Exploring The Mechanism of Action of Herbal Medicine (*Gan-Mai-Da-Zao* Decoction) For Post-Stroke Depression Based On Network Pharmacology And Molecular Docking

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Research

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Abstract

Backgrounds: Post-stroke depression is the most common and serious neuropsychiatric complication occurring after cerebrovascular accidents, seriously endangering human health while also imposing a heavy burden on society. Even so, it is difficult to have drugs to contain the progression of the disease. It's reported that *Gan-Mai-Da-Zao* decoction was effective to PSD, but it is unknown on its mechanism of action for PSD. In this study, we aimed to explore the possible mechanisms of action of *Gan-Mai-Da-Zao* decoction in the treatment of PSD using network pharmacology and molecular docking.

Material and methods: We obtained the active components and their targets of all drugs from the public database TCMSP and published articles. Then, we collected the PSD-related targets from GeneCards and OMIM databases. Cytoscape 3.8.2 was applied to construct PPI and composite target disease networks. In parallel, the DAVID database was used to perform GO and KEGG enrichment analysis to obtain the biological processes involved in drug treatment diseases in vivo. Finally, molecular docking was used to verify the association between the main active ingredients and the targets.

Results: The network pharmacological analysis of *Gan-Mai-Da-Zao* decoction for PSD identified 107 active ingredients with important biological effects, including quercetin, luteolin, kaempferol, naringenin, isorhamnetin, etc. A total of 203 potential targets for drug treatment of diseases were screened, including STAT3, JUN, TNF, TPT53, AKT1, EGFR, etc. They were found to be widely enriched in a series of signaling pathways such as TNF, HIF-1, and the Toll-Like receptor. Meanwhile, molecular docking analysis showed that the core active components were tightly bound to the core targets, further confirming their anti-PSD effects.

Conclusion: This is a prospective study based on the integration and analysis of large data, using the technology of network pharmacology to explore the feasibility of *Gan-Mai-Da-Zao* decoction for the treatment of PSD, and successfully validated by molecular docking. It reflects the multi-component and multi-target characteristics of Chinese medicine, and more importantly, it also brings hope to the clinical treatment of PSD.

Introduction

Post-stroke depression (PSD) is one of the most common and heavy neuropsychiatric complications after stroke [1, 2], which often starts insidiously, with mild symptoms of malaise and drowsiness in the early stages. What's more, if patients with PSD are unable to express their feelings clearly due to language or cognitive impairment, the diagnosis is often compromised and treatment is delayed [3]. This not only poses a great challenge to clinical work but also adds a heavy burden to the society and economy. A recent statistical study showed that approximately 795,000 people suffered from stroke each year in the United States. In detail, approximately 610,000 people of them had a stroke for the first time, and about 185,000 people have recurred stroke, more than 100,000 of those people die from stroke [4]. A meta-analysis of longitudinal studies found that the prevalence of depression was 29% (95% CI 25–32),

and remains stable up to 10 years after stroke, with a cumulative incidence of 39–52% within 5 years of stroke [5]. Studies reported the cross-sectional prevalence of PSD was 18% and 33% [5–8]. So far, tricyclic antidepressants (TCAs) and selective serotonin reuptake inhibitors (SSRIs) were used to treat PSD. However, due to the lack of timely diagnosis, the adverse effects on cardiovascular function of drugs, and increased risk of bleeding, which leads to unsatisfactory treatment of patients with PSD [9, 10].

Traditional Chinese medicine (TCM) for post-stroke depression is characterized by fewer side effects and individualized treatment [11]. Therefore, the search for the treatment of PSD from herbal medicine has become a hot topic in contemporary pharmacological research. *Gan-Mai-Da-Zao* decoction is from *Jingui Yaolue*, which is composed of three Chinese herbal medicines: *Glycyrrhiza uralensis* Fisch. (gancao in Chinese), *Triticum aestivum* L. (xiaomai in Chinese), and *Ziziphus jujuba* Mill. (dazao in Chinese), which is mainly used for sleep disorders and depression-related psychiatric disorders [12]. It's reported *Gan-Mai-Da-Zao* decoction intervention 2 and 4 weeks could significantly improve Hamilton depression rating scale (HAMD) scores in postpartum women with depressive states in a randomized controlled study [13]. Modern pharmacological studies have also confirmed *Gan-Mai-Da-Zao* decoction could increase central excitability, sedation and hypnotism, anti-depressant and other pharmacological activities [14–16]. However, it's unclear about the mechanism of the active ingredients in *Gan-Mai-Da-Zao* decoction to alleviate depressive symptoms.

TCM with multiple components, multiple targets, and multiple pathways can bring very many new possibilities for clinical treatment. Network pharmacology is the result of the integration of multidisciplinary basic theories and research tools such as biology, computer science, multidirectional pharmacology, molecular pharmacology, and medicine, which can systematically and comprehensively reflect the intervention mechanism of drugs on disease networks. This has a strong convergence with the principle of overall dynamics of TCM treatment of diseases and the characteristics of multi-component, multi-target, and multi-pathway interactions. Therefore, network pharmacology can provide a new and powerful technical support for the study of the mechanism of action of TCM compounding, which can help to reveal the scientific connotation of TCM compounding, discover drug targets, inherit and develop TCM theory [17]. This experiment aims to investigate the mechanism of action of *Gan-Mai-Da-Zao* decoction in the treatment of PSD through network pharmacology and molecular docking technology (Fig. 1).

Materials And Methods

Screening of active compounds and prediction of putative targets

Through the traditional Chinese medicine systems pharmacology database and analysis platform (TCMSP, https://tcmspw.com/tcmsp.php), an authoritative public database that collects a large number of active ingredients, related targets, and pharmacokinetic information [18], we searched for the active ingredients of three Chinese medicines and supplemented them with published literature [19, 20]. Oral

bioavailability (OB) is an important indicator for evaluating the rate and extent of drug absorption into the human circulation, and drug-likeness (DL) is an indicator for evaluating the similarity of a compound to a known drug [21, 22]. Based on these two ADME (absorption, distribution, metabolism, and excretion) mode values, we performed a preliminary screening of active ingredients to obtain the active compounds and their protein targets, where OB ≥ 30% and DL ≥ 0.18% were set to obtain the above information [23]. After screening, to standardize protein target information, the Uniprot database (The Universal Protein Resource, https://www.uniprot.org/) was unified to standardize the protein targets on which compounds act, resulting in a piece of more comprehensive target information such as gene IDs and gene symbols.

Identification of disease-related targets and filtering intersecting targets

Using “Post-Stroke Depression” as the keyword, we mined the GeneCards database (https://www.genecards.org/) and the OMIM database (https://omim.org/) for gene targets related to PSD [24]. The targets associated with PSD were obtained by merging the targets of the two databases and removing duplicates. The common targets were then screened by using Venny 2.1 (http://bioinformatics.psb.ugent.be/webtools/Venn/), and the common targets were defined as the potential targets of *Gan-Mai-Da-Zao* decoction for PSD.

Protein-protein interaction construction

A Protein-protein interaction (PPI) network model was constructed by submitting common disease-drug targets to the search tool for the retrieval of interacting genes (STRING, https://string-db.org/) database [25]. Set the organism species as “Homo sapiens”, and the confidence score with correlation degree ≥ 0.950, meanwhile hide disconnected nodes. Then, the interaction information was further visually analyzed by Cytoscape 3.8.2. The CytoNCA plug-in can analyze the topological attributes of the data submitted to Cytoscape [26, 27]. In this plug-in, betweenness centrality (BC), closeness centrality (CC), and degree centrality (DC) are used to estimate the importance of nodes in the network. The higher the quantitative value of these three numerical values, the more important the node is in the network. In the PPI network, the values of BC, CC, and DC are used as variables to screen out the core targets and build a network relationship diagram of the core targets based on the screening results.

GO and KEGG pathway enrichment analysis

The previously collected *Gan-Mai-Da-Zao* decoction targets for the treatment of PSD were imported into the DAVID (https://david.ncifcrf.gov/summary.jsp) database to analyze its main biological processes and metabolic pathways and enrich them set of analysis, which is a comprehensive annotation capability and have updated monthly gene annotation data repository. In species selection, we chose “Homo sapiens”, terms with a $P < 0.05$. The data results were saved and visualized using bioinformatics (http://www.bioinformatics.com.cn/) to finally obtain The Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis bubble map, where GO enrichment analysis covers

three aspects of biology: cellular component, molecular function, biological process, KEGG enrichment analysis can also suggest us the biological mechanism of drug action in the human body and the pathways involved in regulation.

Network construction

(1) Cytoscape 3.8.2 was implemented to draw out the disease-herb-component-target (D-H-C-T) network relationship diagram to visualize the relationship between *Gan-Mai-Da-Zao* decoction and PSD. The different colors and shapes in the diagram represent the disease, drug, component, and target, and the "edges" represent the correlation between this information. (2) To more visually reflect the relationship of target enrichment on the pathway, a more complex but more intuitive network relationship diagram was constructed by combining the previous network relationship diagram, which includes the more comprehensive information of the drug, active ingredient, target, and pathway.

Molecular docking prediction

Preparation of small molecule ligands

To better evaluate the reliability of network analysis predictions, the core active ingredient was then molecularly docked to the core gene target. Firstly, the small molecule ligands were prepared by obtaining the 2D structures of the active ingredients in *sdf* format through PubChem (https://pubchem.ncbi.nlm.nih.gov/) database and then converting the 2D structures to 3D structures in *mol2* format through ChemOffice software. Finally, it was imported into AutoDock-Tools to convert to *pdbqt* format files [28].

Preparation of protein receptors

Firstly, the core gene target was entered into the Uniprot database to obtain its Uniprot ID, and the 3D structure of the core gene target was retrieved and downloaded from Protein Data Bank (PDB, http://www.rcsb.org/) database in *pdb* format by Uniprot ID. Using PyMOL 2.3.4 software, the protein was de-watered and de-liganded, and the core gene target was hydrogenated and charge calculated using AutoDock-Tools [29], and finally saved as a *pdbqt* format file. When everything was ready, ligand-receptor molecular docking was performed using AutoDock Vina.

Results

Active compounds and targets of gancao, xiaomai and dazao

We found a total of 442 drug-related components from the TCMSP database as well as from the available literature. Then the screening was performed according to DL ≥ 0.18 and OB ≥ 30%, meanwhile, the gene targets involved in each active ingredient were obtained, and finally, the duplicate targets were removed after comparison and correction by the Uniprot database. A complete of 109 active ingredients

and 239 gene targets were obtained, among which some active ingredients and targets were derived from multiple drugs, which also reflects the multi-component and multi-target characteristics of TCM. The details about active ingredients are shown in the Supplementary file, Table S1.

Gene targets of PSD

In this study, 4733 PSD-related gene targets were obtained from the GeneCards database, and another 37 gene targets were obtained from the OMIM database, in which there were 8 identical targets, after removing duplicate values, the total number of PSD-related gene targets was 4762. Next, 203 interacting gene targets were obtained by taking the intersection of disease-related gene targets and drug-related gene targets (Fig. 2a). These targets are the potential targets of the *Gan-Mai-Da-Zao* decoction against PSD (Table 1).

PPI network analysis

The 203 intersecting gene targets were submitted to the STRING database, and then the data obtained from this platform were imported into Cytoscape 3.8.2 for visualization and topological analysis (Fig. 2b), which consisted of 139 nodes and 426 edges, and the topological properties of the intersecting gene targets were analyzed using the CytoNCA plug-in, whose median values of BC, CC, and DC were: 22, 0.046184739, and 4. There were 49 gene targets above the median, and these genes were important for the treatment of PSD with *Gan-Mai-Da-Zao* decoction (Fig. 2c). Among them, AKT1, STAT3, TP53, CTNNB1, CDKN1A, ESR1, VEGFA, MAPK1, MAPK3, CASP8, CCND1, MAPK14, RELA, TNF, EGFR, FOS, JUN, CXCL8, STAT1 were greater than the twofold median values of BC, CC, DC (286.3560165, 0.046954747, 11), indicating that these 19 genes are the core targets in the PPI network (Fig. 2d). The details from STRING are shown in the Supplementary file, Table S2.

Table 1 Potential targets of *Gan-Mai-Da-Zao* decoction against Post-Stroke Depression (PSD)

NO.	Gene	NO.	Gene	NO.	Gene	NO.	Gene	NO.	Gene
1	PTGS1	42	PLAU	83	CDKN1A	124	THBD	165	CCNA2
2	DRD1	43	LTA4H	84	MMP9	125	SERPINE1	166	ESR2
3	CHRM1	44	MAOA	85	MAPK1	126	COL1A1	167	CDK2
4	DRD5	45	ADRB1	86	IL10RA	127	IFNG	168	MAPK10
5	SCN5A	46	BCL2	87	EGF	128	ALOX5	169	PYGM
6	CHRM5	47	BAX	88	RB1	129	IL1A	170	GRIA2
7	PTGS2	48	CASP9	89	TNF	130	MPO	171	OLR1
8	HTR3A	49	JUN	90	IL6ST	131	NCF1	172	IL4
9	RXRA	50	CASP3	91	AHSA1	132	ABCG2	173	HSD3B1
10	OPRD1	51	CASP8	92	TP53	133	GSTP1	174	IKBKB
11	SLC6A2	52	PRKCA	93	ELK1	134	NFE2L2	175	MAPK8
12	ADRA1A	53	PON1	94	NFKBIA	135	NQO1	176	PPP3CA
13	CHRM2	54	MAP2	95	POR	136	PARP1	177	AKR1C3
14	ADRA2B	55	CAT	96	ODC1	137	AHR	178	SLPI
15	ADRA1B	56	HAS2	97	TOP1	138	SLC2A4	179	MAPK3
16	SLC6A3	57	DRD4	98	RAF1	139	COL3A1	180	LDLR
17	ADRB2	58	ACHE	99	SOD1	140	CXCL11	181	BAD
18	CHRNA2	59	F7	100	HIF1A	141	CXCL2	182	MTTP
19	SLC6A4	60	CACNA1S	101	STAT1	142	NR1I3	183	APOB
20	DRD2	61	KDR	102	RUNX1T1	143	CHEK2	184	PLB1
21	OPRM1	62	AKT1	103	HSPA5	144	CLDN4	185	HMGCR
22	GABRA1	63	VEGFA	104	ERBB2	145	PPARA	186	CYP19A1
23	NR3C2	64	MMP2	105	ACACA	146	PPARD	187	UGT1A1
24	PPARG	65	MMP1	106	CYP1A1	147	HSF1	188	SREBF1
25	CYP3A4	66	HMOX1	107	ICAM1	148	CXCL10	189	GSR
26	NR1I2	67	CYP1A2	108	IL1B	149	CHUK	190	ABCC1
27	CYP2B6	68	CAV1	109	CCL2	150	SPP1	191	ADIPOR1
28	NOS2	69	CTNNB1	110	SELE	151	RUNX2	192	ABAT

29	KCNH2	70	MYC	111	VCAM1	152	E2F1	193	SOAT1
30	ESR1	71	CASP7	112	PTGER3	153	CTSD	194	BACE2
31	AR	72	F3	113	CXCL8	154	IGFBP3	195	STAT3
32	PRSS1	73	GJA1	114	PRKCB	155	IGF2	196	CDK4
33	PDE10A	74	MMP10	115	BIRC5	156	CD40LG	197	MDM2
34	MAOB	75	FASN	116	DUOX2	157	IRF1	198	APP
35	ADRA2A	76	DPP4	117	NOS3	158	ERBB3	199	PCNA
36	CA2	77	MMP3	118	HSPB1	159	HK2	200	TYR
37	ADRA2C	78	RELA	119	SULT1E1	160	RASA1	201	XIAP
38	ADRA1D	79	EGFR	120	IL2RA	161	GSTM1	202	PTGES
39	PGR	80	CCND1	121	CYP1B1	162	MAPK14	203	MET
40	ADH1C	81	BCL2L1	122	CCNB1	163	GSK3B		
41	AKR1B1	82	FOS	123	PLAT	164	CHEK1		

GO and KEGG Pathway Enrichment Analyses

The information retrieved from the DAVID database was ranked into TOP20 according to the *p*-value, and finally, the data was transformed into a bubble chart for display. When more genes were enriched the larger the bubble was and the deeper the color the smaller the *p*-value.

The biological process mainly involves response to drug (GO:0042493), response to lipopolysaccharide (GO:0032496), positive regulation of transcription from RNA polymerase II promoter (GO:0045944), response to ethanol (GO:0045471) and response to estradiol (GO:0032355) (Fig. 3a), and analysis on cellular components showed that it was similar to extracellular space (GO:0005615), cytosol (GO:0005829), membrane raft (GO:0045121), plasma membrane (GO:0005886) and integral component of plasma membrane (GO:0005887) are strongly related (Fig. 3b). Besides, molecular functions aspects mainly involve enzyme binding (GO:0019899), identical protein binding (GO:0042802), protein heterodimerization activity (GO:0046982), drug binding (GO:0008144), and protein binding (GO:0005515) (Fig. 3c). All of the above data suggest that the *Gan-Mai-Da-Zao* decoction may treat PSD by modulating multiple GO functions.

The KEGG pathway enrichment analysis yielded a total of 122 pathway information, and the same selection rules of GO enrichment analysis were used to obtain the TOP20 pathways for graphic visualization, through the pictures we can visually see that the functions of relevant targets for PSD were mainly enriched in Hepatitis B (hsa05161),

Pathways in cancer (hsa05200), Pancreatic cancer (hsa05212), Bladder cancer (hsa05219) and TNF signaling pathway (hsa04668). Among these pathways, “Pathways in cancer” was identified as an important critical pathway with the highest target enrichment, and similarly, “Hepatitis B” was also an important pathway because it had the lowest *p*-value (Fig. 3d). The details about GO and KEGG enrichment analysis, which from the DAVID database are shown in the Supplementary file, Table S3.

Network construction analysis

There were 314 nodes and 1825 edges in the D-H-C-T network diagram, among which the potential active ingredients icos-5-enoic acid (MOL004985) and gadelaidic acid (MOL004996) were hidden in the network diagram because the corresponding gene targets didn’t overlap with the disease targets (Fig. 4). The details are shown in the Supplementary file, Table S4. The pathway information was imported into Cytoscape 3.8.2 and combined with the previous graph to obtain a new network relationship graph with 335 nodes and 2300 edges (Fig. 5).

Docking results

The nodes with high degree values in PPI analysis were considered as core targets, and the five core gene targets with the highest degree values in PPI, STAT3 (PDB ID: 6NJS), JUN (PDB ID: 5T01), TP53 (PDB ID: 6WQX), AKT1 (PDB ID: 5WBL) and TNF (PDB ID: 2E7A) were correlated with the 10 active ingredients with the highest degree in the "D-H-C-T" network: quercetin (MOL000098), luteolin (MOL000006), kaempferol (MOL000422), 7-Methoxy-2-methyl isoflavone (MOL003896), naringenin (MOL004328), isorhamnetin (MOL000354), formononetin (MOL000392), licochalcone a (MOL000497), beta-sitosterol (MOL000358), and medicarpin (MOL002565) were molecularly docked and their binding energies were calculated (Table 2, Fig. 6). Meanwhile, we graphically demonstrated the specific details of the docking of the most core genes STAT3 and JUN with the most core components quercetin, luteolin, kaempferol, and 7-Methoxy-2-methyl isoflavone (Fig. 7). The lower the binding energy of both ligand and receptor, the more stable the binding is [30]. In general, a docking fraction value of less than − 4.25 $kcal{\cdot}mol^{-1}$ indicates some binding activity, less than − 5.0 $kcal{\cdot}mol^{-1}$ indicates good binding activity, and less than − 7.0 $kcal{\cdot}mol^{-1}$ indicates strong binding activity [31]. The results showed that a total of 50 groups of core components were selected to have good binding activity with the target proteins.

Table 2 Binding energies of 10 main compounds to two potential targets

NO.	Compound	Binding Energy / *kcal·mol*$^{-1}$				
		STAT3	JUN	TP53	AKT1	TNF
1	naringenin	-7.7	-8.5	-8.5	-8.4	-8.6
2	luteolin	-7.8	-8.6	-8.2	-8.7	-8.9
3	quercetin	-7.5	-8.4	-8.3	-8.9	-6.8
4	licochalcone a	-6.3	-7.0	-8.1	-7.0	-6.0
5	Medicarpin	-7.3	-8.0	-8.1	-7.9	-8.4
6	7-Methoxy-2-methyl isoflavone	-7.6	-8.6	-9.0	-8.4	-6.7
7	beta-sitosterol	-7.2	-6.6	-7.0	-7.3	-6.2
8	formononetin	-7.4	-7.8	-8.6	-8.3	-8.4
9	isorhamnetin	-7.3	-7.8	-7.7	-8.7	-9.0
10	kaempferol	-7.3	-7.8	-7.9	-8.4	-8.9

Discussion

Network pharmacology is a method to predict the possibility of drug treatment for diseases by searching for drug-disease shared genes and finding gene enrichment pathways, which are then confirmed by available experimental evidence. And molecular docking can predict the binding power of active ingredients to target proteins, which further confirms the therapeutic effect of drugs. The use of these techniques has largely solved the great challenges posed to research due to the multi-component and multi-target nature of TCM. Therefore, we used network pharmacology and molecular docking to reveal the possible mechanism of action of *Gan-Mai-Da-Zao* decoction against PSD.

In our study, the core active ingredients were screened in the “D-H-C-T” network, including quercetin, luteolin, kaempferol, naringenin, isorhamnetin, etc. In recent years, it has been found that flavonoids have significant effects on the central system, with neuroprotective, antidepressant, and anxiolytic effects [32]. In our study, quercetin is derived from *Glycyrrhiza uralensis* Fisch. and *Ziziphus jujuba* Mill. A relevant animal study confirmed that quercetin could reverse the stress-induced depression and anxiety in the mice [33]. Besides, it’s evidenced that quercetin exerts anti-depressant effects through anti-oxidant, anti-inflammatory, decreasing cytotoxicity, and increasing 5-hydroxytryptamine levels [34]. Luteolin is derived from *Triticum aestivum* L. It’s reported that luteolin showed an anti-depressant effect via suppressing the endoplasmic reticulum [35], inhibiting and downregulating plasma membrane monoamine transporter (PMAT, Slc29a4) [36]. The study also found that luteolin may improve cognitive performance by inhibiting microglial activation and neuroinflammation in older mice [37]. Kaempferol, naringenin, and isorhamnetin are all derived from *Glycyrrhiza uralensis* Fisch. Kaempferol promoted the expression of brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) protein in hippocampal tissue of aged rats in a

chronic stress depression model, which resulted in neuroprotection and improved depression-like behavior [38]. Naringenin is a flavonoid compound with strong anti-oxidant and anti-inflammatory effects. The literature evidenced that naringin may produce functional behavioral effects through enhancement of cholinergic transmission, antioxidant defense systems, inhibition of lipid peroxidation and nitrosative processes [39]. The application of isorhamnetin potentiated the nerve growth factor (NGF) induced neurite outgrowth. In parallel, the expression of neurofilaments was markedly increased in the co-treatment of NGF and isorhamnetin in the cultures. This suggested that isorhamnetin might be used to some extent in the treatment of neurodegenerative diseases, including Alzheimer's disease and depression [40]. These important active ingredients are all sourced from *Gan-Mai-Da-Zao* decoction, and multiple active ingredients work together to exert their effects in the treatment of PSD.

In addition, a total of 19 core targets of *Gan-Mai-Da-Zao* decoction for PSD were screened in the PPI network, including AKT1, STAT3, TP53, CTNNB1, CDKN1A, ESR1, VEGFA, MAPK1, MAPK3, CASP8, CCND1, MAPK14, RELA, TNF, EGFR, FOS, JUN, CXCL8, STAT1. Among them, EGFR is highly expressed in a variety of malignancies, and depression is common in oncology patients, being four times more prevalent than in the general population [41, 42]. Meanwhile, it’s also found that EGFR mutant non-small cell lung cancer produced depression by mediating inflammatory factors [43]. Depressant-like behavior was induced by forced swimming, and MAPK1 was overexpressed in the hippocampus to lead anti-depressant effect [44]. It’s reported that estrogen-regulated neurotransmitter conversion and thus produced anti-depressant effects. It’s thought that the biological function of estrogen is largely mediated by intracellular activation of its primary receptors, estrogen receptor alpha (ESR1) and estrogen receptor beta (ESR2). Thus, genetic variation in ESR played an important role in the susceptibility of women to depression [45, 46]. STAT3 is expressed in both hippocampal neurons and glial cells and is closely related to neurodegenerative diseases. It was demonstrated that pharmacological treatment of PSD and improvement of depressive state may be related to the inhibition of JAK2/STAT3 signaling pathway-related gene and protein expression to promote the neural remodeling in the hippocampal [47].

Among the 20 pathways screened by KEGG enrichment analysis, some of them are closely related to PSD, including TNF and Toll-like receptor signaling pathways. The immune-inflammatory response is one of the important pathogenic mechanisms of PSD. Elevation of various inflammatory biomarkers such as IL-6, TNF-α, increased high sensitive C-reactive protein (CRP) concentration was found to be present in mild to moderate depressive patients six months following stroke [48, 49]. It has been shown that the improvement in depression in rats under acupuncture intervention may be closely related to the Toll-like receptor pathway and TNF signaling pathway enriched by significantly downregulated differentially expressed genes in the hippocampus, frontal lobes, and pituitary gland of rats [50]. Also, there are pathological mechanisms such as neuronal apoptosis and nerve growth disorders involved in some pathways that also play an important role in the development of PSD. The efficacy of current antidepressants has been linked to the Ras signaling pathway, which may be involved in the onset and development of depression-related disorders by indirectly affecting neurotrophic factors or directly affecting neuroplasticity [51]. Furthermore, antidepressants not only upregulate cAMP levels in receptor cells but also activate protein kinase A (PKA) to phosphorylate PKA, which then activates the cAMP-

response element-binding protein (CREB) signaling pathway, altering functional protein activity and gene expression patterns to form new synapses, thus exerting antidepressant effects [52].

Conclusion

In summary, network pharmacological analysis showed that there are as many as 203 possible targets for *Gan-Mai-Da-Zao* decoction in the treatment of PSD. Several pathways may be very closely related to the treatment of PSD, including TNF and Toll-like receptor signaling pathways, and the 19 core gene targets screened from the PPI network map are also enriched in these important pathways. Therefore, the results of this study provide evidence for follow-up research and a basis for the clinical application of *Gan-Mai-Da-Zao* decoction and its prescriptions in the treatment of PSD.

Abbreviations

PSD: post-stroke depression

TCAs: tricyclic antidepressants

SSRIs: selective serotonin reuptake inhibitors

TCM: Traditional Chinese Medicine

HAMD: Hamilton Depression Rating scale

TCMSP: Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform

OB: Oral bioavailability

DL: Drug-likeness

ADME: absorption, distribution, metabolism, and excretion

Uniport database: The Universal Protein Resource

PPI: Protein-Protein Interaction

STRING: Search Tool for the Retrieval of Interacting Genes

BC: Betweenness Centrality

CC: Closeness Centrality

DC: Degree Centrality

GO: Gene ontology

KEGG: Kyoto Encyclopedia of Genes and Genomes

D-H-C-T: disease-herb-component-target

PDB: Protein Data Bank

Declarations

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Authors' contributions

DZC, XFF, and SQD: study design; acquisition of data; analysis of data. DZC and XFF wrote the manuscript. CJH, LB, and LNX: acquisition of data; critical revision of the manuscript. YSZ: revision of the manuscript and study supervision. All the author(s) read and approved the final manuscript.

Availability of data and materials

All data are available in the manuscript and they are exhibited in figures and tables.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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Competing interests

The authors declare that there are no conflicts of interest in relation to this work.

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Figures

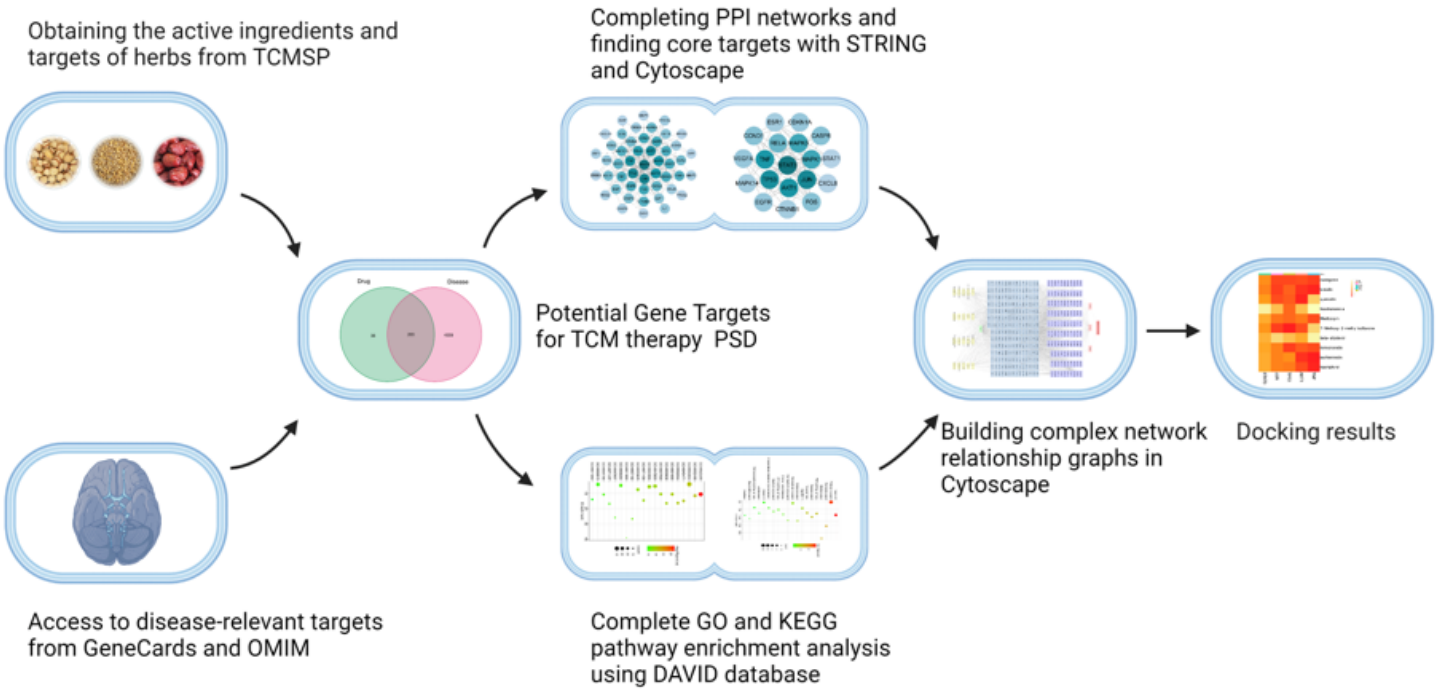

Figure 1

Detailed process of research design

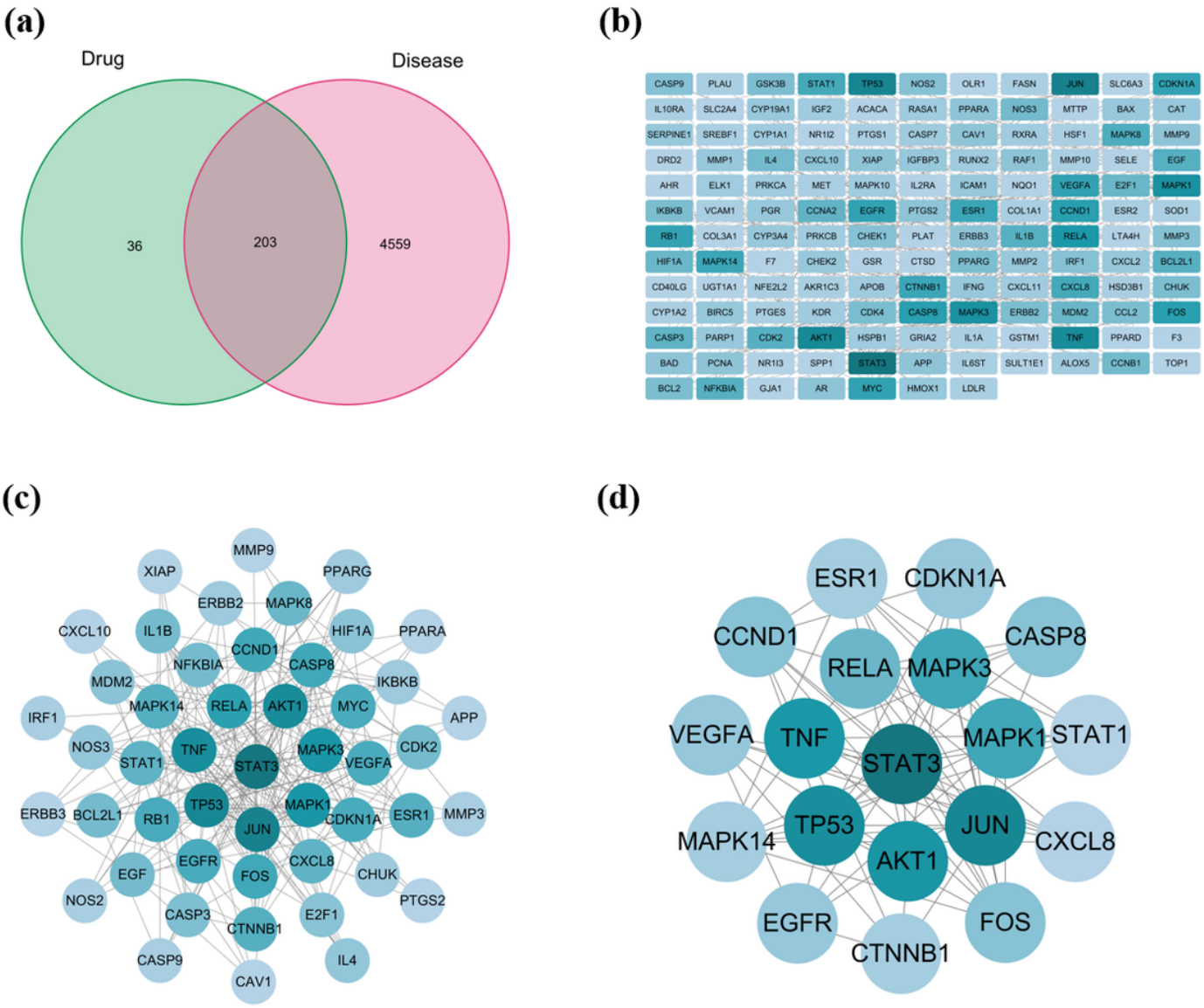

Figure 2

Drug-disease targets intersection Venn diagram and protein-protein interaction network. The darker the color means the larger the degree value, the more important it is in this network a) 203 intersection gene targets; b) a protein-protein network from STRING; c) one protein-protein cluster with 49 nodes and 230 edges; d) one protein-protein cluster with 19 nodes and 76 edges.

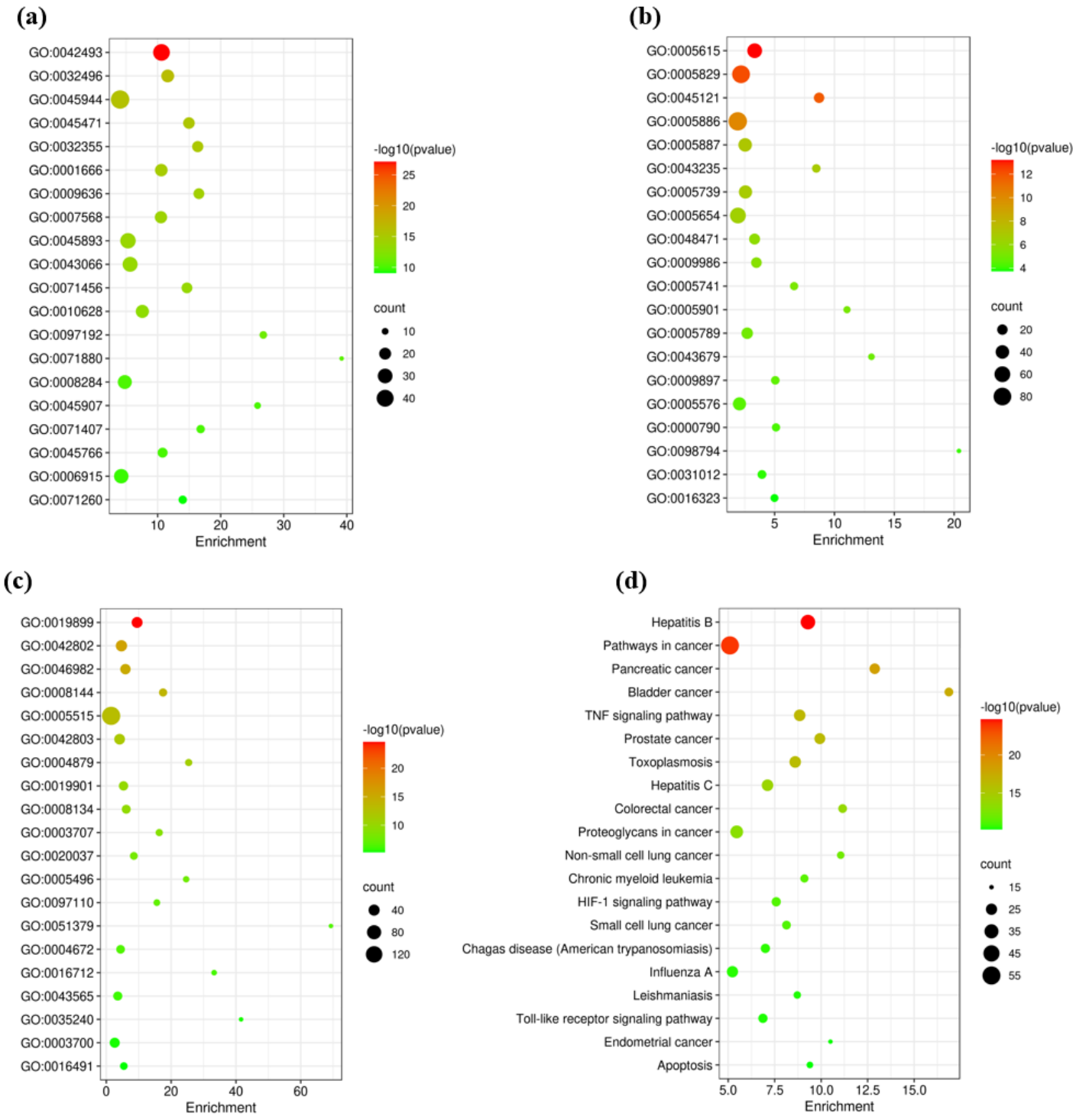

Figure 3

Bubble maps of GO and KEGG pathway enrichment analysis of Gan-Mai-Da-Zao decoction for PSD. a) GO: biological process; b) GO: cellular components; c) GO: molecular functions; d) KEGG pathway enrichment analysis

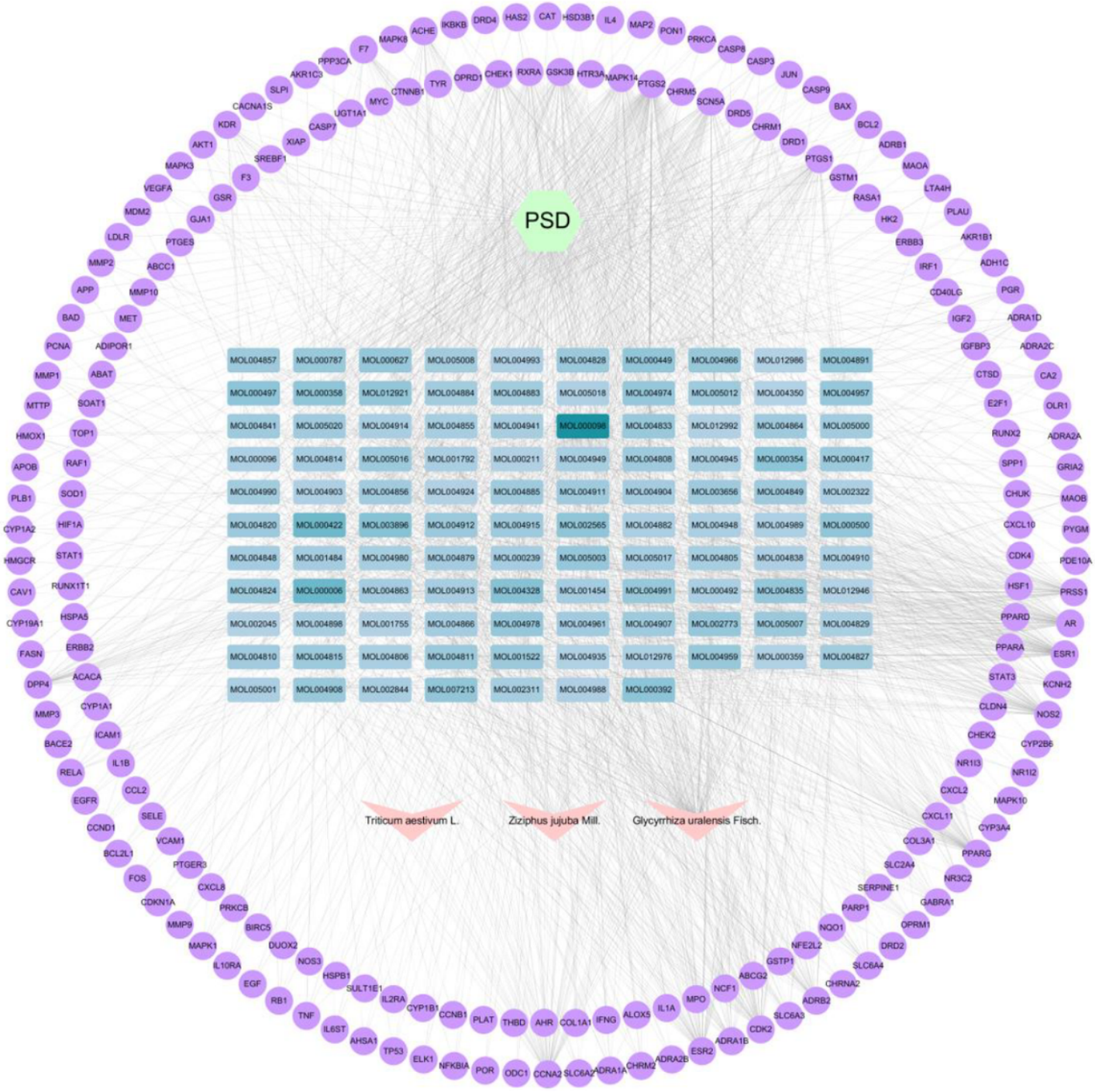

Figure 4

Disease-herb-compound-target (D-H-C-T) network of Gan-Mai-Da-Zao decoction against PSD. The purple circles represent genes, the green hexagons represent diseases, the pink V-shapes represent drugs, and finally, shades of blue rectangles represent active ingredients, the darker the color, the greater the degree value in the network, indicating greater importance.

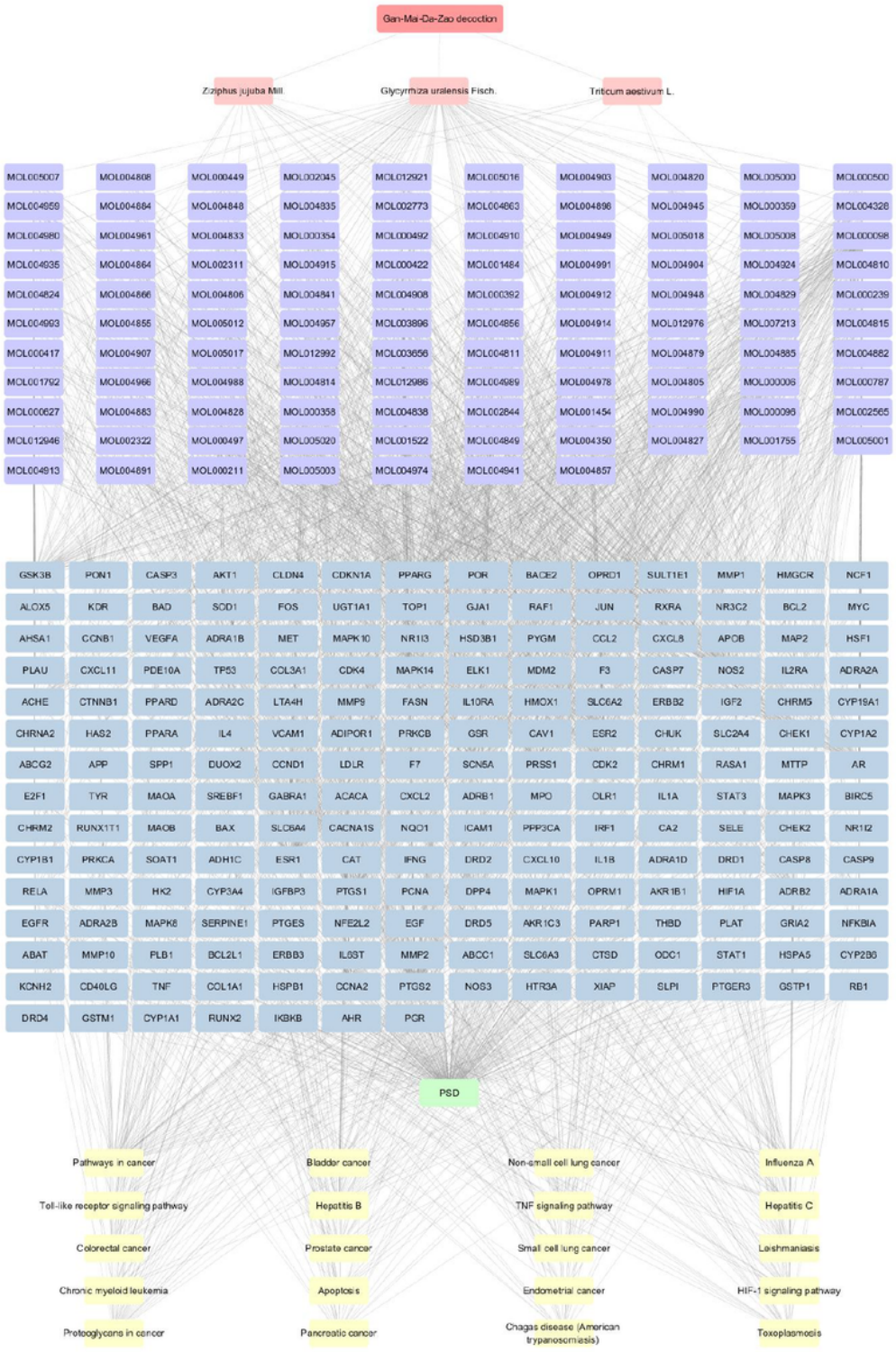

Figure 5

Network diagram containing pathway enrichment analysis. The different colored rectangles from top to bottom represent: herbal medicine, herbs, active ingredients, targets, disease, pathways

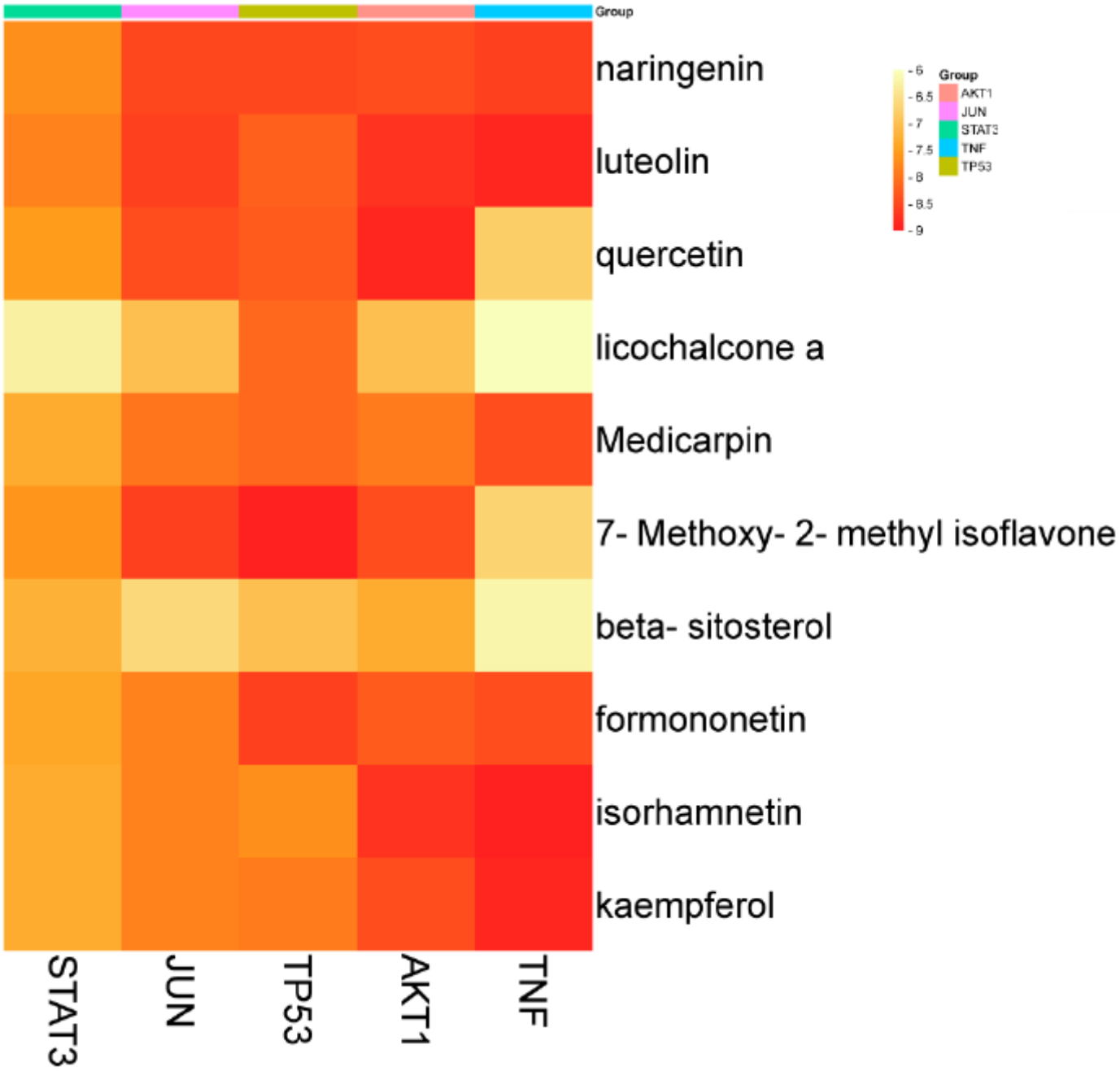

Figure 6

The heat map of the docking score

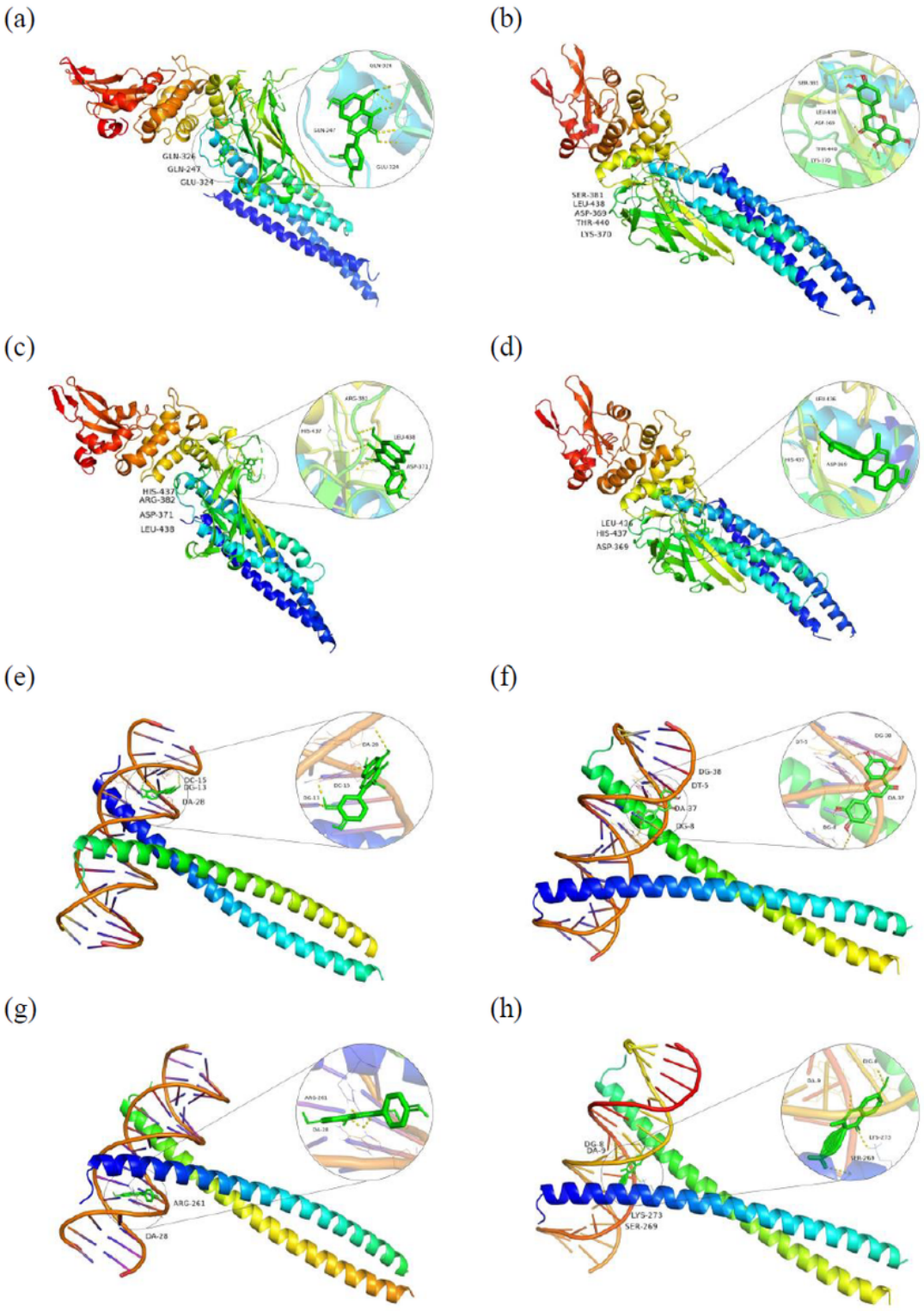

Figure 7

The docking complex consisting of two targets and four components. The colored irregular lines represent proteins, the green rod-like structures represent compounds, and each image shows the details of the docked parts. (a) STAT3-quercetin, (b) STAT3-luteolin, (c) STAT3-kaempferol, (d) STAT3-7-Methoxy-2-methyl isoflavone, (e) JUN-quercetin, (f) JUN-luteolin, (g) JUN-kaempferol, (h) JUN-7-Methoxy-2-methyl isoflavone

Supplementary Files

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