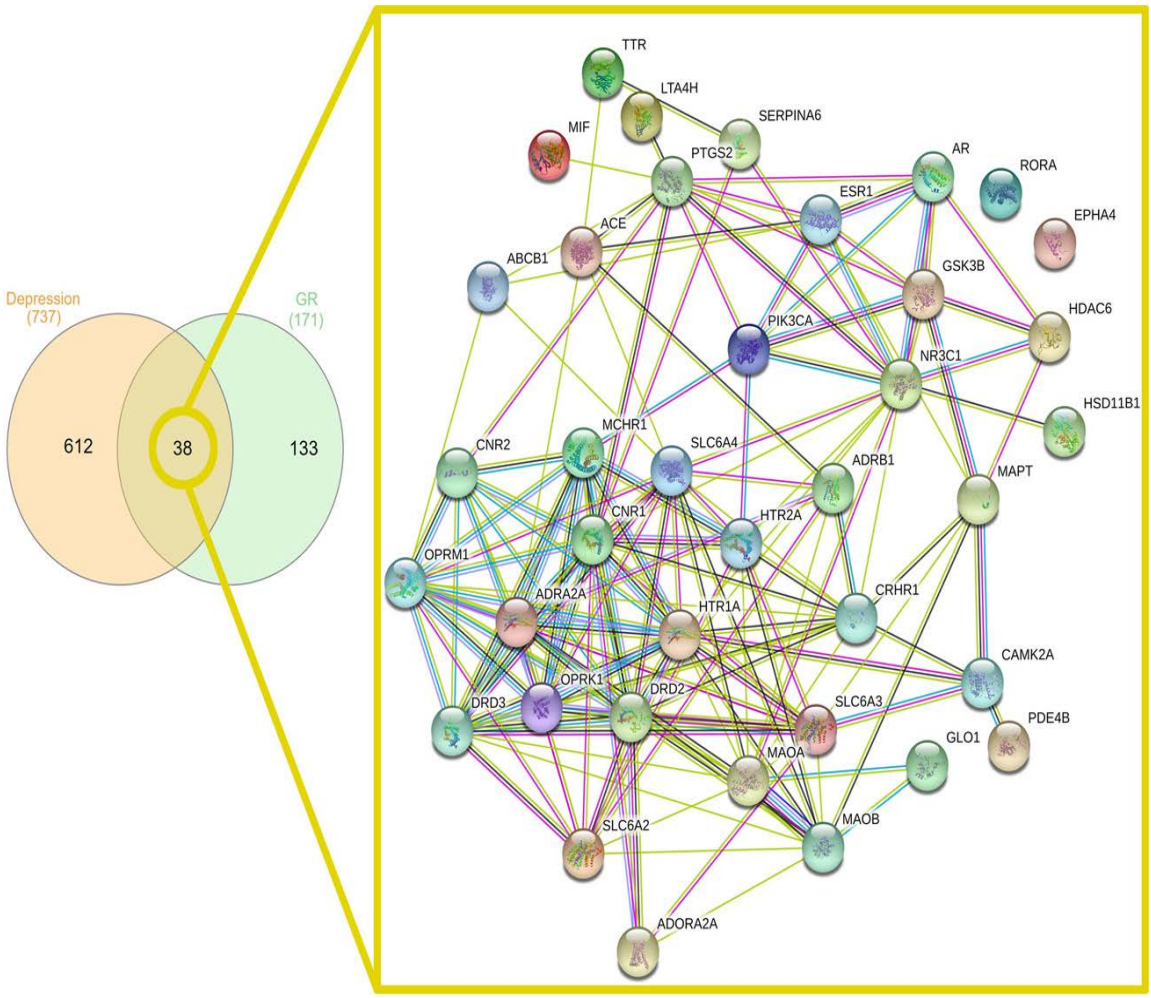

Fig. 4: Anti-depressive targets of GR obtained by Venn diagram and their PPI network

Table 2: Top 10 genes by six ranking methods in cytoHubba

	Ranking methods in cytoHubba					
Category	MNC	Degree	EPC	BottleNeck	Closeness	Radiality
Top 10 genes	***CNR1***	***CNR1***	***CNR1***	NR3C1	***CNR1***	***CNR1***
	DRD2	DRD2	HTR1A	PTGS2	HTR1A	NR3C1
	HTR1A	HTR1A	DRD2	CRHR1	NR3C1	HTR1A
	SLC6A4	***SLC6A4***	***SLC6A4***	HTR2A	DRD2	DRD2
	ADRA2A	ADRA2A	DRD3	***SLC6A4***	***SLC6A4***	***SLC6A4***
	DRD3	NR3C1	ADRA2A	***MAOA***	***MAOA***	***MAOA***
	NR3C1	DRD3	OPRM1	***SLC6A3***	ADRA2A	CRHR1
	MAOA	***MAOA***	OPRK1	***CNR1***	CRHR1	***SLC6A3***
	OPRM1	OPRM1	***MAOA***	CAMK2A	***SLC6A3***	ADRA2A
	SLC6A3	***SLC6A3***	***SLC6A3***	ACE	OPRM1	HTR2A

Bold italic genes were the overlap genes in the top 10 by six different ranking methods in cytoHubba

38 targets were most significantly enriched in neuroactive ligand-receptor interaction (hsa04080), followed by dopaminergic synapse (hsa04728) and cAMP signaling pathway (hsa04024). A total of 26 targets were enriched in the top 10 KEGG pathways (Fig. 6b).

Active components anti-depressive targets network: GR active components anti-depressive targets network consisted of 62 nodes and 106 edges (Fig. 7a). The mean degree values of active components and targets in the network were 4.417 and 2.789, respectively. As illustrated in Fig. 7b, the

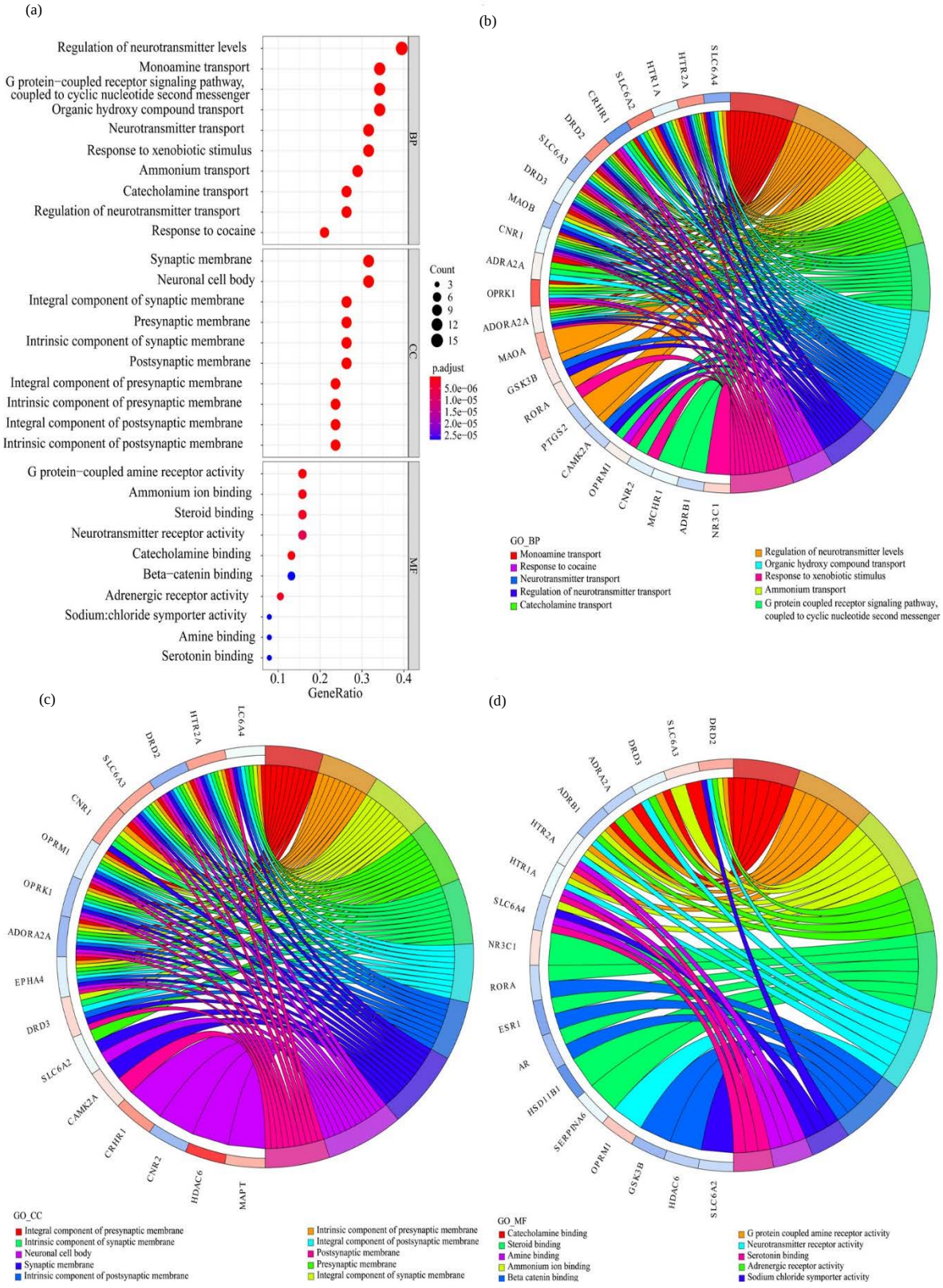

Fig. 5(a-d): Bubble and chord diagrams of top 10 entries of each GO category

(a) Bubble diagram of top 10 entries of each GO category and (b-d) are chord diagrams of top 10 BP, CC and MF entries, respectively

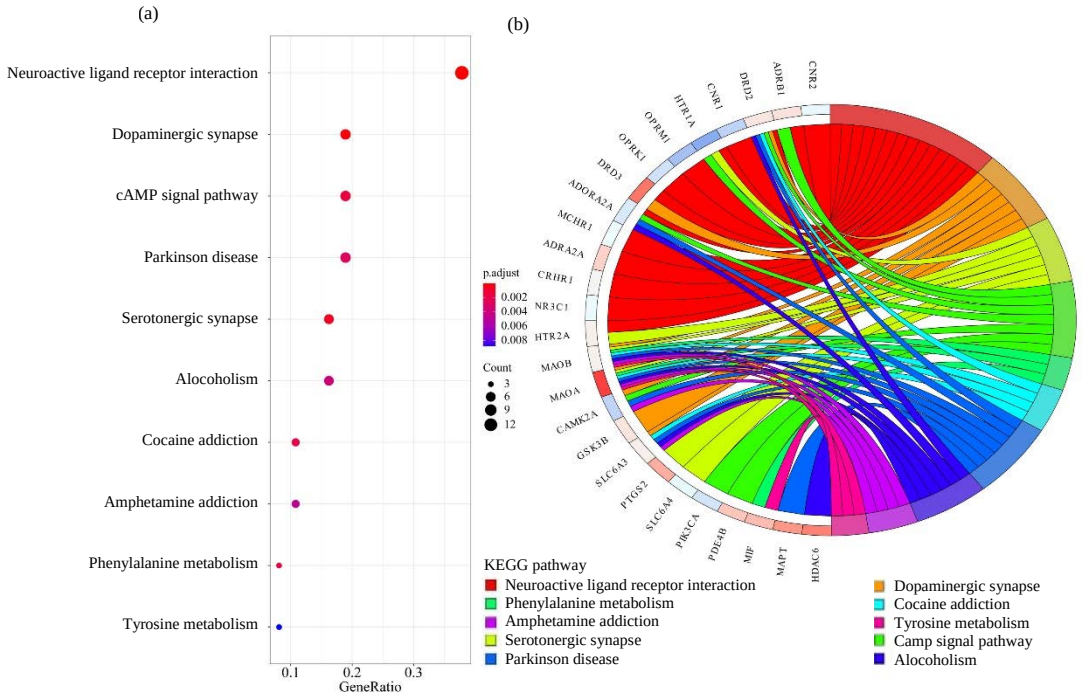

Fig. 6(a-b): (a) Bubble and (b) Chord diagrams of top 10 KEGG pathways

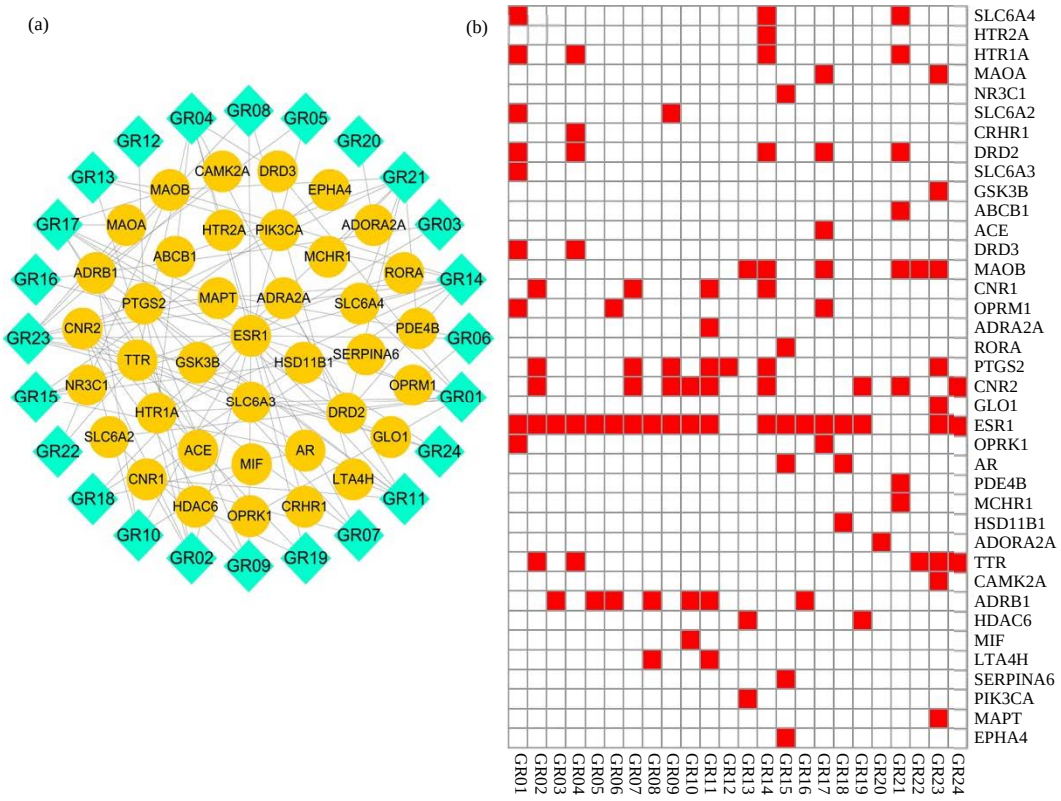

Fig. 7(a-b): (a) Network and (b) Heatmap of GR active components-anti-depressive targets

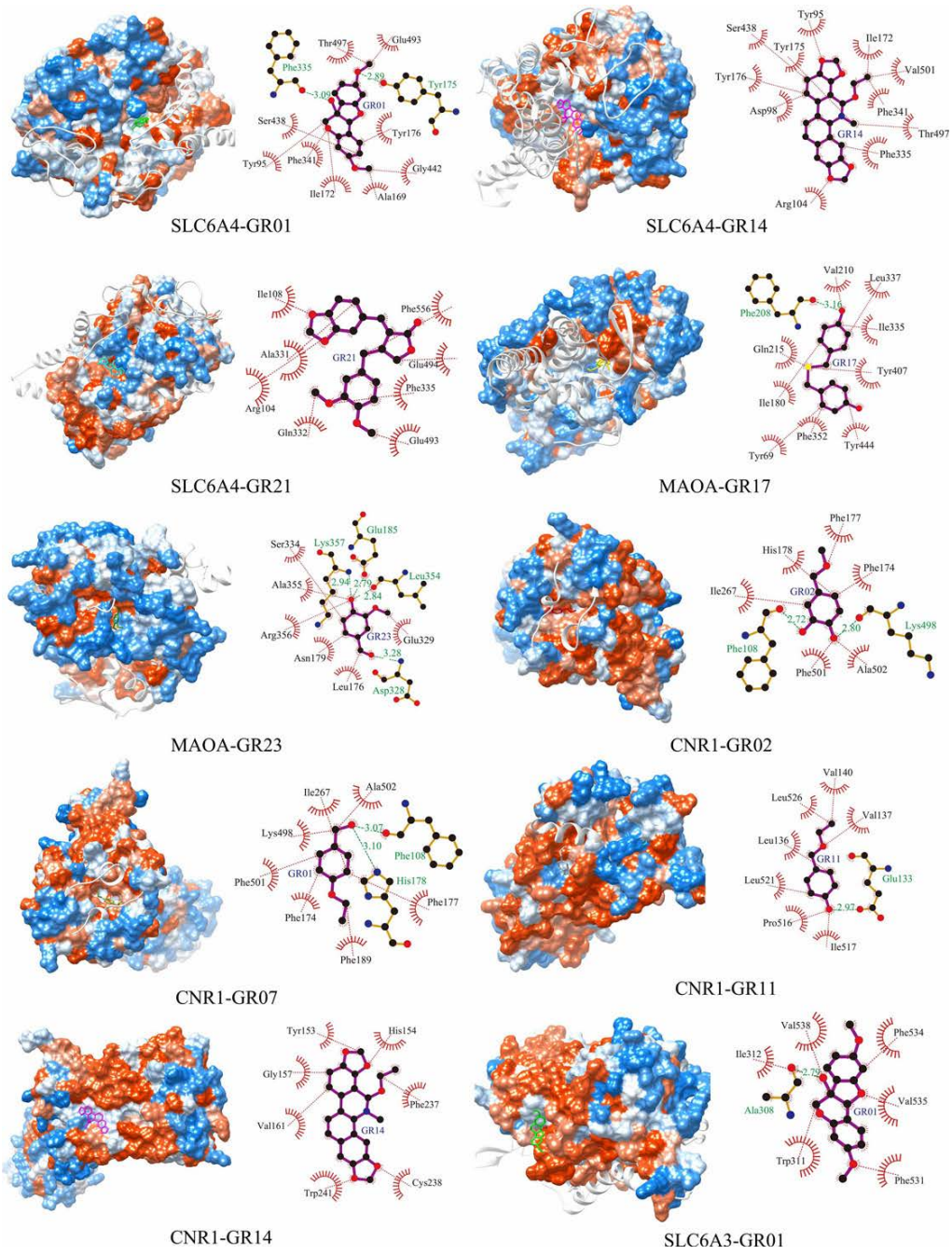

Fig. 8: 3D and 2D molecular docking models of hub genes and the GR active components. In the 2D model, the red dashed line represents hydrophobic interaction and the green dashed line represents hydrogen bond

active components with the highest degree value were the (-)-variabilin (GR01), 6-ethoxysanguinarine (GR14) and vanillin (GR23).

Hub genes and molecular docking simulation: By overlap of the top 10 genes according to six ranking methods in cytoHubba, SLC6A4, MAOA, CNR1 and SLC6A3 were identified as hub genes (Table 2). Correspondingly, the GR01, GR02, GR07, GR11, GR14 and GR21 may be the hub anti-depressive components. The interaction models between four hub genes and their corresponding active components were investigated by molecular docking simulation. Simultaneously, fluoxetine, phenelzine, cannabidiol and bupropion were selected as positive control drugs. Ten active component-target pairs of docking results were obtained (Table 3). Of these, the binding affinities of four pairs (SLC6A4-GR14, MAOA-GR17, CNR1-GR14

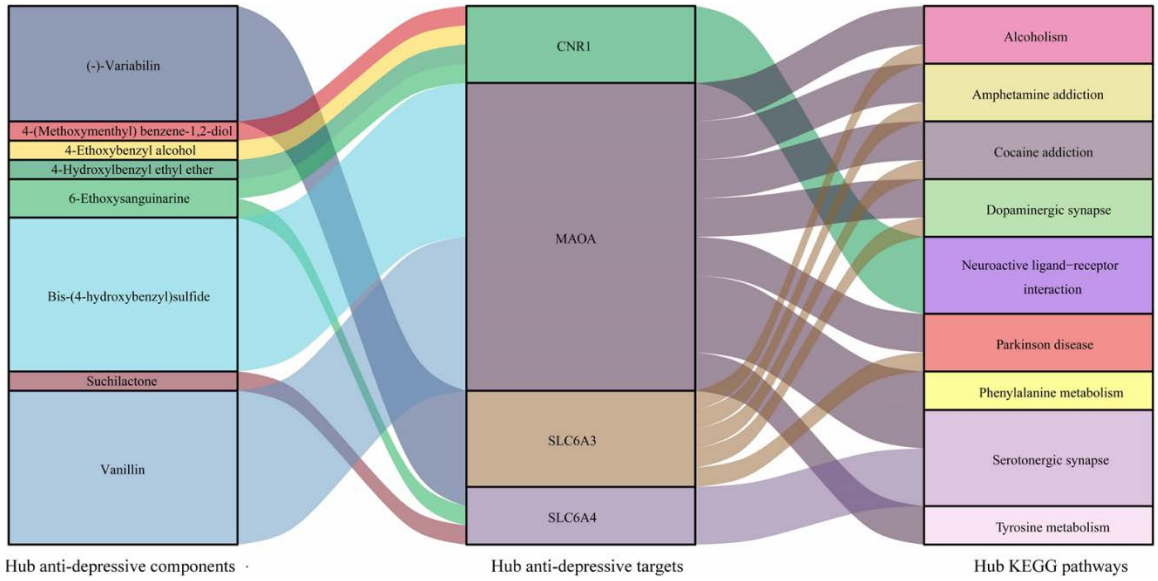

Fig. 9: Sankey diagram of GR active components-hub targets-pathways

Table 3: Docking score of the hub targeted genes and their corresponding active components of GR

Gene symbol	Protein name	Compound (ID)	Affinity (kcal moL^{-1})
SLC6A4	Sodium-dependent serotonin transporter	(-)-Variabilin (GR01)	-9.2
		6-ethoxysanguinarine (GR14)	-10.3
		Suchilactone (GR21)	-7.7
		Fluoxetine (Control)	-9.2
MAOA	Amine oxidase [flavin-containing] A	Bis-(4-hydroxybenzyl)sulfide (GR17)	-8.3
		Vanillin (GR23)	-6.4
		Phenelzine (Control)	-6.6
CNR1	Cannabinoid receptor 1	4-(methoxymenthyl) benzene-1,2-diol(GR02)	-5.6
		4-Ethoxybenzyl alcohol(GR07)	-5.8
		4-hydroxylbenzyl ethyl ether(GR11)	-5.4
		6-ethoxysanguinarine (GR14)	-7.9
		Cannabidiol (Control)	-7.2
SLC6A3	Sodium-dependent dopamine transporter	(-)-Variabilin (GR01)	-7.2
		Bupropion (Control)	-5.9

and SLC6A3-GR01) were stronger than their corresponding positive control (SLC6A4-fluoxetine, MAOA-phenelzine, CNR1-cannabidiol and SLC6A3-bupropion). The 3D and 2D molecular docking models of GR active component-target pairs were illustrated in Fig. 8. All of these active components were observed to enter the interfaced pocket formed by amino acid residues in the proteins. Taking the SLC6A4-GR01 as an example, it showed that GR01 forms two hydrogen bond interactions with Phe355 and Tyr 175 residues on SLC6A4 and forms nine hydrophobic interactions with Thr497, Glu493, Tyr176, Gly442, Ala169, Ile172, Phe341, Tyr95 and Ser438 residues on SLC6A4.

Sankey diagram of GR active components-hub targeted genes-pathways: To comprehensively showing the relationship between GR active components, hub targets and KEGG pathways, a sankey diagram was constructed by using "ggalluvial" package in R software. As showed in Fig. 9, CNR1 was connected with four active components and one pathway, MAOA was connected with two active components and eight pathways, SLC6A3 was connected with one active component and five pathways, while SLC6A4 was connected with three active components and one pathway.

DISCUSSION

To the best of our knowledge, this is the first study to systematically uncover the GR active components and its pharmacological targets against depression based on the network pharmacology method. Disease enrichment analysis showed that 171 targets of 24 GR active components were significantly enriched in tumor and psychiatric disorders.

Recently years, continuous attention has been paid in the area of herbal medicine by researchers. Concerning depression, many herbal medicines, including saffron, turmeric, St John's wort, Korean ginseng, lavender, roseroot, catmint and dodder, had been demonstrated to have anti-depressive effect in clinical trials[45]. However, the multi-component, multi-target and multi-pathway characteristics caused barriers to uncover the molecular mechanism of herbal medicine. Fortunately, the development of network pharmacology offers an effective tool to reveal the active component and molecular mechanism of herbal medicine in the treatment of disease. Previous experimental studies had demonstrated the anti-depressive, antipsychotic and anti-tumor activities of GR, which was consistent with this study[10,46,47].

Further analysis showed that there were 38 genes involved in the anti-depressive effect of GR. The most significant enriched BP, MF and CC entries of these 38 genes were regulation of neurotransmitter levels, synaptic membrane and G protein-coupled amine receptor activity, respectively. The serotonin (5-HT), noradrenaline (NA) and dopamine (DA) transporter are the gene products of SLC6A4, SLC6A2 and SLC6A3, respectively. They are located in the plasma membrane of the presynaptic nerve terminals and are the major pharmacological targets of anti-depressants[48]. Previous studies have documented that GR water extract exhibited an anti-depressive effect by inhibition of the MAOA activity and regulation of monoamine neurotransmitters[14,49]. MAOA is a flavoenzyme that catalyzes the oxidative transformations of 5-HT, NA and DA. Inhibition of MAOA could lead to the increasing of 5-HT, NA and DA in the synaptic cleft. In this study, SLC6A2, SLC6A3, SLC6A4 and MAOA are supposed to be the pharmacological targets of GR. Furthermore, SLC6A3, SLC6A4 and MAOA are regarded as hub genes, which could explain the anti-depressive activity of GR.

It's worth mentioning that three substance dependence pathways, including cocaine addiction, alcoholism and amphetamine addiction, were significantly enriched. Previous studies have revealed that individuals with depression were at high risk for concurrent substance dependence[50,51]. Many genes, including SLC6A3, MAOA and MAOB, were extensively involved in substance dependence, nervous system and neurodegenerative disease pathways. Recent research has indicated that substance dependence pathways were related to the pathogenesis and treatment of depression[52]. Additionally, the cAMP signaling was decreased in the brain of depressed patients and increased after SSRI treatment[53]. Furthermore, it is widely accepted that DA plays an important role both in depression and Parkinson's disease. Compared with the general population, individuals with Parkinson's disease have a higher prevalence of depression[54].

CNR1 was also identified as a hub anti-depressive target. The CB1 receptor, a gene product of CNR1, is widely localized in many brain structures, including the prefrontal cortex, frontal cortex, hippocampus and cerebellum, which implicated in the pathogenesis of depression[55]. Administration of AM251, a CB1 receptor antagonist, exerts antidepressant-like effects in a dose-dependent manner in the mice[56]. Likewise, a recent study indicated that inhibition of the CB1 receptor by AM251 leads to the increased activity of moclobemide, an MAOA inhibitor[57]. This suggests that simultaneous inhibition of MAOA and CRN1 may exert a synergistic anti-depressive effect.

In terms of compounds, a total of eight components have been identified as hub active components. Experiment research has uncovered that vanillin GR01 (vanillin) alleviates depressive symptoms in the rat depression model by elevating both 5-HT and DA levels in brain tissue[58]. In line with the previous study, our study suggests that vanillin could bind with MAOA, which may be increasing 5-HT, NA and DA levels. Additionally, a previous study has also documented that GR17 (Bis-(4-hydroxybenzyl) sulfide) possesses a neuroprotective effect[59].

Overall, our study reveals that GR exerts an anti-depressive effect possibly by regulation of neurotransmitter levels, which is consistent with previous experimental researches and supplementary to study on the anti-depressive effect of GR. (-)-Variabilin (GR01), bis-(4-hydroxybenzyl)sulfide (GR17) and 6-ethoxysanguinarine (GR14) might be promising anti-depressive leading compounds since they have been well docked with hub targets. Our study further proves that the anti-depressive effects of GR might be mainly mediated by the regulation of monoamine neurotransmitters and thereby GR was beneficial in depression treatment as complementary medicine or functional food. However, there are several limitations to this study. Firstly, only already identified GR chemical ingredients were collected in this study, which may ignore the anti-depressive effect of unknown components of GR. Secondly, despite hub targets have good affinity with their corresponding GR active components in the molecular docking simulation, further experiments should be carried out to verify the interaction between them.

CONCLUSION

In this study, the active components of GR and its potential mechanism for the treatment of depression were

revealed based on network pharmacology and molecular docking technology. There were 24 active components and 38 targets involved in the anti-depressive effect of GR. SLC6A3, SLC6A4, MAOA and CNR1 may be considered as hub anti-depressive targets. GR exerts an anti-depressive effect mainly by regulation of neurotransmitter levels via neuroactive ligand-receptor interaction and dopaminergic synapse pathway. It is of great significance to developing GR as a functional food or complementary medicine in depression prevention and treatment.

SIGNIFICANCE STATEMENT

This study uncovers the pharmacological targets and active components of GR against depression by using network pharmacology, bioinformatic analysis and molecular docking. The results of this study will help the researcher to find natural compounds derived from GR as potential antidepressants that many researchers were not able to explore. This study provides a clear direction and evidence for future researches.

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