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Action mechanism of Roman chamomile in the treatment of anxiety disorder based on network pharmacology

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Abstract

Anxiety disorder is a common psychiatric disease. Roman chamomile as medicine or tea has long been used as a mild tranquilizer to reduce anxiety, but the mechanism is unclear. This research is based on network pharmacology combined with database mining to find the ingredients, action pathways and key targets of Roman chamomile for the treatment of anxiety. About 126 common targets related to chamomile and anxiety were obtained, and these targets were involved in 56 KEGG pathways. GEO screened LRRK2 as a key protein, and molecular docking showed that the protein could stably bind to drug components. Roman chamomile has the characteristics of multi-target and multi-pathway in the treatment of anxiety disorder. Its possible mechanism is to intervene anxiety disorder in the process of disease development, such as neuroactive ligand-receptor interaction, serotonin synapse, and cAMP signaling pathway. LRRK2 may be an important gene for Roman chamomile in the treatment of anxiety disorder.

Practical applications

Roman chamomile is well known for its use in medicine and tea making. It contains many nutrients, which can relieve people's anxiety, help sleep, antibacterial and anti-inflammatory. In this article, through network pharmacology combined with Gene Expression Omnibus data mining and molecular docking, the target and mechanism of Roman chamomile in the treatment of anxiety were discussed, and its efficacy was verified by model animals, which not only clarified its mechanism at the systematic level, but also proved to be effective at the biological level. It provides a reference for the further development and utilization of Roman chamomile.

KEYWORDS

anxiety disorder, GEO database, molecular docking, network pharmacology, Roman chamomile

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1 | INTRODUCTION

Anxiety disorder, also known as anxiety neurosis, is one of the most common psychiatric diseases (Ping & Buoyan, 2019; Yan & Shasha, 2019). About a quarter of adults can suffer some form of anxiety disorder. Anxiety disorder is characterized by excessive worry, restlessness, fatigue, inattention, irritability, muscle tension, and sleep problems (Mao et al., 2014). Anxiety disorder can become a morbid disorder that seriously affects normal life and even leads to suicidal ideation, making anxiety disorder an important cause of death (Bystritsky et al., 2008; Lili & Guangze, 2013). Thus, anxiety disorder must be early detected and treated to prevent the worsening of this psychiatric condition.

Among the anxiety disorder treatments, preparations of Roman chamomile (RC) have been used (Keefe et al., 2016; Mao et al., 2014). RC is an annual or perennial herb of the family Compositae produced in many regions although the one used in this study is from the United Kingdom. In the United Kingdom, RC has a long history of cultivation, and a large area of artificial cultivation. The technical system is more complete, more basic research and used for medical purposes. It has a wide range of pharmacological effects, including antibacterial and anti-inflammatory effects, anti-ulcer effects, antispasmodic effects, and emotional relief. RC preparations are also commonly used in several human diseases which include insomnia, inflammation, muscle spasm, menstrual disorders, ulcers, wounds, gastrointestinal diseases, rheumatic pain, and hemorrhoids (Srivastava et al., 2010). In anxiety disorder, the RC had been showed significant clinical effect (Hieu et al., 2019; Kong et al., 2017; Yeung et al., 2018). However, the target of RC remains to be elucidated. The purpose of this study was to explore the mechanism, pathway, and key target protein of RC essential oil in the treatment of anxiety.

Network pharmacology aims to provide a global view of the relationship between drugs, target proteins, and diseases. The view is obtained from high-throughput screening analysis or network analytic techniques for target prediction or mechanism analysis. (Cheng & Gen, 2015; Shixiu et al., 2019; Wenhua et al., 2019; Xia et al., 2019; Yulin et al., 2019). In the present study, we have used data mining techniques to obtain information on drug and disease targets to integrate and explore them in a correlation network of RC and anxiety disorder. To clarify the mechanism of action of RC on anxiety disorder, we queried SwissTargetPrediction, TCMSP component target prediction database combined with DisGeNET and OMIM disease target database, STRING protein–protein interaction (PPI) analysis. Other network pharmacological techniques were employed to predict a component-target-disease pathway (Xiaoxiao et al., 2019). In addition, potential key targets of RC for the treatment of anxiety disorder were identified by data mining of the Gene Expression Omnibus (GEO) database, followed by a network pharmacology analysis. Finally, the key target proteins were subjected to molecular docking with RC chemical components using Discovery Studio 4.0. The docking results were compared with the docking scores of existing positive drugs to provide a framework for follow-up experiments.

2 | MATERIALS AND METHODS

2.1 | Determination of chemical constituents of Roman Chamomile

The RC volatile oil was purchased from Shanghai Poli Company. Chemical composition was determined by gas chromatography-mass spectrometry (GC-MS). The gas chromatography was carried out using an Agilent HP-5 ms (30 m × 250 μm × 0.25 μm) capillary column, where the carrier gas was pure He. The GC conditions were as follows: injection volume 3 μl, shunt ratio 10:1, flow rate 1 ml/min. The initial temperature was 40°C, and increased to 250°C at 6°C/min. Electron energy acceleration was 70 eV, the ion source temperature was 230°C, the MS quadrupole temperature was 150°C, the solvent was delayed 3 min, and the mass scanning was done with the full scan mode at a scanning range 40:450 amu (Chunxue et al., 2018; Guilin et al., 2019). Data were processed with data analysis to obtain the volatile components under this condition. The mixed standard solution of n-alkane (C6-C24) was purchased from sigma Aldrich Shanghai Co., Ltd. the standard product of n-alkane was diluted with n-hexane and filtered with organic filter membrane. Under the above conditions of GC-MS, the mixed standard solution of n-alkane standard compound was analyzed and the Retention Index (RI) of each component was calculated, the calculation formula$RI = 100n + 100\left[tR\,(x) - tR\,(n)\right]/[tR\,(n+1) - tR(n)$. In the formula: $tR\,(x)$, $tR\,(n)$, $tR\,(n+1)$ represent the retention time of the n-alkane to be measured, the carbon number n and the carbon number $n+1$ respectively and $tR\,(n) < tR\,(x) < tR\,(n+1)$. Based on the comparison of retention index and NIST 14L database, the relative percentage content of each component was determined by area normalization (Yangbin et al., 2019; Yonghui et al., 2019).

2.2 | Prediction of chemical composition targets of RC

The total chemical components for RC obtained above were encoded into Canonical SMILES format by querying each component on the PubChem (https://pubchem.ncbi.nlm.nih.gov/). The Canonical SMILES were submitted to the SwissTargetPrediction (http://www.swisstargetprediction.ch/) online platform to obtain the predicted targets for each component. The SwissTargetPrediction website is a web server that can accurately predict the targets of bioactive molecules based on the combination of 2D and 3D similarity measures and known ligands, and is updated regularly (Gfeller et al., 2014). Next, according to the CAS (Chemical Abstracts Service) number of chemical composition the predicted target of chemical composition was obtained through traditional Chinese medicine systems pharmacology (TCMSP) database, TCMSP database is a unique systematic pharmacological platform of Chinese herbal medicine, which contains 499 herbs and the compound components of each medicine, as well as the targets of potential active molecules and their disease information,

which provides a new platform for studying the action mechanism of traditional Chinese medicine at the systematic level (Zhao et al., 2020). The predicted target of SwissTargetPrediction and (TCMSP) database was integrated, that is, the predicted targets of RC composition was obtained (Feng et al., 2019).

2.3 | Construction of the component-Target network

In order to visualize the relationship between drug components and targets, the above data was imported into Cytoscape 3.7.1 software, and the RC component-target network was constructed. The components and targets are called nodes, and the line connecting two nodes are referred to as edges.

2.4 | Obtaining target genes for anxiety disorder

To obtain the genes associated with anxiety disorder, the DisGeNET (http://www.disgenet.org/) and OMIM (https://omim.org/) databases were queried. DisGeNET database, which integrates the gene-disease associations of multiple databases and a large number of literatures, and uses text mining technology to analyze the correlation among Mendelian diseases, complex diseases, and environmental diseases (Piñero et al., 2020). OMIM is a continuously updated database on human genes and genetic disorders, mainly studying the relationship between phenotype and genotype (Baxevanis, 2012). Using the keyword anxiety, the results obtained were merged and integrated into an Excel table to delete duplicates, which resulted in the list of target genes related to anxiety disorder (Guifeng et al., 2019; Qingya et al., xxxx

2.5 | Gene mapping of drug diseases

The RC target and anxiety disorder related target gene lists were introduced into Venny 2.1.0, to obtain their intersection. In order to visualize more clearly the interaction between drug component target and disease target, the data were uploaded into Cytoscape 3.7.1 to construct the component-target-disease (C-T-D) network using the merge function provided by the software.

2.6 | Construction of the PPI protein network

Shared elements were integrated into an Excel table and uploaded to the STRING (https://string-db.org/) platform to construct an interaction between target and target function related proteins. The results of target–target interaction were saved in RTF format. The information of node and combination fraction were imported into Cytoscape 3.7.1 software, and the analysis tools option was used to analyze the size and color of nodes to reflect the number of degrees. This analysis showed more clearly and intuitively the interaction between the target proteins.

2.7 | Correlation path and annotation analysis

The Gene Ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment of intersecting targets were carried out by using R language (Cluster Profiler package) (Guangchuang et al., 2012). Biological processes associated to RC active components acting on anxiety disorder target proteins and the regulatory pathway were analyzed.

2.8 | Verification of target protein GEO dataset

Keywords "anxiety disorder" on species "Homo sapiens" were searched in the GEO database, producing two data sets with IDs GSE61672 and GSE78104, for subsequent analysis. Data set GSE61672 is composed of 546 samples (451 control and 95 patient samples). Dataset GSE78104 is composed of 60 samples (30 controls and 30 patients with the disease). The raw dataset was read using the Affy package for the R language (Version 1.50.0, http://www.bioconductor.org/packages/release/bioc/html/affy.html). To identify significant differentially expressed genes, the anxiety disorder cases (disease) were compared to the gene expression profiles in the control group (healthy). The limma package (Version 3.26.9) (http://bioconductor.org/packages/release/bioc/html/limma.html) was used to calculate the differential expression, the *p*-value and the fold Change values. *p*-values less than .05 and logFC > logFC_cutoff were selected as threshold to screen differentially expressed mRNA. Finally, the platform annotation file was used to annotate the probe by removing the probes that did not match the gene (based on Gene symbols). The different genes obtained from the two samples were intersected with common drug-disease targets (Yanhong et al., 2018).

2.9 | Molecular docking

In order to verify the binding between drug components and their potential key targets, the chemical components were docked using Discovery Studio software v 4.0. The 2D structure of the RC compounds were downloaded from PubChem database. At the same time, the 2D structure of positive control drugs for the target protein were obtained from the DrugBank database. The structure of the target protein was downloaded as pdb format on SWISS MODEL, and used as a receptor in Discovery Studio 4.0. The structure of the protein was edited by deletion of water and prepared using the option "Prepare protein" for conventional pretreatment to complete the missing residues, delete the conformation of the excess protein, hydrogenate and distribute the related

charges, identify the active site, and define it by the software. "Small Molecules" option was selected to pretreat the ligand, select the "Full Minimization" module to minimize the energy of small molecules followed "Libdock" to select the docking mode using default docking parameters. Results of the scoring function were analyzed (Jiahui et al., 2020; Kumar et al., 2019; Peng et al., 2018; Qin et al., 2016).

2.10 | Animal grouping and modeling

30 Specific Pathogen Free grade Sprague Dawley (*SD*) rats, 180 ± 10 g, half male and half female, were purchased from Chengdu Dashuo Experimental Animal Co., Ltd., Chengdu, China. The experimental animal license number is SYXK (chuan) 2019-189. This experiment was approved by the Ethics Committee of Shaanxi University of Chinese Medicine. After 7 days of adaptive feeding under standard feeding conditions, the rats were randomly divided into three groups. Except for the control group, anxiety models were established by stress stimulation, as described (Xiong, 2019). One type of daily stimulation was performed during 28 days, and each stimulus was randomly used three to four times to ensure that no consecutive stimuli were the same. After the modeling was completed, the three groups of rats were placed in three spaces of the same size but isolated from each other. Each space had vents to ensure ventilation. The treatment group was fumigated with 2% RC essential oil for 45 min. Control and model groups were fumigated with distilled water for 45 min during 14 days (Aponso et al., 2020; Sánchez-Vidaña et al., 2019).The open field test was carried out at the end of the treatment, when the complete hippocampal tissue was quickly isolated after anesthesia and fixed with formalin.

2.11 | Open field test

The rats were placed in a square open box (100 cm × 100 cm × 38 cm), and spontaneous activity was analyzed during 5 min. During that period, the environment was quiet and the temperature was suitable. The indices recorded were average speed, total distance of movement, rest time and number of times entering the central area (50 cm × 50 cm). The average speed was used as an index to evaluate the autonomous activity of animals. The number of times entering the central area is an indicator of animal anxiety behavior and the smaller this number, the more serious the anxiety state. After each test, the bottom and side walls of the box were cleaned to make it clean and odorless (Seibenhener & Wooten, 2015).

2.12 | Immunohistochemistry

Expression of 5-HT2A in rat hippocampus was detected by immunohistochemistry. The hippocampal tissue was fixed in formalin for 48 hr and embedded in conventional paraffin. Sliced of 4 μm were deparaffinized and hydrated, and added 3% after antigen retrieval H_2O_2 blocks endogenous peroxidase and blocks non-specific antigens. The primary antibody was added and incubated overnight at 4°C. The sample was placed at room temperature for 40 min and rinsed with PBS for 3 × 3 min. The secondary antibody was incubated for 60 min and rinsed with PBS for 3 × 3 min. The sample was counterstained with hematoxylin, dehydrated with an ethanol gradient, xylene to transparent, and mounted in neutral gum. The expression of positive particles in the hippocampus were observed by randomly choosing five fields of view for each slice. The average optical density (OD) was calculated with Image-Pro Plus 6.0 image analysis software, and the average was taken as that result of the slice.

2.13 | Statistical analysis

The experimental data were expressed as means ± *SD*. The comparison of more than two groups was performed with one-way analysis of variance (ANOVA).

3 | RESULT

3.1 | Determination of chemical constituents in chamomile and identification of its targets

GC-MS analysis showed that the proton flow diagram for the RC volatile oil (Figure 1). The threshold of the data was set to 19.5 by data analysis software. A total of 63 chemical components were measured under this condition; 55 after deletion of repeated components (Table 1). Our measured GC-MS results are highly consistent with the standards provided to us by the manufacturer. The 55 chemical constituents of RC were submitted to the PubChem and SwissTargetPrediction databases. The targets identified from the two databases were integrated and duplicates were deleted. After this data treatment a total of 669 RC component targets were obtained.

3.2 | Construction of component-target network

The component-target network (Figure 2) consisted of 727 nodes, 55 RC components, and 672 targets. The edges between nodes represent the interaction between components and targets, with a total of 5,073 edges.

3.3 | Anxiety related targets

A total of 1,212 pieces of information about anxiety disorder targets were obtained from database DisGeNET and 273 pieces from OMIM. The information from those two databases data was further

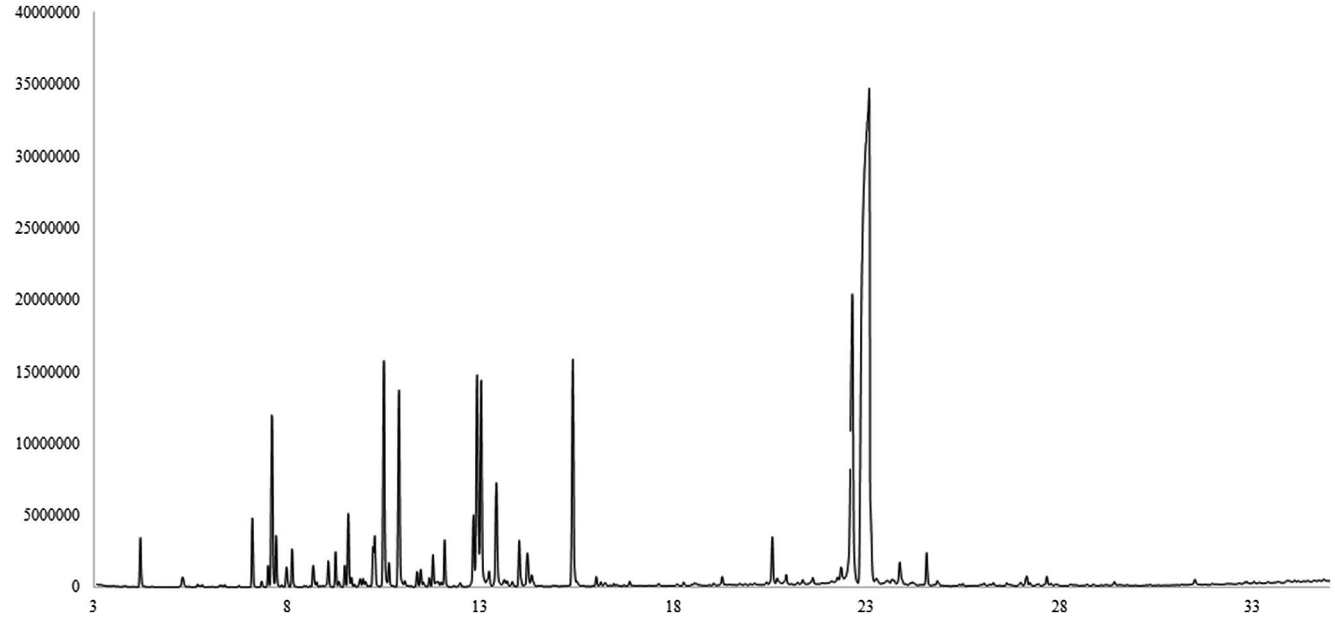

FIGURE 1 GC-MS chromatogram of volatile oil from Roman chamomile

merged, and after deleting the duplicate genes, a total of 712 related targets were obtained.

3.4 | Target protein cross validation

The targets of the RC components and the anxiety disorder-related genes were intersected with the help of Venny map. As a result, 126 common proteins were obtained. These 126 target proteins are target proteins of RC acting on anxiety disorder (Figure 3). In the network image of RC component target and anxiety disorder target, green represents the target protein of anxiety disorder, the left circle represents RC component and target protein, and red in the middle represents their intersection, which may be involved in the therapeutic pathway of anxiety disorder (Figure 4).

3.5 | Construction of PPI protein Network

The protein–protein interaction diagram of 126 intersecting target proteins using STRING is shown in Figure 5(a), Red is evidence of fusion, green is evidence of proximity, yellow is evidence of text mining, light blue is evidence of database, blue is evidence of coexistence, black is evidence of co-expression, and purple is evidence of experiment, and the protein interaction map is shown in Figure 5(b). The size of the circle in the image varies according to the (degree) value of each protein; thus, the larger the circle is, the higher the (degree) value of the protein. The higher the Degree value is, the more pathways are the protein participating. According to the value of degree, the first five proteins are BDNF, AKT1, APP, CREB1, and IL6. The literature shown that BDNF protein is involved in the neural pathway, which is related to neurological diseases (Eiji et al., 2003; Shuxian et al., 2014), and APP protein also plays a role in the treatment of anxiety disorder by participating in the cAMP pathway (Chunhui, 2015; Lundegaard et al., 2015).

3.6 | Correlation path and annotation analysis

GO analysis showed that there were 1,591 pathways related to the biological process (BP), which included the regulation of neurotransmitter levels, transport of metal ions, the regulation of membrane potential, learning or memory, and the behavioral response of cocaine. The enrichment results for the first 20 pathways with a significant p value is shown in the Figure 6(a), the horizontal axis is the number of genes of the BP term, and the vertical axis is the description of the term. Depending on the size of the P.adjust value, the color changes from red to blue. Figure 6(b) is an interactive network constructed by R language, each node is an enriched BP term, and the node size corresponds to the number of genes enriched under the term red dots show different terms and show the relationship between each term. Figure 6(c) is the core path, term gray dots represent genes, yellow dots represent term, from which we can see the enriched targets on each path.

There were 81 pathways related to cellular components (CC) ontology, including presynaptic membrane, postsynaptic membrane, excitatory synapse, neuromuscular connection, neuronal cell body, and dopaminergic synapse, which are involved in the pathological development of anxiety disorder. The enrichment results of the most significant 20 pathways are shown in the Figure 7.

The results of molecular functional (MF) analysis suggested 149 pathways, including neurotransmitter receptor activity, G protein coupled peptide receptor activity, neurotransmitter binding, neuropeptide receptor activity, serotonin receptor activity, and so on. In

TABLE 1 Constituents of volatile oil from Roman chamomile

No.	RT	Compound	Retention index	Pct. total
1	4.2267	Toluene	756	0.821
2	5.3203	1,1-Diethoxyethane	724	0.262
3	7.1307	Butylisobutyrate	956	1.12
4	7.3696	2-Methylbut-2-en-1-yl acetate	922	0.112
5	7.5351	Propanoic acid, 2-methyl-, 2-methyl-2-propenyl ester	928	0.337
6	7.6361	(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-en	–	3.284
7	7.7464	2-Propenoic acid, 2-methyl-, 2-methylpropyl este	–	0.871
8	8.0129	Bicyclo[2.2.1]heptane, 2,2-dimethyl-3-methylene-, (1S)-	–	0.401
9	8.16	Methallyl methacrylate	955	0.637
10	8.7022	2(10)-Pinene	965	0.425
11	8.7941	3-Methylpentyl acetate	981	0.088
12	9.0973	2-Butenoic acid, 2-methyl-, propyl ester, (Z)-	995	0.483
13	9.2811	Butanoic acid, 2-methyl-, 2-methylpropyl ester	1,004	0.563
14	9.3638	Isobutyl isovalerate	1,007	0.105
15	9.5201	Butanoic acid, 3-methylbutyl ester	1,055	0.343
16	9.612	Propanoic acid, 2-methyl-, 2-methylbutyl ester	1,018	1.23
17	9.6947	2-Methylbutanoic anhydride	–	0.163
18	9.9152	Benzene, 1-methyl-4-(1-methylethyl)-	1,022	0.169
19	9.9887	D-Limonene	–	0.148
20	10.0623	Eucalyptol	1,028	0.104
21	10.2553	Cyclopropanecarboxylic acid,3-methylbutyl ester	-	0.594
22	10.531	2-Butenoic acid, 2-methyl-, 2-methylpropyl ester, (Z)-	1,056	4.437
23	10.6596	Butanoic acid, 3-methylbut-2-enyl ester	–	0.452
24	10.9261	2-Butenoic acid, 2-methyl-, 2-methyl-2-propenyl ester, (Z)-	1,069	3.958
25	11.0732	3-Ethyl-4-methylpentan-1-ol	1,024	0.099
26	11.3948	(E)-2-Methylbut-2-en-1-yl methacrylate	1,088	0.246
27	11.4867	Butyl angelate	1,092	0.301
28	11.7072	Butanoic acid, 2-methyl-, 3-methylbutyl este	1,102	0.146
29	11.7991	Butanoic acid, 2-methyl-, 2-methylbutyl ester	1,105	0.522
30	12.102	Propanoic acid, 2-methyl-, 2-methylpentyl ester	1,119	0.791
31	12.8468	3-Methylpentyl methacrylate	1,148	1.362
32	12.9387	Pentan-2-yl 2-methylbut-2-enoate	1,153	4.673
33	13.2511	Tridecenyl tiglate, 2E-	–	0.435
34	13.4441	Pinocarvone	1,165	2.277
35	13.6463	Borneol	1,167	0.191
36	14.2436	Bicyclo[3.1.1]hept-2-ene-2-carboxaldehyde, 6,6-dimethyl-	1,177	0.759
37	14.3539	2-Pinen-10-ol	1,183	0.035
38	15.4199	2-Butenoic acid, 2-methyl-, 3-methylpentyl ester, (Z)-	1,259	4.297
39	16.0265	cis-3-Hexenyl tiglate	1,275	0.154
40	16.8903	Cyclohexasiloxane, dodecamethyl-	–	0.077
41	20.5846	(–)-Germacrene D	1,480	0.95
42	20.713	Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4a.alpha., 7.alpha., 8a.beta.)]-	1,488	0.219
43	21.3749	.delta.-Cadinene	1526	0.109
44	21.6323	Propanoic acid, 2-methyl-, 3,7-dimethyl-2,6-octadienyl ester, (Z)-	1,488	0.177
45	22.2755	Diethyl Phthalate	1595	0.211

(Continues)

TABLE 1 (Continued)

No.	RT	Compound	Retention index	Pct. total
46	22.3675	Phthalic acid, ethyl 2-phenylethyl ester	–	0.52
47	22.6523	Diethyl Phthalate	1595	50.511
48	23.2772	1,4-Methanoazulene, decahydro-4,8,8-trimethyl-9-methylene-, (1S,3aR,4S,8aS)-(+)-	1,403	0.262
49	23.8838	Cyclohexene, 4-methyl-1-(1-methylethyl)-	1,016	0.644
50	24.5822	Geranyl tiglate	1701	0.597
51	24.8579	Hexanoic acid, 3,7-dimethyl-2,6-octadienyl ester, (Z)-	1733	0.13
52	27.1737	2-Pentadecanone, 6,10,14-trimethyl-	1847	0.234
53	27.6884	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	1869	0.164
54	31.5205	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-	2,123	0.142
55	36.2349	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl) -4-methyl-	–	0.738

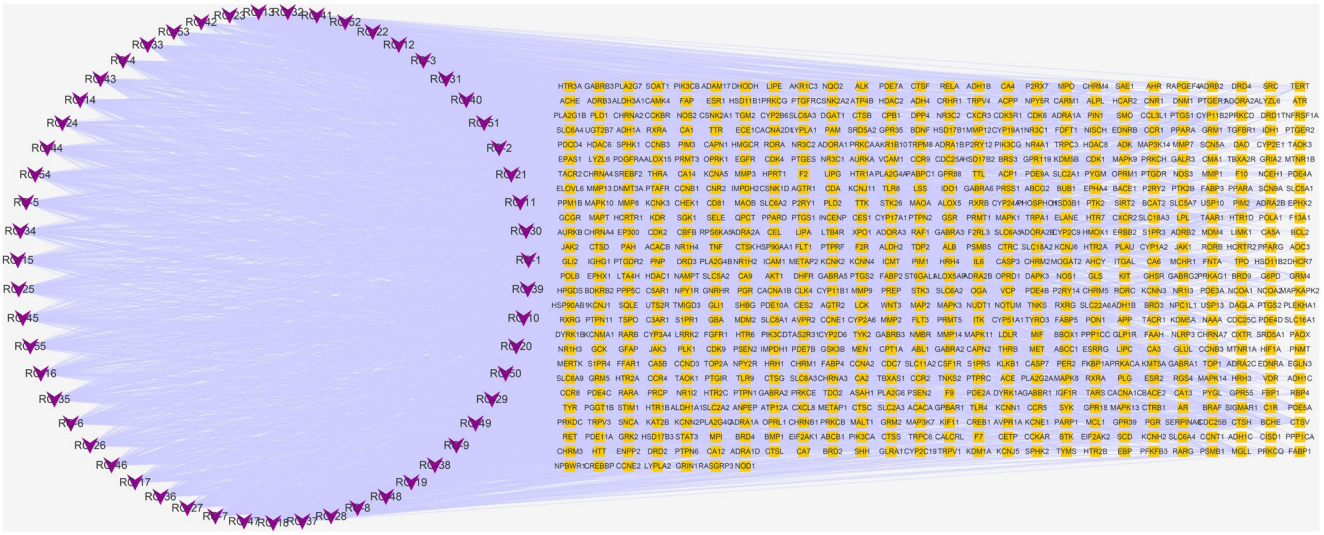

FIGURE 2 Component-target network. The purple V shape on the left represents the composition of RC, and the orange rectangle on the right is the target protein corresponding to the component

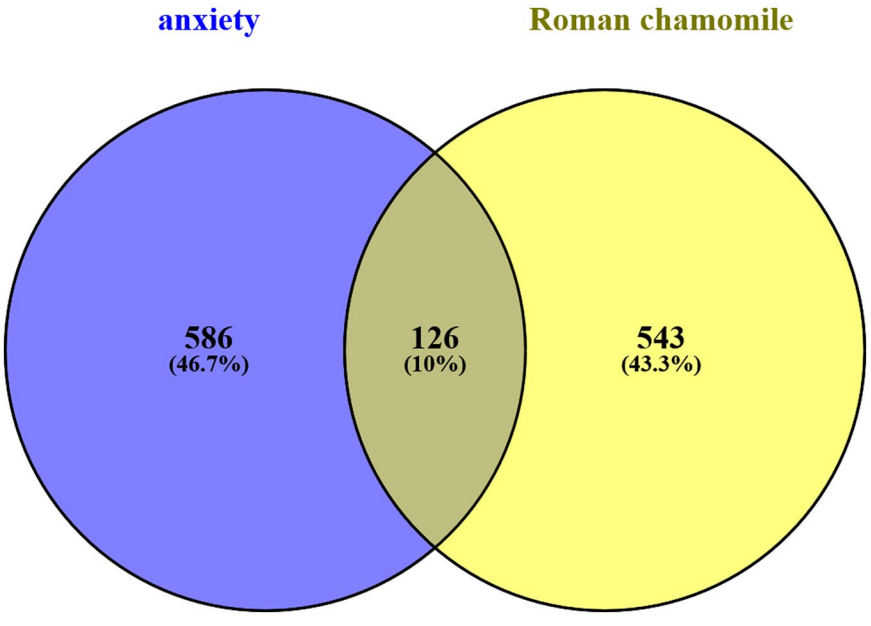

FIGURE 3 Venny diagram of component targets and disease targets

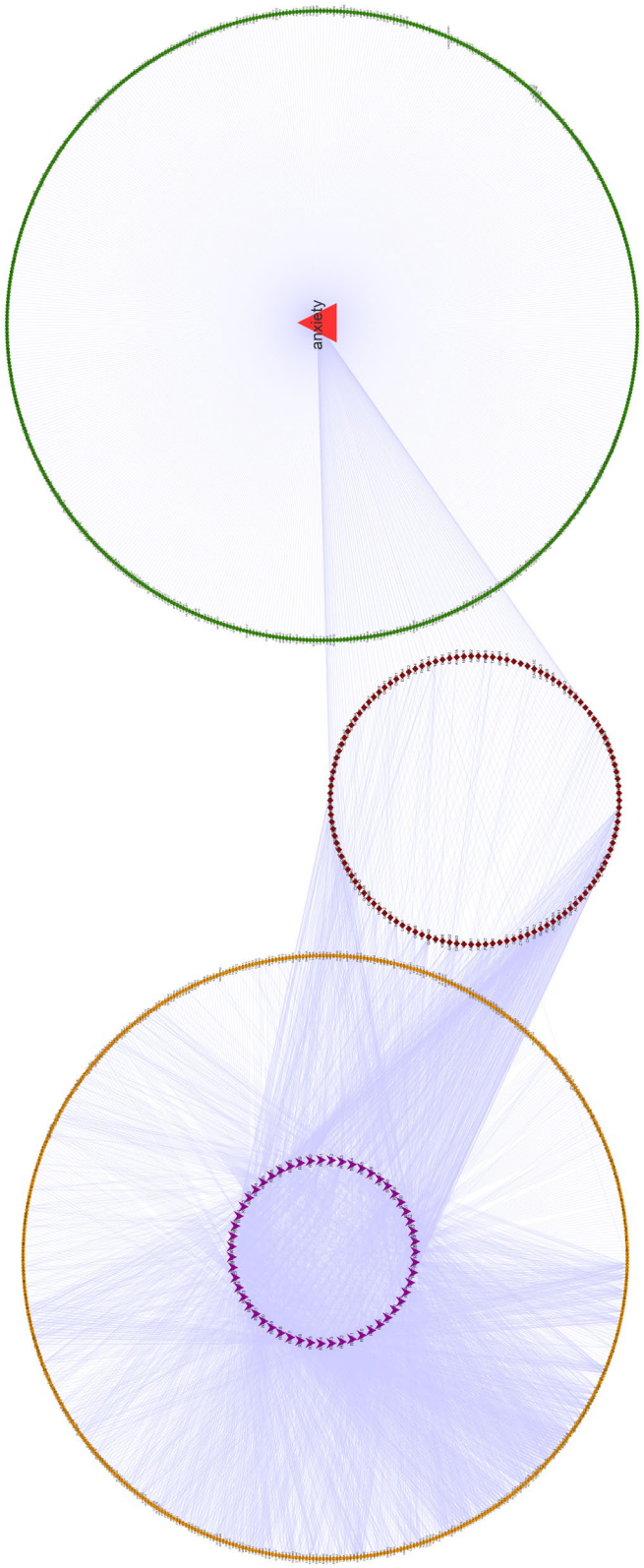

FIGURE 4 Component-target-disease pathway. This is a direct view of Figure 3. On the left is the composition target map, on the right is the disease target map, and in the middle are 126 intersecting targets. The names of each protein are marked on the map

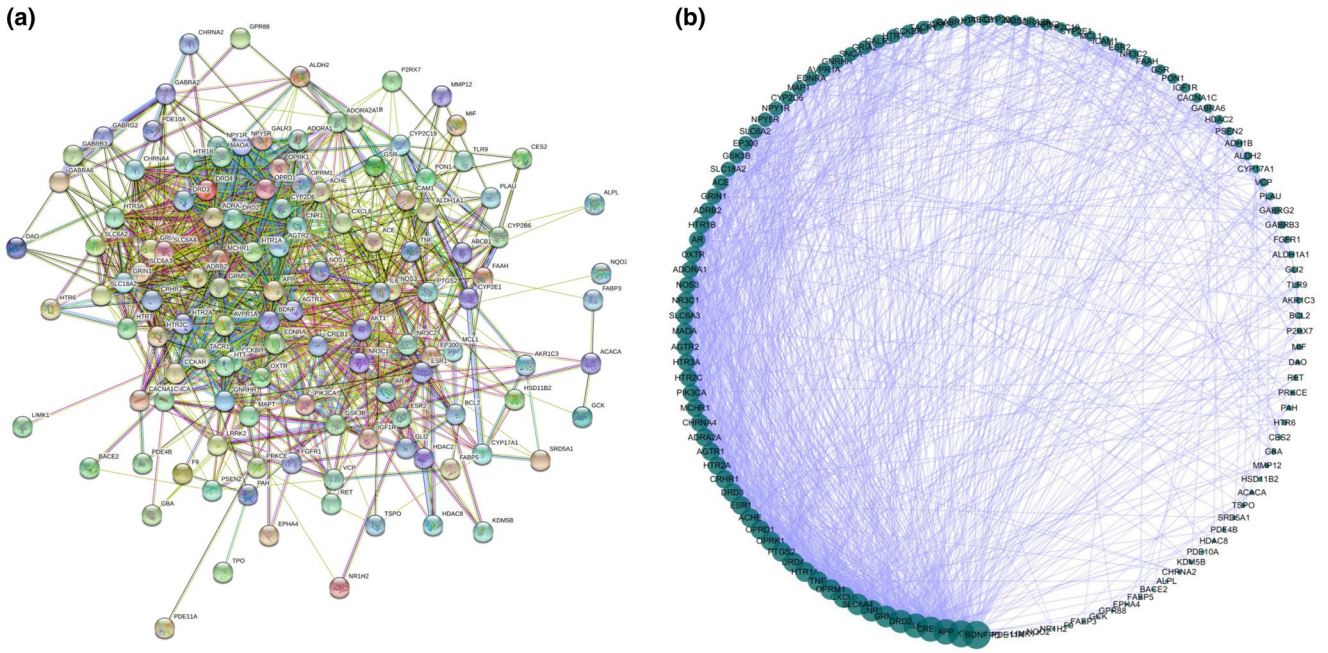

FIGURE 5 Component-disease intersection gene PPI network. (a) is the protein interaction network diagram exported from the STRING website, (b) is the arrangement from Large to Small according to Degree

the Figure 8, the enrichment result for the first 20 pathways with significant *p*-value are shown.

A total of 56 KEGG pathways, including neuroactive ligand–receptor interaction, serotonin synapse, cAMP signaling pathway, calcium signaling pathway, and drug metabolism-cytochrome P450, were related to the anxiety disorder. According to the most important 20 pathways (Figure 9(a)), the serotonin synaptic pathway was closely related to anxiety disorder (Figure 9(b)). The serotonin, also known as 5-HT, is a messenger that produces pleasant emotions. 5-HT is also associated with anxiety disorder, which affects almost every aspect of brain activity: from regulating mood, energy, memory to shaping an outlook on life. 5-HT reuptake inhibitors are effective for anxiety disorders. The target proteins acting on this pathway were present on the diagrams.

3.7 | Verification of target protein GEO dataset

The two groups of samples were downloaded from the GEO database and processed with affy package and limma package of R language, respectively, and the differential genes of the two groups of samples were sorted out. There were 466 differential genes from the GSE61672 data set and 532 differential genes from the GSE78104 data set, as shown the volcano plots (Figure 10(a, b)), where blue is the down-regulated gene and red is the upregulated gene. The two groups of differential genes were inspected through Venn diagrams and 126 were shared, among them the key gene LRRK2, was obtained (Figure 10(c)). LRRK2 can be regarded as a key target protein for RC in the treatment of anxiety disorder. The results of previous GO analysis showed that LRRK2 target protein was involved in the biological process (BP), 273 entries in total, which is related to the regulation of neurotransmitter levels, response to ammonium ion, signal release, and other pathways involved in the regulation of disease development. The five molecular functional (MF) related to this gene were ion channel binding, tubulin binding, actin binding, etc. Involved 30 cellular components (CC), include presynaptic, neuronal cell body, neuronal projection end, and so on. Table 2 shows the first five processes selected from each level of GO according to their *p*-value.

3.8 | Molecular docking

LRRK2 was docked with RC components and its own positive drug, respectively. The docking results show that the RC components can stably bind into the active site of the target LRRK2. The docking scores (Diller & Merz, 2001; Li & Liao, 2013) for the RC components were higher than that for the positive drug. The best three components and positive drug scores are shown in Table 3. A two-dimensional diagram of the three ligands with the protein was made, as shown in Figure 11. The small circles represent amino acid residues, and the amino acid name abbreviation is marked on them. The color represents the type of interaction. The lightest green represents carbon–hydrogen bonds, light green represents van der Waals forces, and dark green represents conventional hydrogen bonds, purple is the alkyl group, and the dashed line is the interaction between the receptor and the ligand. Ligand components and proteins interact through these chemical bonds. Figure 11(a) is the positive drug

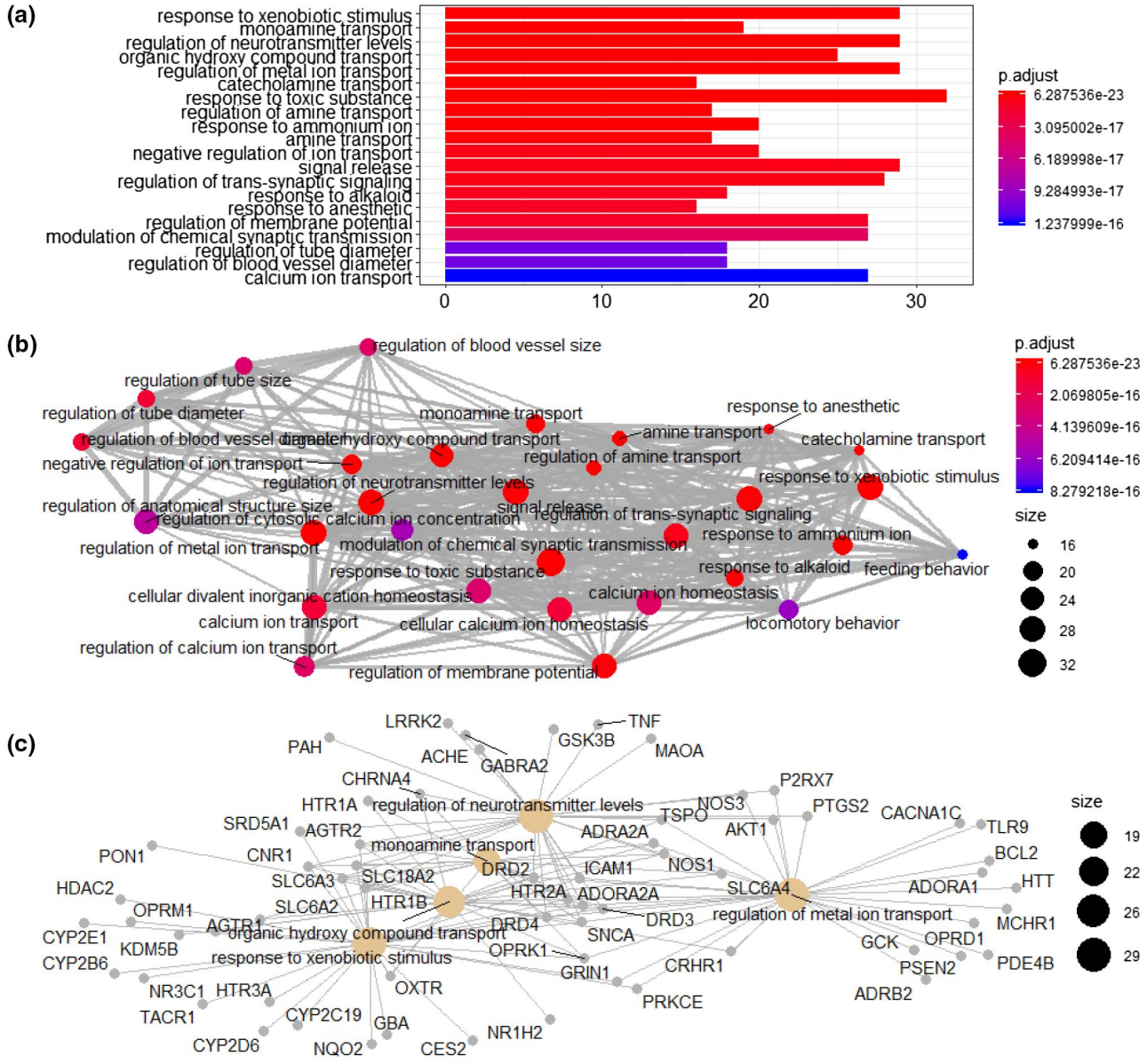

FIGURE 6 BP path analysis. (a): enrichment results of the first 20 pathways (b): path interaction network (c): core pathway

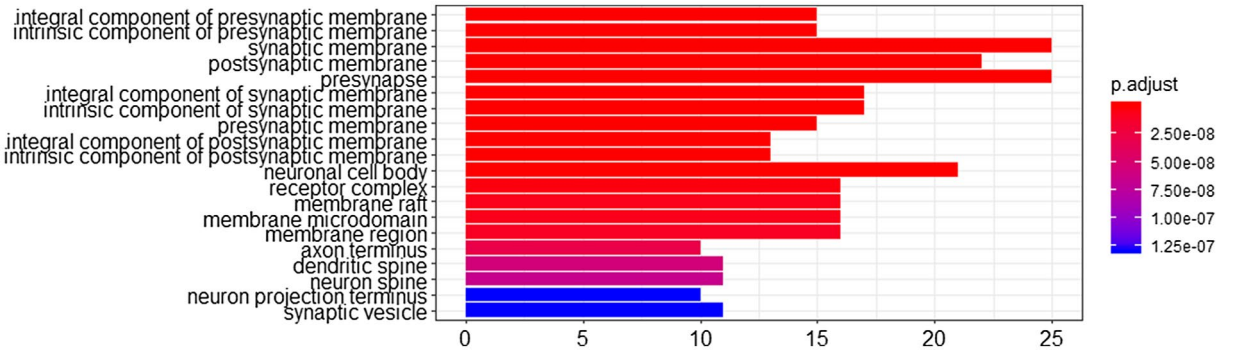

FIGURE 7 The first 20 items with significant P value in CC analysis

docking results. It can be seen that alanine and methionine interact with functional groups through conventional hydrogen bonding, serine acts through carbon-hydrogen bonding, glycine acts through carbon-hydrogen bonding and electron-withdrawing, and leucine, valine, and Histidine interacts via van der Waals forces, and lysine interacts with alkylation groups via electron-withdrawing forces.

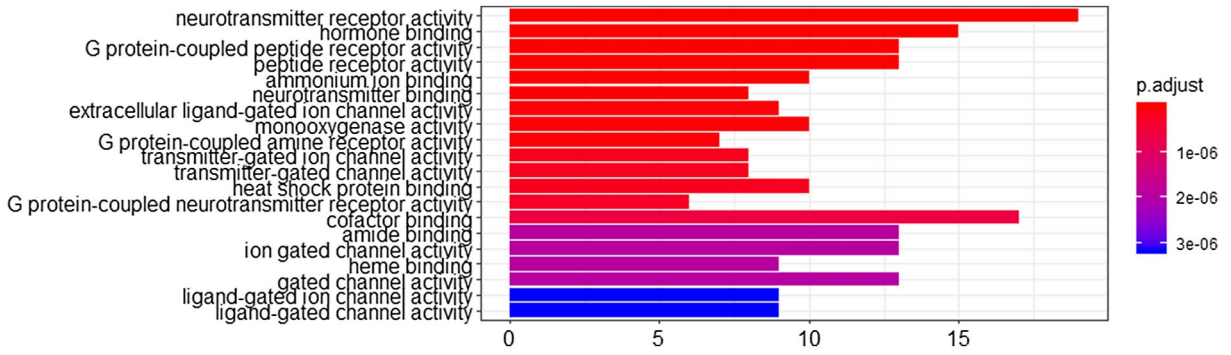

FIGURE 8 The first 20 items with significant P value in MF analysis

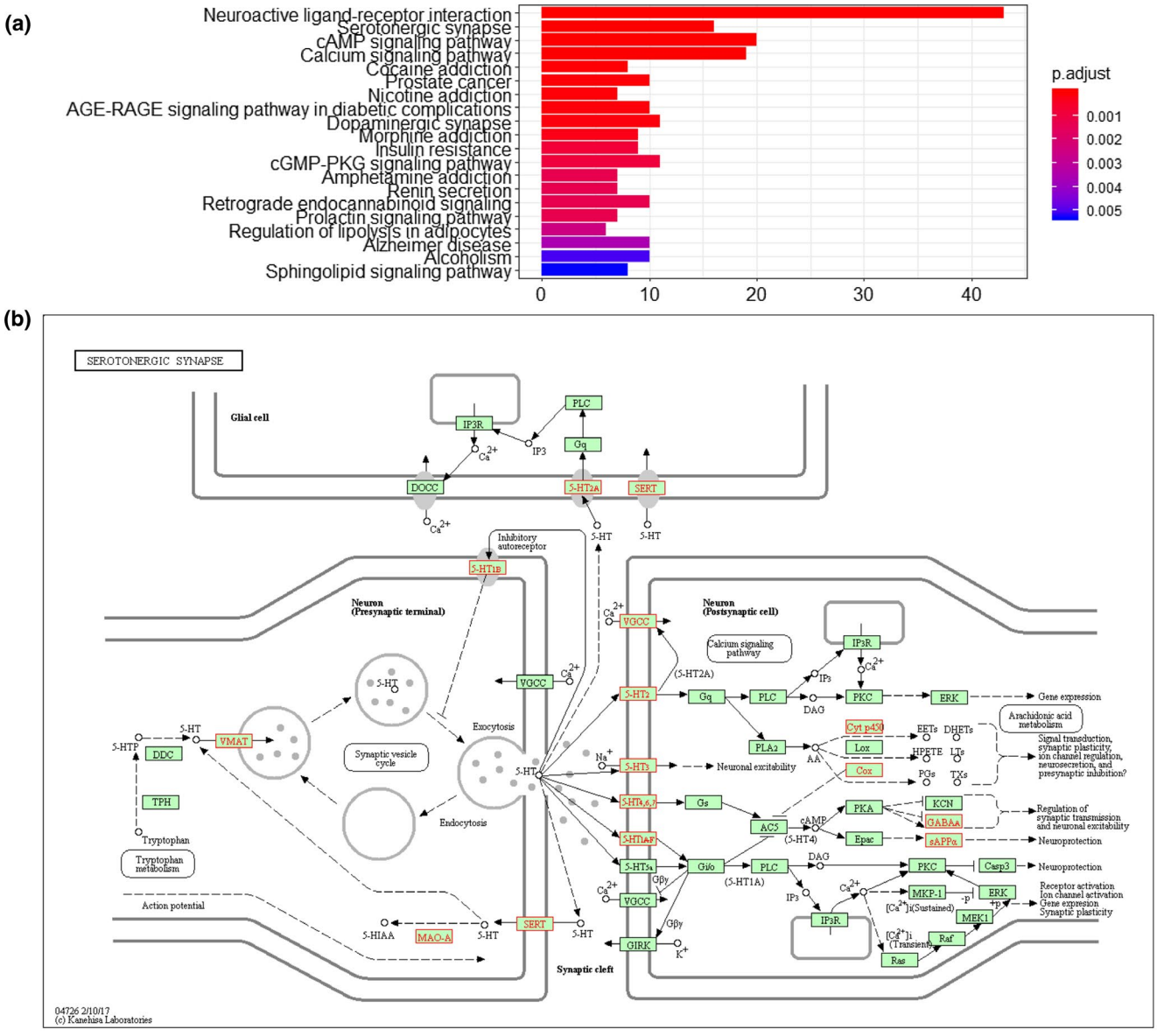

FIGURE 9 KEGG enrichment results. (a): The first 20 significant *P* values of KEGG pathway (b): Serotonin synaptic pathway. The green rectangle represents the gene on the node, the solid line is the direct effect, and the dotted line is the indirect effect

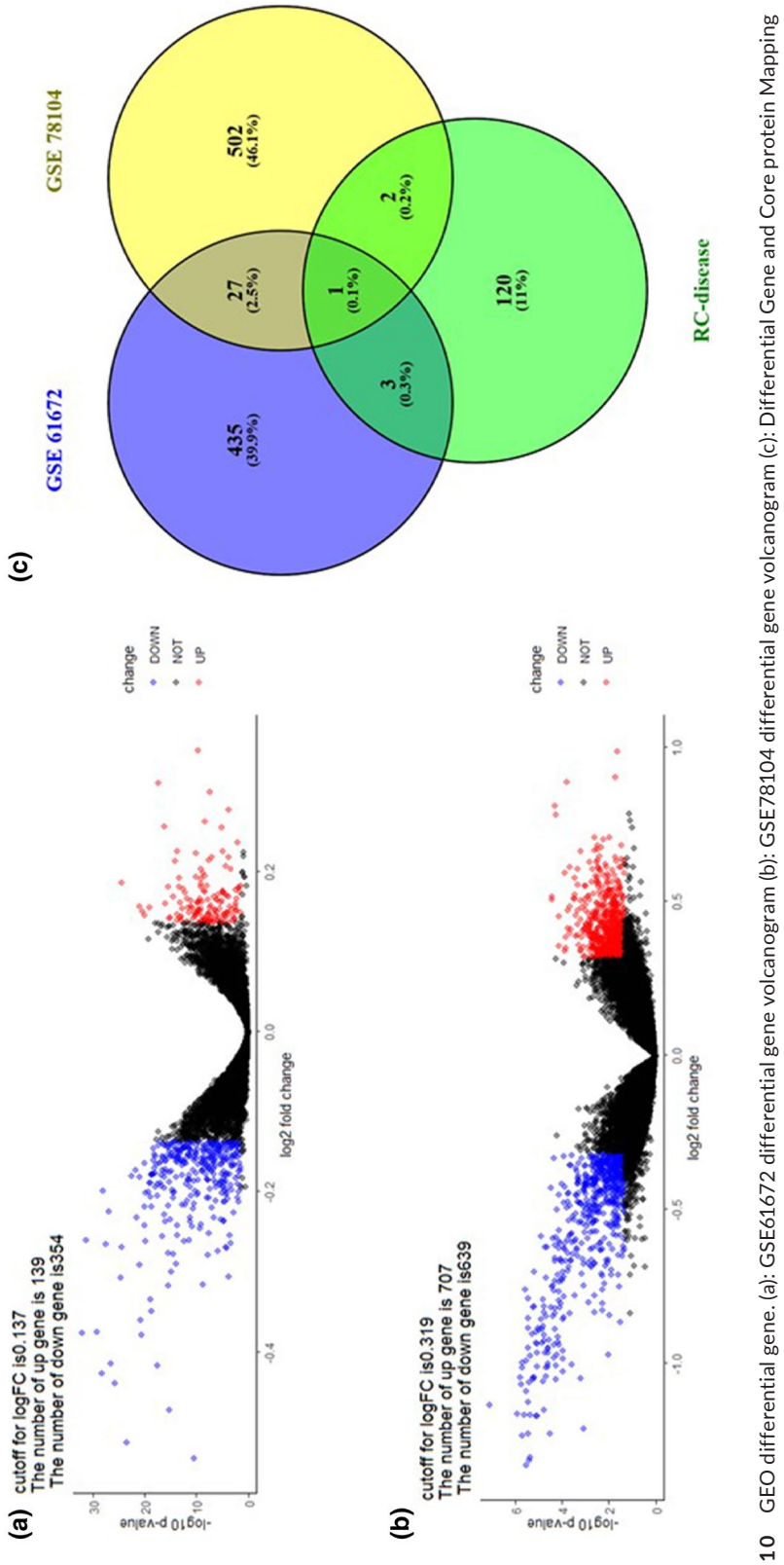

FIGURE 10 GEO differential gene. (a): GSE61672 differential gene volcanogram (b): GSE78104 differential gene volcanogram (c): Differential Gene and Core protein Mapping

TABLE 2 partial GO analysis of LRRK2 participation

	Description	*p*-value	geneID
BP	regulation of neurotransmitter levels	2.87E-24	DRD2/HTR1A/MAOA/PAH/SLC6A4/ADORA2A/CNR1/DRD4/HTR2A/ACHE/HTR1B/SLC6A3/AGTR2/TSPO/CHRNA4/GABRA2/PTGS2/SLC18A2/SRD5A1/TNF/**LRRK2**/DRD3/P2RX7/ICAM1/NOS1/GSK3B/SNCA/NOS3/AKT1
BP	response to toxic substance	3.65E-22	DRD2/SLC6A4/ADORA2A/CNR1/OXTR/DRD4/HTR1B/SLC6A3/CREB1/CYP2E1/IL6/OPRM1/CHRNA4/HTR3A/PTGS2/SLC18A2/SRD5A1/TACR1/TNF/**LRRK2**/HDAC2/DRD3/BCL2/PRKCE/ICAM1/OPRK1/TPO/OPRD1/KDM5B/NOS3/GSR/GRIN1
BP	response to ammonium ion	7.13E-22	DRD2/CNR1/OXTR/DRD4/HTR2A/HTR1B/SLC6A3/OPRM1/CHRNA4/GABRB3/GABRG2/HTR3A/TACR1/**LRRK2**/HDAC2/DRD3/PRKCE/OPRK1/SNCA/GRIN1
BP	signal release	1.32E-20	DRD2/FGFR1/HTR1A/CRHR1/ADORA2A/CNR1/HTR2A/CACNA1C/HTR1B/CREB1/HTR2C/IL6/OPRM1/ADORA1/AGTR1/AGTR2/CHRNA4/SLC18A2/TACR1/TNF/**LRRK2**/ADRA2A/P2RX7/PRKCE/OPRK1/GCK/KDM5B/GSK3B/SNCA
BP	regulation of trans-synaptic signaling	1.36E-20	DRD2/APP/EPHA4/MAPT/SLC6A4/ADORA2A/CNR1/OXTR/DRD4/HTR2A/ACHE/HTR1B/CREB1/ADORA1/ADRB2/CHRNA4/FABP5/PTGS2/TACR1/TNF/**LRRK2**/DRD3/GRM5/NPY5R/PRKCE/GSK3B/SNCA/GRIN1
CC	presynapse	1.67E-16	DRD2/APP/EPHA4/SLC6A4/ADORA2A/CNR1/HTR2A/HTR1B/SLC6A3/HTT/OPRM1/ADORA1/CHRNA4/GABRA2/HTR3A/PDE4B/SLC18A2/**LRRK2**/P2RX7/NOS1/OPRK1/OPRD1/PSEN2/SNCA/GRIN1
CC	neuronal cell body	3.25E-12	DRD2/APP/EPHA4/MAPT/ADORA2A/HTR2A/CACNA1C/SLC6A3/OPRM1/ADORA1/CHRNA4/CYP17A1/HTR3A/SRD5A1/PDE10A/**LRRK2**/PAM/P2RX7/OPRK1/SNCA/RET
CC	membrane raft	2.96E-10	APP/MAPT/SLC6A4/CNR1/HTR2A/SLC6A3/OPRM1/PTGS2/SLC6A2/TNF/**LRRK2**/ICAM1/NOS1/OPRD1/NOS3/RET
CC	membrane microdomain	3.11E-10	APP/MAPT/SLC6A4/CNR1/HTR2A/SLC6A3/OPRM1/PTGS2/SLC6A2/TNF/**LRRK2**/ICAM1/NOS1/OPRD1/NOS3/RET
CC	membrane region	5.18E-10	APP/MAPT/SLC6A4/CNR1/HTR2A/SLC6A3/OPRM1/PTGS2/SLC6A2/TNF/**LRRK2**/ICAM1/NOS1/OPRD1/NOS3/RET
MF	ion channel binding	0.001615	HTT/TSPO/PDE4B/**LRRK2**/NOS1
MF	syntaxin-1 binding	0.008351	SLC6A4/**LRRK2**
MF	tubulin binding	0.010536	MAPT/HTT/PDE4B/SLC6A2/**LRRK2**/SNCA
MF	SNARE binding	0.012474	SLC6A4/**LRRK2**/SNCA
MF	actin binding	0.016824	MAPT/SLC6A4/SLC6A2/**LRRK2**/PRKCE/SNCA/NOS3

TABLE 3 Molecular docking fraction

Name	PubChem CID	LibDockScore
Fostamatinib (Positive drug)	11,671,467	78.5389
Geranyl tiglate	5,367,785	86.5483
Propanoic acid, 2-methyl-, 3,7-dimethyl-2,6-octadienyl ester, (Z)-	5,365,991	84.9253
3-Methylpentyl methacrylate	18,361,659	74.7241

Figure 11(b) is the interaction diagram of PubChem CID (5,367,785) and protein. The relationship between glycine and functional groups is conventional hydrogen bond, carbon–hydrogen bond and van der Waals force, glutamic acid is carbon–hydrogen bond, and lysine is alkylation and van der Waals force. Methionine and alanine are alkylation. Figure 11(c) is the interaction diagram of PubChem CID (5,365,991) and protein. The relationship between alanine and lysine and functional group is alkylation, glycine is carbon–hydrogen bond and van der Waals force, glutamic acid is carbon–hydrogen bond, methionine. It is a conventional hydrogen bond, and leucine is van der Waals. Figure 11(d) is the interaction diagram of PubChem CID (18,361,659) and protein. The relationship between alanine and functional groups is alkylation and van der Waals force, glycine is carbon–hydrogen bond and van der Waals force, glutamic acid is

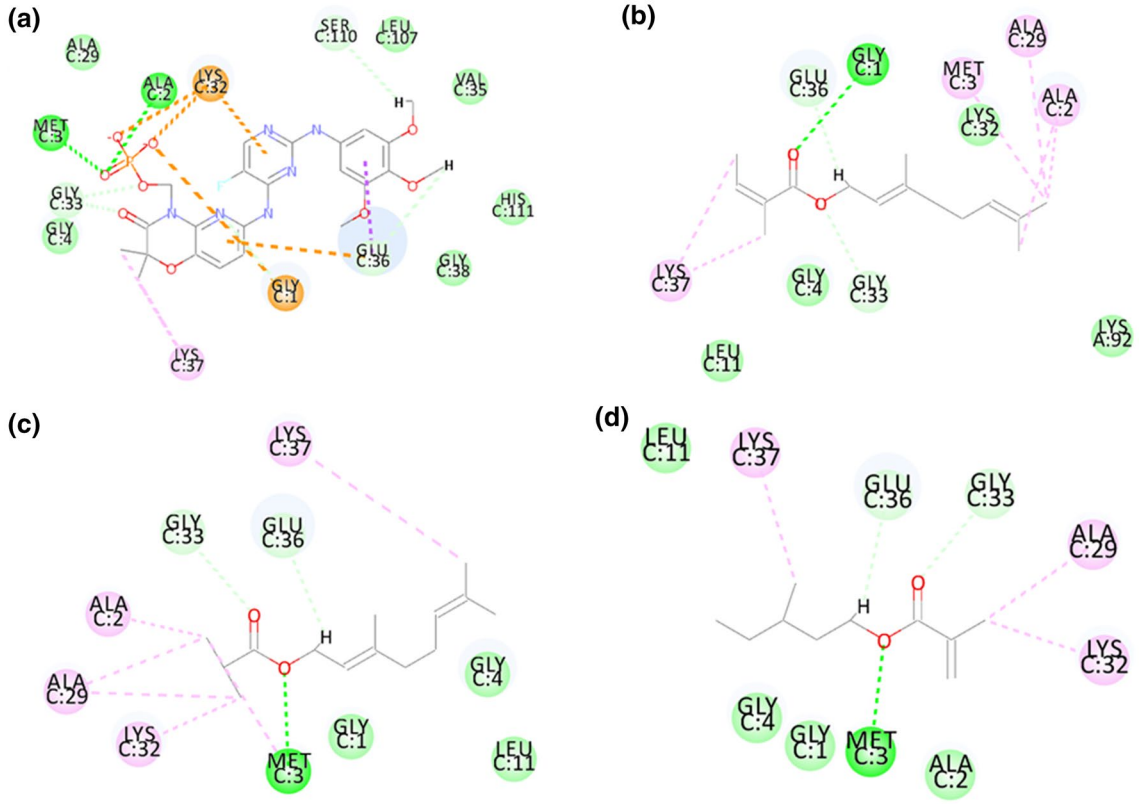

FIGURE 11 Molecular docking two-dimensional diagram. (a).11671467(Fostamatinib) with LRRK2 2D diagram (b).5367785(Geranyl tiglate) with LRRK2 2D diagram. (c). 5,365,991(Propanoic acid, 2-methyl-, 3,7-dimethyl-2,6-octadienyl ester, (Z)-) with LRRK2 2D diagram. (d). 18,361,659(3-Methylpentyl methacrylate) with LRRK2 2D diagram

carbon–hydrogen bond, lysine is alkylated, methionine is a conventional hydrogen bond, and leucine is van der Waals force.

3.9 | Open field test

The results of the open field test are shown in Figure 12. Compared with the control group, the total moving distance and average speed of rats in the model group were significantly reduced ($p < .001$), and compared with the model group, the rats in the RC group were significantly increased ($p < .01$). Entered the central area times in the model group was significantly lower than that of the control group ($p < .001$), and the RC group was significantly higher than the model group ($p < .05$). The rest time of rats in the model group was significantly higher than that of the control group ($p < .001$), and the RC group was significantly lower than model group ($p < .01$). It shows that the anxiety model is successful and RC can lighten anxiety-like behavior in rats.

3.10 | Immunohistochemistry

As shown in Figure 13, the expression of 5-HT2A in the hippocampus of the model group was significantly higher than that of the control group ($p < .01$), and the RC group was significantly lower than that of the model group ($p < .001$). It is confirmed that RC can lighten the anxiety symptoms of model rats by reducing the expression of 5-HT2A in the hippocampus of model rats.

4 | DISCUSSION

Anxiety disorder is a common disease today, and its incidence is increasing year by year. The effect of chemical drugs is not significant, and there are many side effects, strong dependence, poor compliance of patients (Chaoying, 2006; Runchen et al., 2017). RC is a traditional drug with mild effects and remarkable effect on anxiety disorder; however, its mechanism of action remains unknown. This study used network pharmacology and gene expression data mining to clarify and predict the potential protein targets and its relationship and on pathways related to RC and anxiety disorder. Molecular docking technology is used to verify the docking of key targets. Using model animals to test the efficacy in vivo.

During the study, the method of network pharmacology was used to reveal the synergistic effect of multi-target, multi-component and multi-pathway at the molecular level. Through the prediction of drug target disease targets, 669 RC targets and 712 anxiety disorder targets were analyzed. The important targets of RC in the treatment of anxiety disorder were identified. After PPI analysis, the more important proteins BDNF and APP were selected and verified

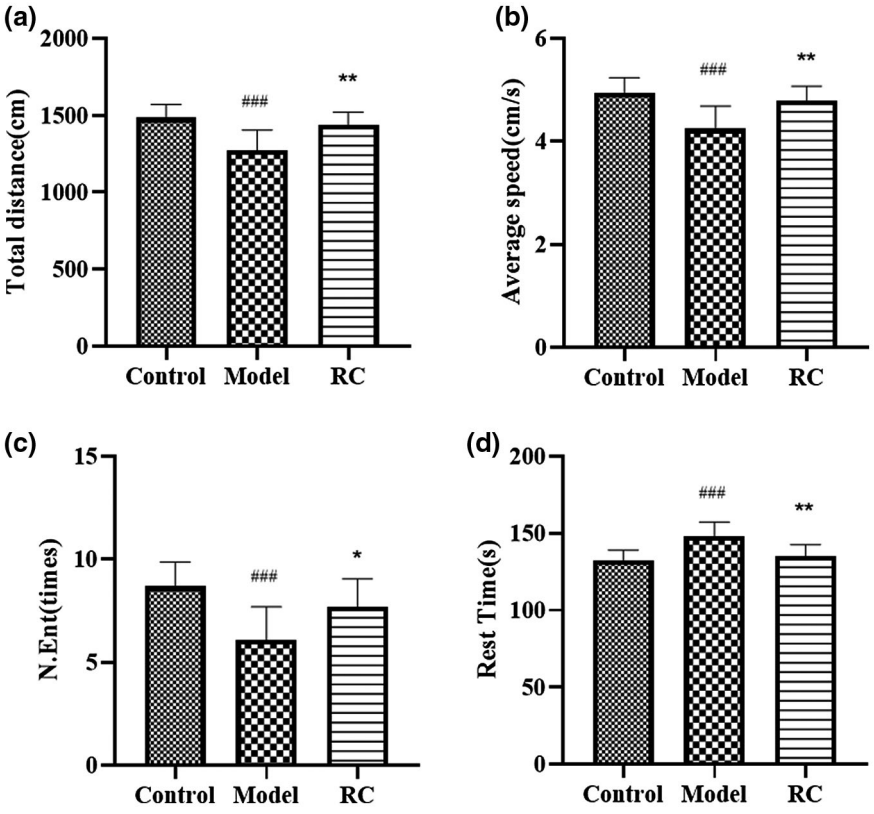

FIGURE 12 Open field test. (a): Total distance (b): Average speed (c): Number of times to enter the central area (d): Rest time. Data were expressed as means ± *SD*. Differences were assessed by ANOVA and denoted as follows: $^{*}p < .05$ versus model group; $^{**}p < .01$ versus model group; $^{###}p < .001$ versus control group

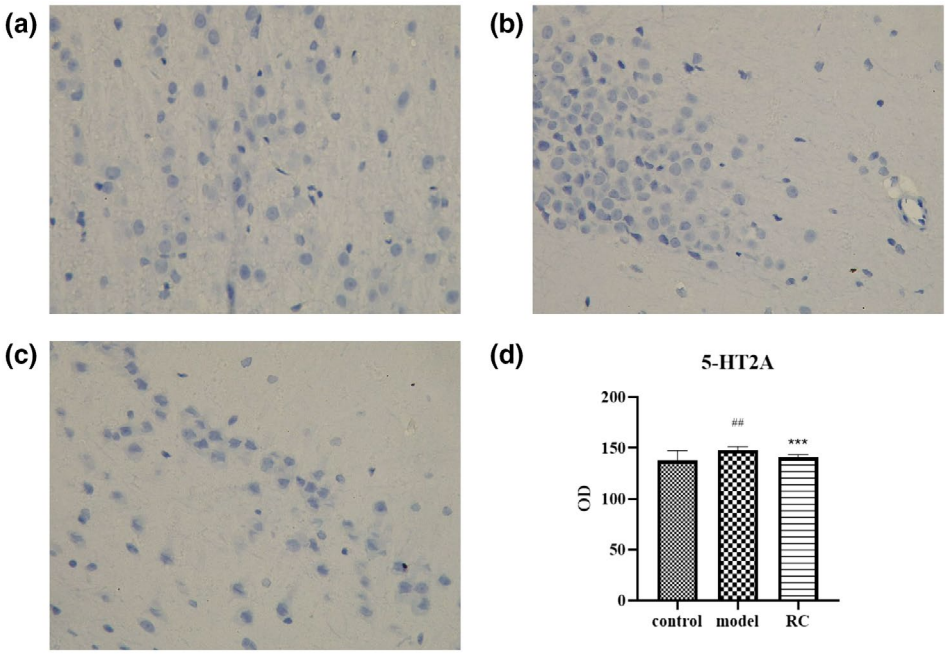

FIGURE 13 Immunohistochemistry (a): control (b): model (c):RC (d): OD value of each group $^{##}p < .01$ versus control group; $^{***}p < .001$ versus model group

in the literature (Eiji et al., 2003; Jianguo et al., 2012; Pervolaraki et al., 2019; Shuxian et al., 2014). These proteins played a role in the treatment of anxiety disorder by participating in the relevant neural pathways. Through the construction of network pathways, GO enrichment analysis and KEGG pathway enrichment analysis we found that the active components of RC may play a role in the treatment

of anxiety by participating in neuroactive ligand–receptor interaction, serotonin synapse, cAMP signaling pathway, neurotransmitter binding pathway, etc. It is known from the literature that the receptor biogenic imines contained in the neuroactive ligand–receptor interaction signal pathway is an essential stimulating nerve tissue molecule, which controls and regulates many important biological functions after binding to the corresponding receptors. For example, emotion, memory, endocrine, etc., the disorder of this pathway or the down-regulation of the receptor will cause anxiety (Lingzhen et al., 2011; Su et al., 2009). The serotonin synaptic pathway was also found, the serotonin well-known molecule that produce pleasant mood, can participate in regulating mood, energy, memory, and serotonin is also related to the occurrence of anxiety disorder (Jingxiong et al., 2018; Long et al., 2014). The cAMP signaling pathway is also involved in the anti-anxiety effect through the regulation of cAMP, reducing the level of intracellular cAMP can produce a specific anti-anxiety-like effect (Chunhui, 2015; Lundegaard et al., 2015).

Gene expression studies made on patients with anxiety disorder were analyzed in combination with key targets obtained before, and protein LRRK2 was obtained. Its gene is closely related to the treatment of Parkinson's disease (Jennifer et al., 2005; Smith et al., 2006), Parkinson's disease is usually accompanied by anxiety, and may also develop due to anxiety (Meng et al., 2018; Zhengqin et al., 2017). GO analysis shows that LRRK2 is involved in a number of GO enrichment pathways. Therefor this gene may be important for the development and treatment of anxiety disorder. Finally, LRRK2 was docked with the RC component and docked with the positive drug for the gene in DrugBank. The results showed that RC components could bind to the target LRRK2 and the docking scores were higher than that of the positive drug.

We used an animal anxiety model to detect the efficacy of RC. Behavior and histopathology results showed that the drug was effective. RC could effectively reduce the anxiety in model rats and the expression of 5-HT2A in the hippocampus of anxious rats. 5-HT2A is mainly located in hippocampal glutamate neurons. The activation of 5-HT2A receptor increases anxiety behavior in stress response to environmental changes (Ju et al., 2020). It is known from the KEGG results that the drug participates in the 5-HT pathway, 5-HT is considered to be an important substance that induces anxiety, and it is an important detection indicator in anxiety diseases (Bellia et al., 2020; Wang et al., 2019). According to the literature, in anxiety disorders LRRK2 is related to 5-HT, and mutations in LRRK2 induce 5-HT changes (Lim et al., 2018; Wile et al., 2017).

The limitation of the present approach is that we only based on systematic network pharmacology and database mining. Network pharmacology suffers from heterogeneity. Due to the relatively single network big data, the number of small molecule compounds and their targets are limited. They are generally derived from published articles and are updated in real time. The complete pharmacological effects of the drug cannot be revealed. The experimental data obtained through network pharmacology requires special computer virtual analysis software, and the accuracy of the results obtained will be questioned. The research results need to be further verified by pharmacological experiments. The next research will focus on the conclusions of the article, carry out experimental verification at the cell level in vitro, related pathways and targets, and combine network pharmacology with pharmacokinetics and pharmacodynamics to improve the credibility of the article.

5 | CONCLUSIONS

In the study, we have used network pharmacology combined with database mining to study the main target proteins of RC in the treatment of anxiety disorder, constructing a target protein network. It was found that the Roman chamomile mainly participants in the neuroactive ligand-receptor interaction, serotonin synapse, cAMP signaling pathway, calcium signaling pathway, and other pathways, while regulating the key target proteins of LRRK2. Give play to the treatment of anxiety. The mechanism of Roman chamomile regulating anxiety through multiple targets and channels was discussed.

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CONFLICTS OF INTEREST

The authors declared that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

Data curation; Formal analysis; Resources; Writing-original draft: Jia
Data curation; Software; Supervision: Zou
Data curation; Writing-original draft: Wang
Conceptualization; Funding acquisition; Project administration; Writing-review & editing: Zhang
Funding acquisition; Methodology; Validation: Shi
Investigation; Validation: Liang
Project administration; Supervision: Guo
Methodology; Supervision: Yang

AUTHOR CONTRIBUTION

Yanzhuo Jia and Yao Wang collected data and write manuscripts. Junbo Zou and Dongyan Guo analyzed the data. Yanzhuo Jia, Junbo Zou, and Yulin Liang carried out animal experiments. Xiaofei Zhang

designed the study and revised the paper. Yajun Shi and Ming Yang designed the search strategy, and Yanzhuo Jia, Junbo Zou, and Yao Wang contributed equally to the work.

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