

Research Article

Biomedical Analytics of Four Chinese Medicinals in Treatment of Insomnia Based on Network Pharmacology

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Aim. Our aim is to recommend the appropriate Chinese medicinals in clinical treatment of insomnia, which are *suānzǎorén* (Semen Ziziphi Spinosa), *chuānxiōng* (Rhizoma Chuanxiong), *fúlíng* (Poria), and *báisháo* (Radix Paeoniae Alba). **Method.** Based on network pharmacology, the active molecules and mechanism of these four Chinese medicinals treating insomnia were sought and analyzed. The components of the four Chinese medicinals with potential activity were collected and screened. Moreover, the recollected human disease-related targets were correlated through Cytoscape 3.8.2, and the network diagram of drug component disease targets was drawn. Based on the human protein-protein interaction database, the above network diagram was imported to establish the protein-protein interaction (PPI) and composite target pathway (C-T-P) networks. After selecting important information, the pathway analysis was carried out to show the biological process, core target, and core pathway of insomnia treatment. **Result.** In this study, 44 active components and 81 drug-disease common targets were obtained; 307 key targets were found in the PPI network; a core cluster composed of 14 nodes and 50 functional associations was found. **Conclusion.** In summary, the four Chinese medicinals' effective components and main mechanism of in the treatment of insomnia may be related to their participation in the regulation of endocrine. Compared with the existing network pharmacological analysis results of *SuānZǎoRénTāng* (Sour Jujube Decoction), which is commonly used in insomnia, they have similar effects on the immune system and HPA axis, while the focus of the four Chinese medicinals is mainly on endocrine regulation, and *SuānZǎoRénTāng* (Sour Jujube Decoction) is mainly on anti-inflammatory effect.

1. Introduction

Insomnia, as a standard clinical symptom associated with neurological or psychological disorders, describes sleep latency or sleep disturbance, as well as the condition of subjective poor sleep quality [1]. It is defined as a sleep disorder that affects daytime dysfunction [2].

At present, there are three main ways to treat insomnia: cognitive behavior therapy, drug therapy, and traditional

Chinese medicine (TCM) intervention [3]. Among them, cognitive behavioral therapy, including psychotherapy, physical therapy, and comprehensive therapy, is usually recommended and highly valued. Of the three cognitive behavioral therapies, psychotherapy aims to improve patients' confidence of self-control over insomnia by improving their improper cognition and behavior. Physical therapy includes phototherapy and repeated transcranial stimulation. As for drug therapy, benzodiazepine receptor agonists are often used in clinical

treatment. However, for drugs such as diazepam, eszopiclone, and zolpidem, there are many reports of adverse reactions, lacking sufficient control trials for the long-term efficacy of their pharmacological effects. In contrast, the treatment of insomnia by traditional Chinese medicine has a large number of positive reports, which are also recommended for clinical application in some guidelines, such as Chinese medicinals or acupuncture. In the clinical guidelines for the management of insomnia [3], classic formulas such as *SuānZǎoRénTāng* (Sour Jujube Decoction), *GuīPíWán* (Spleen-Restoring Pill), and *ĀnShénDìngZhìWán* (Spirit-Mind Calming Pill) are recommended. Chinese medicinal is more effective in the treatment of insomnia, and there are fewer reports of negative effects.

This study is based on the in-depth discussion of the molecular mechanism of TCM formulas and medicinals for the effective treatment of insomnia. After analyzing 6765 TCM prescriptions for the treatment of insomnia [3], medicinals were ranked according to their frequency of prescription. It was found that the ones with the highest rankings in descending order are *suānzǎorén* (Semen Ziziphi Spinosae), *zhǐqiào* (Fructus Aurantii), *báizhú* (Rhizoma Atractylodis Macrocephalae), *fúlíng* (Poria), *báisháo* (Radix Paeoniae Alba), *chuānxiōng* (Rhizoma Chuanxiong), and *zhīmǔ* (Rhizoma Anemarrhenae). According to the domestic literature, the most frequently used drugs for insomnia are *suānzǎorén* (Semen Ziziphi Spinosae), *gāncǎo* (Radix et Rhizoma Glycyrrhizae), *yèjiāoténg* (Caulis Polygoni Multiflori), *cháihú* (Radix Bupleuri), *fúlíng* (Poria), *dāngguī* (Radix Angelicae Sinensis), *chuānxiōng* (Rhizoma Chuanxiong), and *báisháo* (Radix Paeoniae Alba) [4]. It is obvious that the combination of the above Chinese medicinals is the derivative of *SuānZǎoRénTāng* (Sour Jujube Decoction), which plays a role under the guidance of TCM theory. However, the main components and mechanism of the relevant combinations are still unknown, making it difficult to make a direct comparison with *SuānZǎoRénTāng* (Sour Jujube Decoction). This results in a lack of evidence for clinical choice. Based on the theory of “sour medicinals boost yin,” on which *SuānZǎoRénTāng* (Sour Jujube Decoction) is based, four commonly used Chinese medicinals, namely, *suānzǎorén* (Semen Ziziphi Spinosae), *chuānxiōng* (Rhizoma Chuanxiong), *fúlíng* (Poria), and *báisháo* (Radix Paeoniae Alba), were selected in our study. The network pharmacological flow chart in Figure 1 was to show the method of this manuscript.

The network pharmacology of Chinese medicinals provides a new method to study the protective effect of medicinals during the course of the disease and its possible mechanisms based on network biology and multidirectional pharmacology and makes it possible to explore the medical biological networks and further clarify the human complex network system. The construction of networks of drug and drug targets not only provides a prospective framework for clarifying the relationship between medicinal plants and diseases but also promotes drug research, development, and improvement to a certain extent [5].

Therefore, this study is aimed at analyzing the biomedical targets and mechanism of the four Chinese medicinals

for insomnia treatment by network pharmacology and to clarify the components and related properties of them to provide a reference for further pharmacological research and a new perspective for clinical treatment and drug research and development.

2. Method

2.1. Screening Medicinals' Compounds and Gene Targets. From the platforms of TCMSP [6] (<http://lsp.nwu.edu.cn/tcmsp.php>) and PharmMapper [7] (<http://www.lilabecust.cn/pharmmapper/>), we searched for all chemical components of the four Chinese medicinals. The molecular structures were determined by literature mining and comparison, and the drug compound database was established after screening. Extracting the active target proteins of drugs from the UniProt [8] (<https://http://www.uniprot.org/>) database, the target information database was established after processing such as duplicated data elimination.

Clinically, these four Chinese medicinals are mostly administered orally. They need to go through absorption (*A*), distribution (*D*), metabolism (*M*), and excretion (*E*) to reach the target cells, tissues, and organs to play their roles. Oral bioavailability (OB) and drug-likeness index (DL) are the key parameters of ADME. OB, reflecting drug utilization of oral administration, is the relative amount and rate of absorption of drugs into the blood circulation. As it is the key index to determine the drug characteristic of molecules with pharmacodynamic effects (i.e., bioactive molecules), $OB \geq 30\%$ was taken as one of the screening conditions. DL, a data combing drug compounds and drug database, can be used not only to evaluate whether a compound is suitable for drug design but also to indicate the pharmacodynamic and pharmacokinetic characteristics of drug-like molecules. A compound with $DL \geq 0.18$ (the average value of the whole similarity) is considered similar to the drugs in the DrugBank database. Therefore, $OB \geq 30\%$ and $DL \geq 0.18$ were taken as the filter conditions in this study. The target compounds of the four Chinese medicinals were predicted from TCMSP, PharmMapper, and UniProt databases. Then, preliminary screening was carried out, such as removing the compounds whose corresponding targets have not been determined, deleting the repeated targets and diseases, etc. Then, using GeneCards [9] (<https://www.genecards.org/>) platform, the relevant targets of insomnia were predicted. After processing, association and analysis were made between these relevant targets and the target compounds of the four Chinese medicinals for the drug-disease targets, the basis of later pathway analysis, and topology network establishment.

2.2. PPI Network Construction. From HPRD [10] (<http://www.hprd.org/>), the human protein-protein interaction database was obtained. And it was imported into Cytoscape [11] 3.8.2 software for processing to obtain the background database. Drug-disease common targets were introduced, and the target gene and its neighbor nodes were selected to obtain a PPI network. The Network Analyzer tool was used for the analysis to obtain various network topology

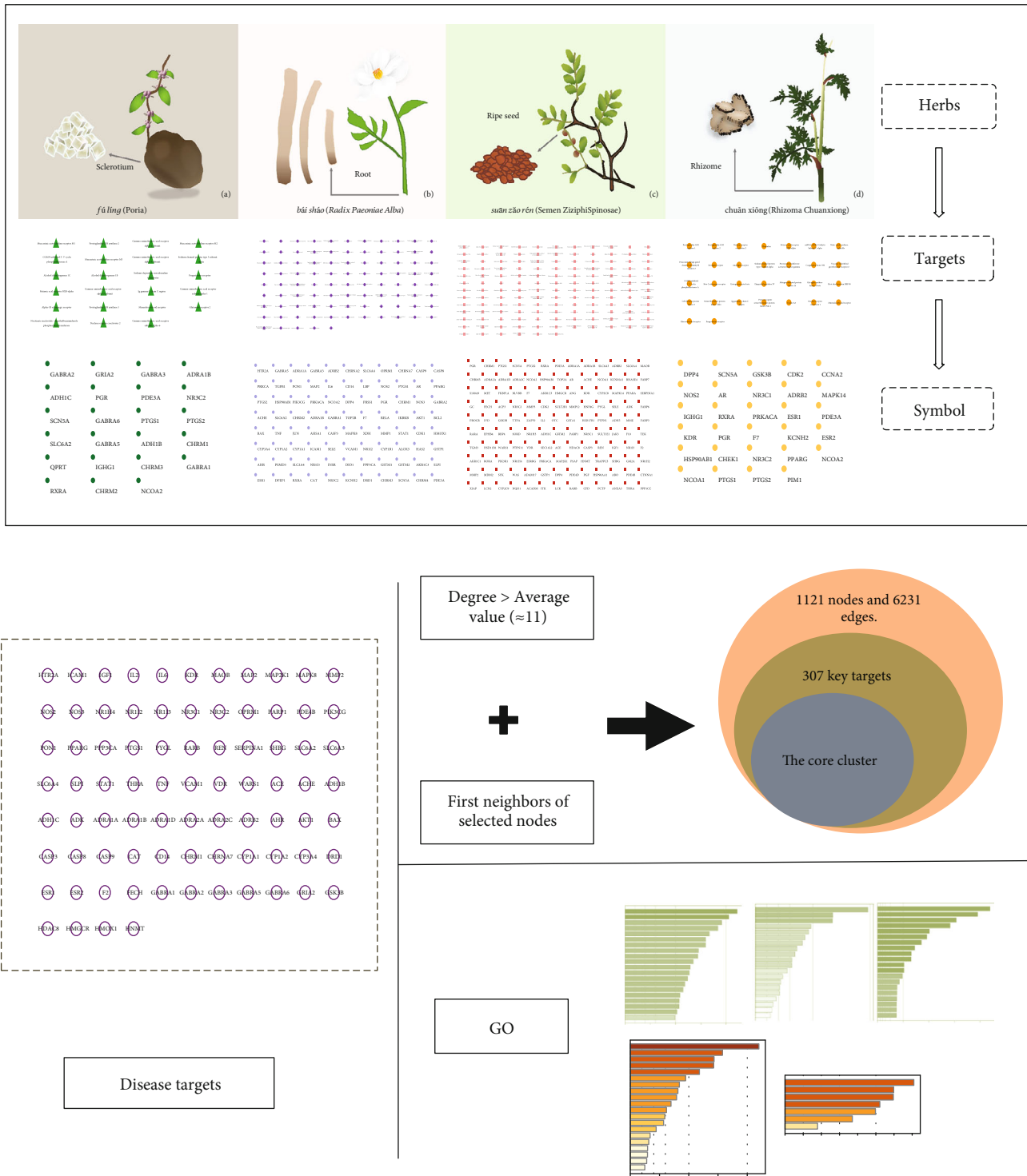


FIGURE 1: Four herbs ((a) *fúlíng* (Poria), (b) *báisháo* (Radix Paeoniae Alba), (c) *suānzǎorén* (Semen Ziziphi Spinosae), and (d) *chuānxiōng* (Rhizoma Chuanxiiong)) of treatment of insomnia network pharmacological flow chart.

parameters. The research suggests that the node degree value (degree) [12] stands for the connectivity of nodes in the network. The greater the node degree, the more important the node is in the network. Therefore, the targets with node degree above the average value were selected as the key

targets, and the top 10 targets were listed as the key targets of this study. Using the plug-in of MCODE, modular cluster analysis of the PPI network was done, and the cluster with the highest result score was selected as the core cluster to determine the high connectivity genome.

2.3. Pathway Analysis. Metascape [13] platform (<https://metascape.org/gp/indexhtml#/main/step1>) containing multiple databases such as GO and KEGG can enrich biological pathways and infer protein complex functions. Confirming the species setting (*Homo sapiens*), the key targets of the four Chinese medicinals for insomnia were imported into the database, and GO enrichment analysis was performed, including biological process analysis (GO-BP), biological cell component (GO-CC), and MF. Next, key targets and core clusters were introduced for analysis of KEGG metabolic pathway. The signal paths of the top 10-log₁₀ (*P*) value were selected as the core path.

3. Results

3.1. Active Compounds and Targets of TF. A total of 44 active ingredients were found in the four Chinese medicinals, including 9 in *suānzǎorén* (*Semen Ziziphi Spinosa*)—American tea acid, carotene, wild Jujuboside A, and phytosterol; 15 in *fúlíng* (*Poria*)—*Poria neoacid B*, *Poria acid A*, and ivy saponin; 7 in *chuānxiōng* (*Rhizoma Chuanxiong*)—Yang Chuanxiong quinone, Chuanxiong indole, and Chuanxiong nafurolactone; and 13 in *báisháo* (*Radix Paeoniae Alba*)—paeoniflorin, benzoyl paeoniflorin, and sitosterol. After processing, the predicted targets of the four Chinese medicinals were obtained: 213 in *suānzǎorén* (*Semen Ziziphi Spinosa*), 31 in *fúlíng* (*Poria*), 43 in *chuānxiōng* (*Rhizoma Chuanxiong*), and 124 in *báisháo* (*Radix Paeoniae Alba*). After deleting the duplicated ones, the aggregate amount of predicted targets was 331.

3.2. Disease Targets and Network. Insomnia-disease targets were retrieved through GeneCards platform, and 528 disease targets were obtained. A total of 81 drug-disease common targets were obtained after the 331 active component targets of the four Chinese medicinals were intersected with 528 disease targets. The drug-disease common target PPI network was derived by Cytoscape software, with 1121 nodes and 6231 action associations, as shown in Figure 2. After further correlation analysis, 307 key targets in the PPI network are obtained, which are illustrated in dots in Figure 3.

The top 10 key nodes in the PPI network suggested that the mechanism of the four Chinese medicinals in the treatment of insomnia is related to genes such as ESR1, AKT1, SRC, AR, MAPK1, TP53, NR3C1, CREBBP, and EP300. A core cluster composed of 14 nodes and 50 functional associations was exported through the plug-in of MCODE, and the result suggests that these targets play a key role in PPI network connection, including targets like gene RET, PTK2, ERBB2, PTPN2, MET, SHC1, PDGFRB, STAT5B, INSR, PIK3R1, IRS1, CBL, GRB2, and PTPN6, as shown in Figure 4.

According to the HPRD information, drug-disease targets and their neighbor targets were introduced to obtain 1121 nodes. The colors from dark orange to light orange were showing the degree value of nodes in descending order.

Selecting nodes above the average degree value, we got 307 key targets. Among the 307 targets, diamonds are the top 10 in regard of the degree value, and the others are cir-

cular, of which colors appear from dark green to light green in descending order.

The 14 circular nodes represent the core cluster, whose colors appear in green black to azure in descending order of degree value. And the 50 edges are shown in gray.

3.3. Gene Ontology Enrichment Analysis. The results are shown in Figures 5(a)–5(c). The results of gene function enrichment of key targets showed that they were significantly enriched in biological processes such as response to toxic substances, blood circulation, response to lipopolysaccharides, cellular response to organic cyclic compounds, and chemical synaptic transmission (chemical synaptic transmission). In terms of molecular function, nuclear receptor activity, neurotransmitter receptor activity, and adrenergic receptor activity were involved. In terms of cellular components, association with kinase binding, transcription factor binding, protein domain specific binding, and protein kinase activity were indicated.

3.4. KEGG Enrichment Analysis. As shown in Figures 5(d) and 5(e), the results exported by Metascape were sorted by $-\log_{10}$ (*P*) values from large to small, and the top 10 were selected as the core path. It was indicated that the key targets notably abounded in the AGE-RAGE signal pathway and cGMP-PKG signal pathway in neuroactive ligand-receptor interaction, cancer pathways, tuberculosis, and diabetes complications.

By introducing core clusters for pathway analysis, it was found that the four Chinese medicinals played a therapeutic role in the ErbB signal pathway, cancer pathway, EGFR tyrosine kinase inhibitor resistance, and insulin signal pathway to treat insomnia.

4. Discussion

Epidemiological evidence shows that insomnia is not only a trigger factor for many accidents but also a contributing factor to human diseases with high incidence rates such as diabetes, hypertension, and malignant tumors [14]. Furthermore, it is also an early symptom of mental disorders such as anxiety disorders, depression, and schizophrenia. The survey shows that on average, one in three adults suffers from sleep problems [15]. According to different diagnostic criteria, the prevalence of insomnia in the natural population is approximately 10%–15%, with an annual incidence rate of about 5% [16].

Chinese medicinals have been reported to have a good curative effect and less side effects in the treatment of insomnia [17]. Therefore, increasingly in-depth researches have been done to explore its components and pathways [18]. Combining with network pharmacology, traditional Chinese medicine transformed into evidence-based medicine from empirical medicine. Not only can we accelerate the understanding and analysis of Chinese medicinals but also can we explore the potential of Chinese medicinals in the treatment of multifield diseases. By constructing the pharmacological network, analyzing the interplay between biologically active compounds and targets, and further

CYP3A4 FECH ADH1C PYGL ABCB7 MACB UGT2B7
 AKR7A2 HGFCAC PRQZ SERPINC1 ELVRB CYP1A2NOSTRINKIFBP2 IGFBP6 WSP2 GABRA3 PEBN4 CYP11AC16H174RCAN2 SNTB1 SNTB2 SNTG1 SAG ABR ANKS1B GLUL MRPL2NDUF10 NME3 PON2 CSF1 PDK1L PRIMA1 LGTN LAMB1 COL6 C6
 CD33 FOXK2 MPR RENEP ATP6A2 POSK1 COL3A3 THBS2 LCN2 TIMP1 OCL22 OCL17 CTRB1 DERL3 DERL2 KLK5 MMP11 PARP3 SWAP70 TPCP2 NUCB2 FAB1 CASL RGN SLC18A2EF1AD AHNAKCCDC8AOLC1 UX3I THEM4 TOL6 BIRC6
 SLC37A1 SORD ZC3H12AMXD4 CLDN4 MTIF MFOAMD7-PLA25DKY3 CEP1B2 CEOR5 C9orf12 C17orf119P0CEC3CAFH AKC QGT2 PSTIP2 KLK4 MT1G NDM1FYTTD1 ACIN1 DSG3 EFS3 EIF4G2 GOLGA3 MYL3 RSP1 SUK PDE5A PDE10A PAR6
 GORASP1 OXT DNAJB4SKR35 KAMP COMT PTMS ECO GALLYARHGEF15MLL2 MTCB2POLR2DPQL4F1POLU4F2NF398 TAP1 RFK4 POLR1BHO3CR2AHTF4 CPEB1 PTGS1 SMPD2SEC1L2PK3R5 PDE4B CABYR FRA11 UPR34MEM132AFRAT2 SNA1
 IUT1 DNA1 CPE2 LRP6 BDNF NOSP KSSP1 IGFBP1 MMP26 IGFBP4 NOV IGFBP7 RAB3D RABEP1 MTCO1 TOLB1 TCLA1 LEP APOA5ABARAPLGRIP2 MAPKB9P5SLC6A2PLXNA1 SAFB2 CD177 CRTG2 SOD1 NAIIP PPR3R1 WNK1 MCM5SERPINE2
 F2RL3SERPINEB1PRSS1 KCTD15 CELA1 KISS1 CLDN1 TIMP4 SPOCK1 MMP25 IL13 ITGADSERPINC1PRSS3 PCSK5 PTGDS CAST CENPA CENPB UDG ERCC6 ZNF423 PRSS3ADYNCH11INSIG1STARD13STK24 MYOCDTHAR11 XBP1 NPPA HMGN3CUEDC2
 RBM23 ATAD2 H2AFY RMDND5 TTBK1 TPPP SARF2 ZNF467 IL27RA APLR2 EZH2 IMPDH2METTL1 PDE3B AKTIP PIFSK1A SRF72 ACP2 ATP5A1DNM1L STK3 AIFM1 NCOA7 TRIM59 REXO4ONECUT1 PBX1 SLC30A8NAJC14EPH8 ADRB3 VEGFC FGF
 MARK2 ACLY GABRP2 DSTN PTHLH IRF9 F13A1 AANATSERPINEBAP2M1 CY58A TBP1 SLC6A4AKT1S1 HSF1 STK4 KDN3 PRDM2HST4H4IGFALS BIRC7 CD55 PON1LGLA3SBRBARAPL2CNJ5 DFFA UE46B MYP PCK2 PLG3 TRIB1 GSDA
 SEM5AKFAP11 ARNTL RCAN1 MYO23 BMP1 PAX2 MCM3 IFNG GCKX THBD GFS SLC3A2 AMPB BCAN MT2ACOL1BAHAPLN1LGLA3MMP17 EFP3 TIMP2SERPIND1 CTS5 PRSS2 HEXIM1 ADCY6CHRNA7 IL18 BUB3 MED6 NMT2 PKC2
 ELK3 BICD2 MARK1 PAK6 KRT10NOTCH2 SFI RYER CRADD TCF20 USF1 HFI PPARGG1MED24 BCAS2 MED21POY1A UBE2Q ANXA5 FGF4 TLR3 CAMK1L TTF MYO7A CABIN1 EIF2B5 GYS1 SLC25A4DUSP7 COPG2 COPG AGTR2
 ACE SNAIP SLOC2 GFRAS2OCHRMI1ADRA2CNUC1GABRG2 ADA DES MMS19 GRN CXCL12 PRS1 MYO22CAMK2D POR HMOX1 SGK3 ADAM9 IL16 BME HDAC8 GNA14 EDRBR AXIN2 UBQLN1FKBP1A EIF3F APPL1 APTX KCN9 PKM2
 CAT TLR10 FLRT1MAPKSP1 KSR2 CUL4B CLU FKBP8 GABRA1DUSP22 AP1B1 ARRS SH2D2A CSEIL LAMA1 HAND1 F9 F11 POLA1 RBM14 IL6R WIP1ADAMTS4CCL7 FZ2 TIMP3 P2RY2GRIPAP1 RBM9 FLIM CARD8 FLP2 CDC32
 EDAR2 DCTH2 HXCR3 MAP6CASP8AP2NOL3 DEDD2 CASP14PCALM1TOPBP1DUSP10 KIF1A CNOT1 MP3 BTG1 KPNAB CITED1COBRA1 MLH1PAPAHB1 FLJ1 SIPR1 PFL EIF4B PARVA F2RL2 DRYSL2MKNK2 KRAS HSF4 SNG3SMARCA2RNF14
 POU2F2 DCAF6SMARCD1 IDE GRIP1 MAPK15DDX54 CHD9 FGFR3 DRD4 AKAP12 TPM2 ADD1 GSTP1 FANCC IL6 MAD2L1 KSR1 AIP BEC3 ATP2B4 KPN1 CAD CXCR1 CTS3D TRIB3 GRK6 TRAP2 BIRC6 ABL2 RPS9A2PP91R2 SDCBP
 DNT1P2 TAF10 SPAG9 BGN COL4A1 SN ALB ACAN ADRB2 MIDK LDB1 ISL1 AP2A1TOPORS UO1 MAP4K4 IRF1 RNF4 OGT NR2C1 PROX1 BRD8 NCOA4 NSD1 ITGB3BP RBM39 UBE3A TRRAP TUBA19ACTA2 PKN1 CENPE ILF2
 MYB2 NR2F6 PRRF6 TXN INF3R2P8MCSF1ADCR1P1NRC1SYNAPSH3GL2 EIF2B21 CCNH RGG FLAU CTSL1 MMP7 ADAM17GDC27 TAF4 TAF7 SARB RPS9A4 IRF2 BIRC3 SH3GL1PMAF1 MOAP1 CCR1 NMT1 RHEBL1 OCC WWOX SLC3A3
 NRPI1 BLM NEDD8 AKAP8 KCNIP3 RGS3 DUSP16 PKN2 ST13 F8 IL2RA COL7A1 BACE1 JTGAM IL1B NLRP1 PARP2 MAP4 FAF1TNFRSF109NOD1 GMEB1 DUSP1 PHB2 WEE1 PMA3 RFC1 NMI1 COX2HIST2H2BERAC2 CPN6A
 EIF2S1 GAV2 ARHGDBMAP1AEPB4L1PK3R3 CALM2 CALM3 STRN GRK5 DUSP4 DAP3 SYTT1 MED1SMARCB1FKBP4 TRIM28 CD28 SNTA1 ITGA2B CAPN2 ITGB7 HCLST1 SPP1 FLNC CYCS YAP1 TLR4 DIABLOBL2L1BCL2L11 WRN ADRA1D
 GNA11 SNAPIN FEN APOE F5 SERPINE1LMO2 NFE2L2CDK11B HIP1 NIN BICD1 EEF2K PNRC2 XROCI1 ATR MAP1B NOS2 PRM1A RALBP1 IFNGR1 ACPI NR2F2 POLR1FITGBF1HKDMSA DCTN1 SYNJ1 IFNAR2ADRA2A ADRB1 DSP FANCA
 KLK3 IL2 IL2RG PBOC TNFSF11HNRNP2 FLS SLC9A3R2BRC5 VDACC1 GNA18P2P26A TSC1 CD14 DRD2 GRIA3 ARAF1NFRSP16 NSF CCND3AKAP1MAPKB9PKKARFADD45PRT3 ACHE XSBF5 TRPA RANBP9 F2R SLR1 CCNT1 ELP2
 MYBL2PLA2G4RHAP31DEED ZBTB11NFRSF14E2RL1 EPN1 NR13 PRMT2 LNB AURKA MAP3K4 FGA AKAP9 SPB TSG101 PDLF1SMARCB1MAP3K4SMTA2 MTA1 DNMT2 FLT4 GNAQ LMNB1 BACR1 A2M PRR36 STX1A BIRC2EIFE6P1GTF2F2
 HIST3H3FOXO4 GR12 MEF2A VEGFA SFRS12 SMAD2 CULL1 PSMOS ACTN1PPP1R8MAP3K8SERPINE3 CTS5 COL1A1 TGFBI FLECI MARK1 PEAS1 NR1H4 KRT28NFRSF1MAPKAP1AROB1 RAB7 TLR2 PAK2CTNND1 EDPI1 CONE1 LETS2 BCL2A1GPRASP1
 GNAO1 FGR CDK7 GTF2H1 ATN1 STAT2 IAMD1 BCL10 UDR2 HSPD1 CXOR9 APC ACVR1 GZMB MAP2K2BORB2 BMX GADD45DRAT4 DCN VCAM1PLSCR1 GATA1 GRIA1 TOPI MAPK11 TDG RUNX111 MITF COPS2MAR3K11DDX5 KPN2
 DRD1 YES1 R32 ROCK1 CDH5 ESRRA GATA2ADRA1B BRAF SGK1 FOXO3 XIAP BAK1 RASSF1 AXIN1 NTRK3 PICK1 TRAF3PPKAP2MAP3K8 KNG1 SREBF1 SF9 HSP90AB1 ILK HMGAI SREB2 NR2C2 PRKQ3 MNAT1 RAE5SMARCC3ACTG1
 CASP4 TCF4 FSL CAMK2G CALR NFKB2 COPPE NCL SH2B2 SH3 FGRFL NCF1 GNAI1DEKRB2DPFD1 TGM2 GTF2I L6ST ARRB2 ACTN2 MAPK8 YBX1 PTENSLD9A3FHART PTMA NOS3 PTPN2MAPKB9PPFP2R1A TNS PRKD1 ITGAV
 APAF1 XPO1 HSP44 NR2F1 NR1H2 TRIP4 PRMT4 JUND GSN MSN OPRM1ICGAP1PTGS9NFRN1NFR3C2MAP4K1PRKCE NR0B2 STUB1 CDC25MAPKB9MAP2K7PLCQ2 IGFBP3 MMP11TFAP2A BID GADPH BAG1 NPM1 GAB2 MUC1 IGF1
 TERT THBS1 PIAS3 SNW1 PRKCI CEBPA SRN ARRB1 AGTR1 NCF1 FBP1 SPTAN1HMGH1TRADD MTO8 RPSKARESKAN MCL1 MDM1MAP2K4XROCS CTS3 CDH1 CHD3 RVR3 RORR PLD2 MAP2 MST1B NOS1 ITGB2 ELANE GSK3A
 AKT2 CHEK1 HTR2A NOTCH1 PSEN1 NR1H2 TUBB EBF1AT1 ETS1PARGGTAFRS3 ELK1 EPHB2 GNAI2 COPRS TYK2 GRB10 CXCPHSPRNATRAF1 RPK2 FOXO1 HDAC2RPS9BILASIP10PDU2R1TRIM24 MET FLT1 BCAR1 PKG3G CBR1 KPN2
 CSF2RB ACTA1 BCL3 CDK5 BOSTM1 ATXN1 FHL2 CSNK2B ITGB6 FAS SMARCA4LUMO1 GAB1CDKN1B PAK2 HSF1 BAX IRAK1 FLAR KRT16 NR4A1 GTF2B RPS9KAT1 FET IL2RB SOS1 DLG4 GRIN2D TP73 PCNA CDKN1A RPK1 E2F1
 MED1 ITGB1 CSK ATF2 TSC2 RPP3C2MAP3K14 KAT5 PPIR EIF2AK2 BAD FADD EZR NCOF1 PTPN1 ERFB2 FKBP1CDKN1MAP3K4SPTX2CA CDC24 NCK1 CCND1 ZBTB16HSPA1A FRKDC GRIN1 SFF MAP3K7 LRP1 PSEN1 PPP1CA FLNA
 VWHAG CREB1 NR1P1 KIT HDAC3 AHR1 KKBK1 TRAF3 HTT SNCA NCOA3NFRSF10RQPKY ACTB CASP1 TRAF6 PIAS1 CASP3 VIM1 ADRB2 NDIM1 RAC1 NIF41 YRCD1 MAP1 BASA1 PXN1 LEB NCOA8BCL2L11 FOS IGF1R VWHAG
 KKBK1 PML THRB CEBF3 SUMO1STAT5B VAV1GNB21 MYC THRA VDR WHA2 PAK1 GALT1 MMP1MAP2K11RARS RARF TEP1 BEB2 PRKCE STAT3ARPOGFFH CRK RST1 NCOA3 CASP9 NCOF2 TAPP LYN NFKB1 CBL IAK1
 PTPN9 F2 INSR RARA SYK CHUK RARF1 SMAD3 BCL2 AK2 KDR SPI1 NCOA1 FLOG1 GAV1 LCK TYK2 S4B3 RPKAGAS1MAD2 ESR2HSP90AA1 ABL1 RAF1 MAPK13VWHAG RB1 PKCRL1 BRCA1PTEN1HTN1NPK3CDK FYN
 RBLA PRKCA JUN RXVA CSNK2A1SMAD3 SHC1 MAPK3 STAT3 MAPK6 CASP6 GPR2 EGFR GSK3B STATT1EP300 CREB3 NR3C1 TP53 MAPK1 AR SFC AKT1 CASP9 ESR1

FIGURE 2: The drug-disease network.

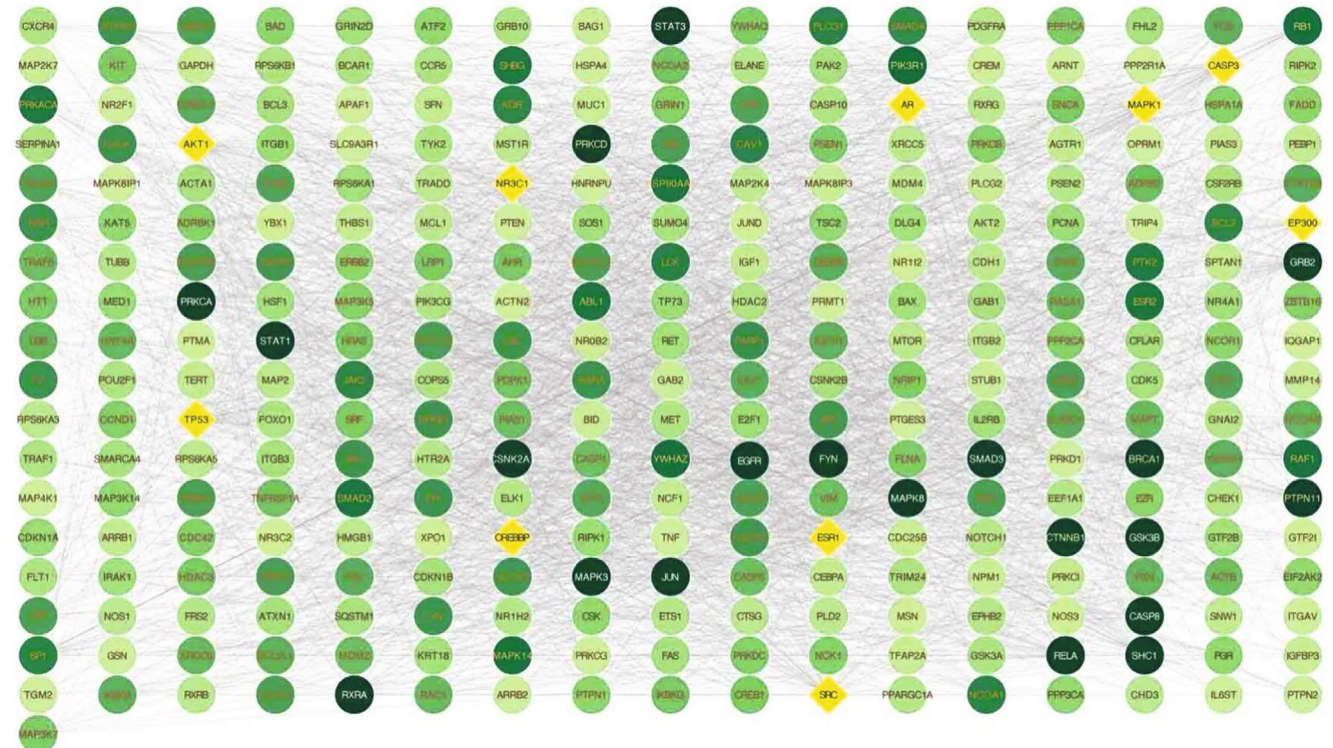


FIGURE 3: The key target network.

determining the key targets, we learned the biological pathways related to the treatment of insomnia by the four medicinals, which can provide novel directions and view for the research of TCM formulas [19].

In this study, 44 active components and 81 drug-disease common targets were obtained; 307 key targets were found in PPI network; a core cluster composed of 14 nodes and 50 functional associations was found. In addition, gene

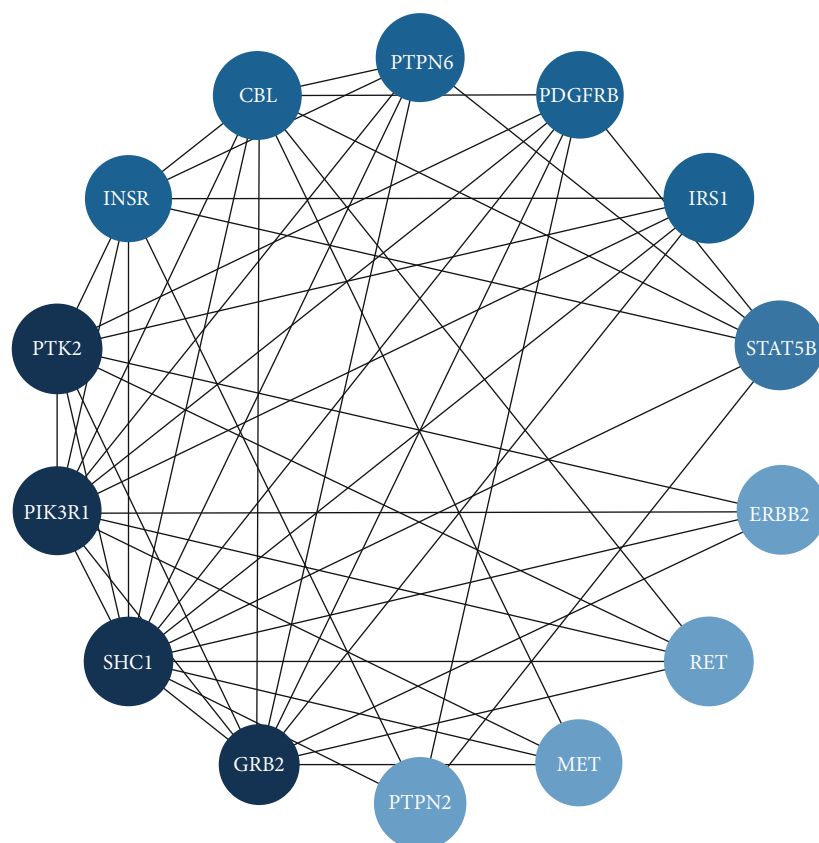


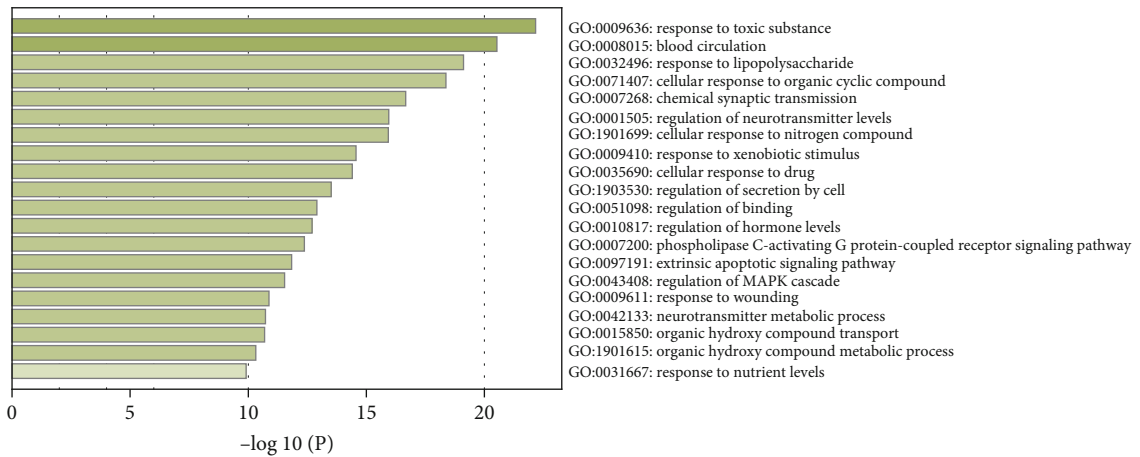
FIGURE 4: The core cluster.

function annotation and signal pathway analysis were carried out on them.

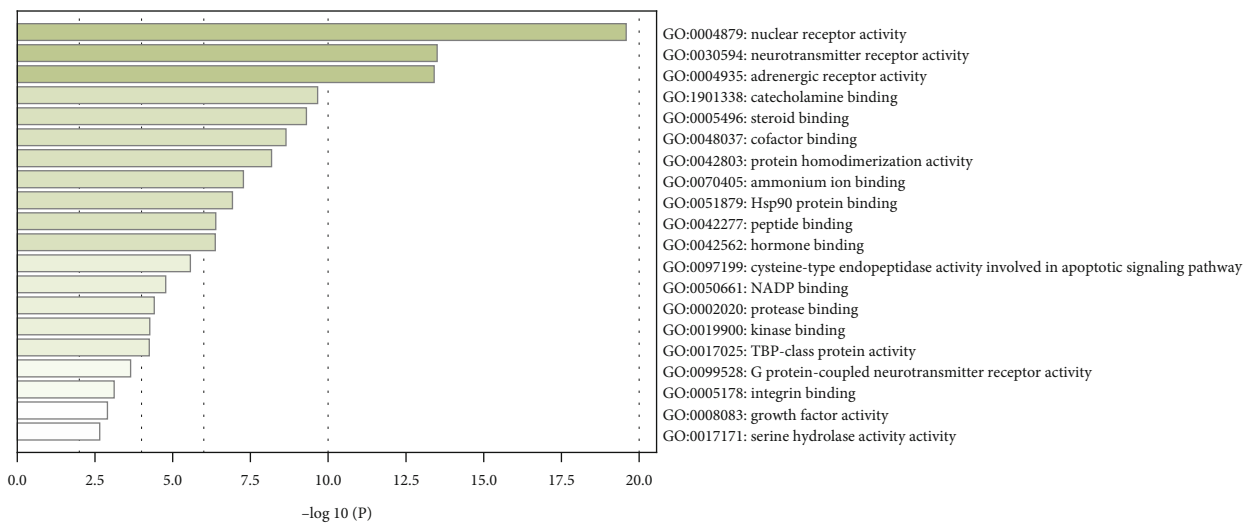
Referring to the node degree value, ESR1, AKT1, SRC, AR, MAPK1, TP53, etc. were chosen as the key targets. ESR1 [20] gene encoding proteins regulate many estrogen-induced gene transcriptions, and these genes play a role in growth, sexual development, and pregnancy and are expressed in many non-reproductive tissues. Their encoding receptors play a key role in breast cancer, endometrial cancer, and osteoporosis and may be of great significance in promoting angiogenesis, reducing inflammation, and improving adipose tissue function. AKT1 [21] gene may be expressed in the neuroprotective effect mediated by nerve growth factor and may also indirectly respond to nutritional and hormone signals to regulate cell growth and survival. SRC [22] proto-oncogene may play a role in embryonic development and cell growth. The activity of its coding protein, tyrosine protein kinase, can be inhibited by the phosphorylation of c-SRC kinase. The mutation of this gene may relate to the malignant progression of colon cancer. MAPK2 [23] also regulates the levels of insulin and glucose by inhibiting downstream pathways or DNA expression to produce antitumor activity. This means that Chinese medicinals comprehensively promote the immune ability of the body in aspects like fighting against tumors and regulating the growth and metabolism of the body by regulating the hypothalamic pituitary adrenal axis (HPA axis) including estrogen [24] and androgen [25].

In the KEGG pathway analysis, it was found that the key targets were significantly abundant in the neuroactive ligand-receptor interaction, and the core clusters had a mutual relationship with the ErbB signal pathway. Neuroactive ligand-receptor interaction [26] is a collection of receptors and ligands related to all intracellular and extracellular signaling pathways on the plasma membrane, and it can relate to endocrine, physiological rhythm, brain function regulation, etc. ErbB [27], a collective name for four tyrosine kinase receptors, relates to cell activities such as proliferation, differentiation, and apoptosis, and its low expression promotes neurodegeneration such as multiple sclerosis and Alzheimer's disease, while its high expression is associated with a variety of solid tumors, such as breast cancer and gastric cancer. ErbB can assist in the treatment of anxiety, depression, and Alzheimer's disease to a certain extent [28]. Through the enrichment analysis of target genes and pathways, it is also found that some targets and signal pathways of the four Chinese medicinals are closely related to tumor growth and have considerable potential to regulate the expression of oncogenes. Clinically, it not only can improve the secondary insomnia of the tumor but also can improve related tumor symptoms [29]. The target and signal pathways still need to be confirmed by further experimental studies *in vivo* and *in vitro*.

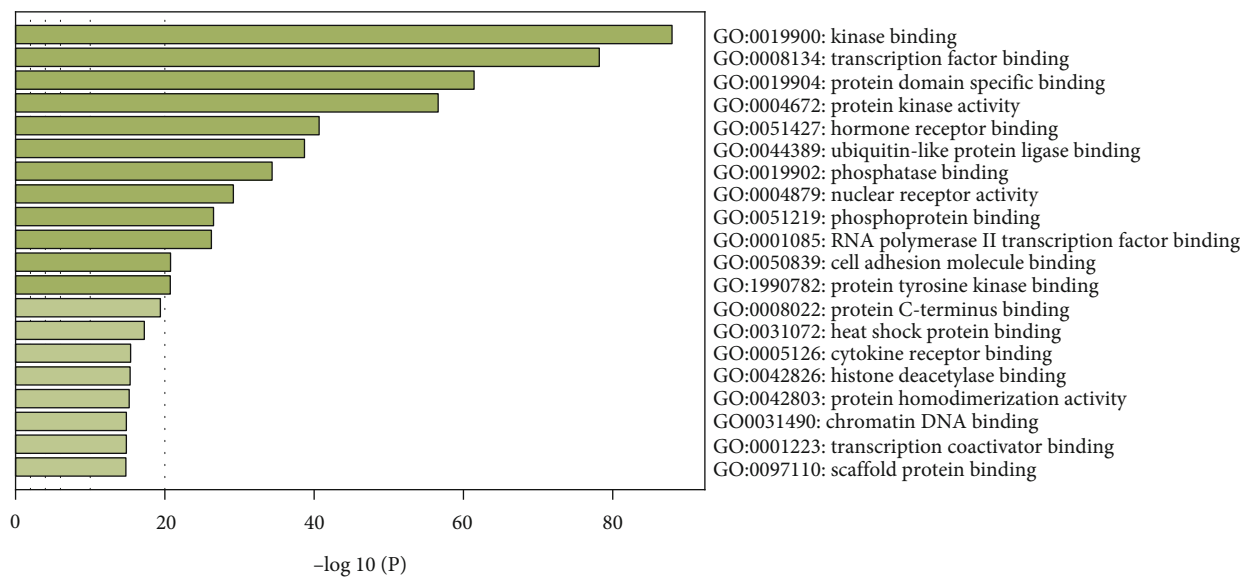
From the perspective of TCM, the combination of the four Chinese medicinals of *suānzǎorén* (Semen Ziziphi Spinosa), *chuānxiōng* (Rhizoma Chuanxiong), *fúlíng* (Poria),



(a)



(b)



(c)

FIGURE 5: Continued.

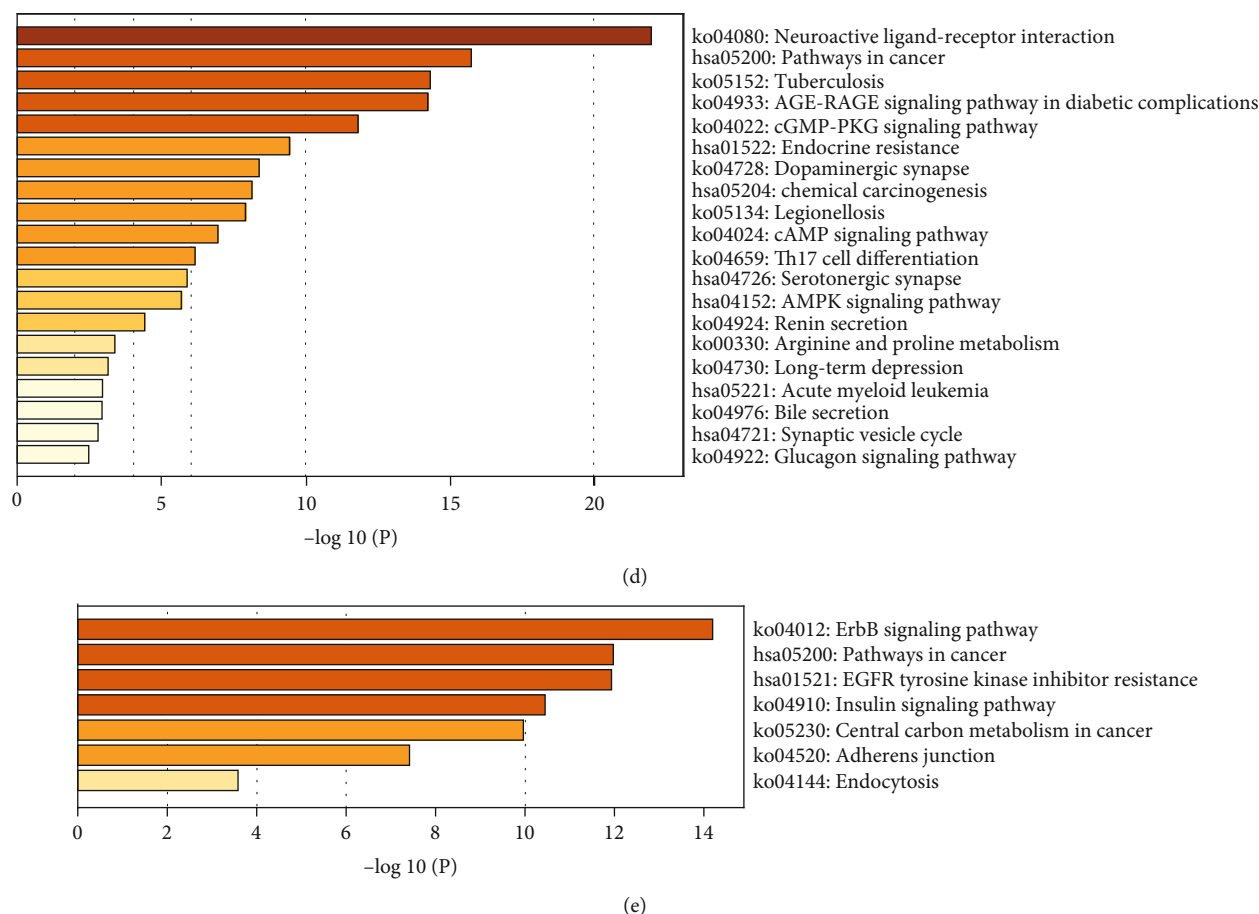


FIGURE 5: (a) Biological process, (b) molecular function, (c) cellular component, (d) KEGG pathway enrichment analysis of targets, and (e) KEGG pathway enrichment analysis of the cluster.

and *báisháo* (*Radix Paeoniae Alba*) is related to *SuānZǎoR-énTāng* (*Sour Jujube Decoction*). Yet, compared with *Suān-ZǎoRénTāng* (*Sour Jujube Decoction*), the heat-clearing effect of this combination is weaker, and the yin-enriching and blood-nourishing effect is stronger. In other word, the combination of the four Chinese medicinals calms the heart and mind by regulating liver qi and nourishing liver blood. From this perspective, it can relate to the premenopausal syndrome featured by deficient blood and floating yang, which is in accordance with the dysfunction of HPA axis and autonomic nerve [30].

Comparison was made between the network pharmacology of these four Chinese medicinals and of *Suān-ZǎoRénTāng* (*Sour Jujube Decoction*). *SuānZǎoRénTāng* (*Sour Jujube Decoction*) contains 139 active components, among which jujube kernel saponin is one of the core components, and 1386 targets with 27 significant correlated diseases [31]. Correlative to biological processes such as cell cycle, *SuānZǎoRénTāng* (*Sour Jujube Decoction*) plays an effect on cell junction tissue paths like regulation of TRP path, adhesion junction, and vitamin B6 metabolism by inflammatory mediators. What is more, this formula had the regulation of ischemic heart disease, psychiatric diseases, neuropathic pain, and other diseases.

Flavonoids such as quercetin, kaempferol, and 7-methoxy-2-methyl isoflavone account for the largest proportion and are predicted to be the core components of *SuānZǎoR-énTāng* (*Sour Jujube Decoction*). For target and mediation center degrees above the mean value, estrogen receptor, calmodulin, androgen receptor, and heat shock protein 90 (HSP90) were associated with more than 70 compounds. It is suggested that the mechanism of *SuānZǎoRénTāng* (*Sour Jujube Decoction*) lies in the multicomponent and multitarget interactions and has many potential therapeutic effects [32]. Studies suggest that flavonoids have antioxidant, anti-inflammatory, analgesic, and immune-regulating effects [33]. Due to its complex effects on a variety of receptors and signal pathways, flavonoids play a role in the central nervous system. Anti-depression and anti-anxiety, they are central nervous inhibiting, can treat schizophrenia, protect the nervous system, relieve pain, improve memory, and affect neuroendocrine. This is consistent with the mechanism of insomnia and its influencing factors [34–39].

In *SuānZǎoRénTāng* (*Sour Jujube Decoction*), the main inflammatory factors related to sleep are tumor necrosis factor (TNF), interleukin-1 (IL-1), interleukin-2 (IL-2), etc. These inflammatory factors can promote sleep to a certain extent, but their high expression level will affect the sleep

quality [40, 41]. The imbalance of the HPA axis will lead to depression and insomnia [42], while *SuānZǎoRénTāng* (Sour Jujube Decoction) can produce an antidepressant effect. The mechanism for this may be to reduce the performance of hippocampal TNF- α and IL-1 β to regulate the function of the immune system, restrain the apoptosis of hippocampal neurons, and lower brain injury [43]. The secretion of cytokines related to immune activation is also closely related to mental diseases [44–46]. In conclusion, *SuānZǎoRénTāng* (Sour Jujube Decoction) may affect the immune system by downregulating the expression of cytokine related pathways to achieve anti-insomnia and antidepressant effects.

The correlation analysis between the conclusion of this study and *SuānZǎoRénTāng* (Sour Jujube Decoction) shows that they have similarities in downregulating the expression of cytokine related pathways, affecting the immune system and regulating the HPA axis. The combined use of the four Chinese medicinals in this study is more likely to play a role in antitumor effect and endocrine regulation, while *SuānZǎoRénTāng* (Sour Jujube Decoction) may be more related to anti-inflammatory effects and central nervous system regulation. However, the network method has its own limitations, and there are differences in the analysis of its target and interaction relationship, so practical research is still needed to verify the information, such as key nodes.

5. Conclusion

In conclusion, the related use (the combination of the four Chinese medicinals with other Chinese medicinals) of *suānzǎorén* (Semen Ziziphi Spinosae), *fúlíng* (Poria), and *báisháo* (Radix Paeoniae Alba) and *chuānxiōng* (Rhizoma Chuanxiong) may entail the regulation of the central nervous system and endocrine system and protection of brain function and immune system in patients with insomnia. Compared with *SuānZǎoRénTāng* (Sour Jujube Decoction), these four Chinese medicinals may be more targeted for insomnia patients complicated by endocrine disorders such as climacteric syndrome and insomnia patients complicated by tumor.

Data Availability

All data and models generated or analyzed during this study are included in this published article.

Conflicts of Interest

The authors declare no conflicts of interest.

Authors' Contributions

Qiyue Deng collected materials, wrote the paper, and edited the images of the article. Libing Huang and Fanjun Yu helped in organizing the information. Jiahui Lin, Lijuan Hu, Jing Zhao, and Litao Pan prepared and polished the manuscript. Yu Kui and Shiheng Wu designed this experiment and cocorrespond to this study. The authors contributed to the article and approved the submitted version.

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Supplementary Materials

The 307 and top 10 key targets are saved in table form. The production source files of Figures 2–4 are shown in S-cys (*Supplementary Materials*). (*Supplementary Materials*)

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