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Exploring the Therapeutic Mechanism of Pingxin Dingzhi Decoction Through Network Pharmacology and Molecular Docking

ABSTRACT

Objective: Studies have demonstrated that the combination of antipsychotics and Pingxin Dingzhi Decoction (PXDZD) can effectively enhance treatment efficacy for schizophrenia (SCZ), while simultaneously reducing the adverse reactions associated with antipsychotic treatment. However, the exact mechanism by which PXDZD exerts its therapeutic effects is still unknown. The aim of this study is to investigate the action mechanism of PXDZD using network pharmacology and molecular docking techniques.

Methods: The primary components and their protein targets of PXDZD were extracted from TCMSP, SYMMAP, and HERB databases. The targets related to SCZ were acquired from OMIM and DisGeNET databases. The overlapping targets between composite targets and disease targets were used to construct a protein–protein interaction (PPI) network in the STRING database. The identified targets underwent GO and KEGG enrichment analysis, followed by molecular docking studies of the core target proteins and active compounds.

Result: The screening process yielded 285 PXDZD component targets and 1982 disease targets, ultimately leading to the identification of 120 shared targets. The Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis revealed that PXDZD treatment for SCZ engages a diverse range of biological mechanisms, including inflammatory responses and apoptotic processes, while also modulating various signaling pathways such as the PI3K-Akt, mitogen-activated protein kinase (MAPK), and tumor necrosis factor (TNF) signaling pathways. The molecular docking results revealed a strong affinity of Estrogen Receptor 1 (ER1) toward both β-sitosterol and stigmasterol, while kaempferol, β-sitosterol, and stigmasterol demonstrated significant binding potential against TNF-α.

Conclusion: Pingxin Dingzhi Decoction can play a role in treating SCZ through its multi-component, multi-target, and multi-pathway approach.

Keywords: Molecular docking, network pharmacology, Pingxin Dingzhi Decoction, schizophrenia

Introduction

Schizophrenia (SCZ) is a severe and chronic psychiatric disorder that afflicts approximately 1% of the global population.[1] It manifests gradually and often relapses, causing significant psychological distress to patients and imposing a substantial financial burden on their families.[2] The Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) outlines 3 primary dimensions of symptomatology in schizophrenia: positive symptoms (e.g., delusions and hallucinations); negative symptoms (e.g., anhedonia, blunted affect, and decreased speech); and cognitive symptoms (e.g., impaired concentration, deficits in working memory, and disturbances in thought processes).[3] The aetiology of SCZ remains unclear, and the clinical cure rate is low.[4] In clinical practice, risperidone, olanzapine, and clozapine are frequently used in the treatment of psychiatric disorders. However, they have been demonstrated to be ineffective, exhibit strong drug resistance, and are associated with a range of adverse side effects.[5] The concurrent administration of pharmaceutical agents such as risperidone with

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traditional Chinese herbal medicine has been demonstrated to mitigate the adverse effects associated with pharmacological intervention while enhancing the overall therapeutic efficacy.[5]

Traditional Chinese Medicine (TCM) possesses a profound historical heritage and plays a pivotal role in the management and regulation of human health.[6] With its distinctive theoretical framework and therapeutic approaches, it has garnered global attention. However, it is important to note that not all TCM preparations demonstrate efficacy, as many lack empirical validation. In the treatment of mental illnesses, TCM also possesses special therapeutic methods and concepts. It emphasizes the concept of holistic health and regards mental disorders as manifestations of imbalances between body and mind.[7] The distinctive concepts and methods of TCM can be integrated into comprehensive rehabilitation plans to help restore physical and mental equilibrium and attain a state of holistic well-being. Dingzhi decoction, a traditional Chinese medicinal prescription, is documented in Volume 7 of the book "Xingyuan Shengchun."[8] Dingzhi decoction is said to produce cognitive clarity and spiritual tranquility and provide heart-soothing and nourishing effects by replenishing qi and blood circulation while reducing counterflow.[8] It is primarily indicated for treating symptoms of "deficient heart energy" such as incoherent speech and excessive jesting without madness. The key constituents of Pingxin Dingzhi Decoction (PXDZD) are *Citri Reticulatae Pericarpium*, *Rhizoma Pinelliae*, *Acori Tatarinowii Rhizoma*, *RadixStellariae*, *Bambusae Concretio Silicea*, *Bambusae Caulis In Taenias*, *Scutellariae Radix*, *Poria, Citrus sinensis*, *Aristolochia contorta Bunge*, *Aquilariae Lignum Resinatum*, *Curcumae Radix,* and *Ziziphi Spinosae Semen*.[9] Studies have shown that risperidone combined with PXDZD can effectively enhance treatment efficacy while simultaneously reducing the adverse reactions associated with antipsychotic treatment and improving the quality of life of patients.[9-11] However, the underlying mechanism of action of PXDZD against SCZ was not clear. With the advent of network pharmacology, it has become possible to predict the active ingredients in TCM compounds through database analysis and computer simulations. The objective of this study was to investigate the mechanism of action of PXDZD in the treatment of core symptoms associated with SCZ, and its potential for mitigating adverse effects caused by antipsychotics, by utilizing network pharmacology and molecular-docking techniques.

Methods

To explore PXDZD-related core symptoms in the treatment of schizophrenia and the mechanism of action and adverse reactions of antipsychotic drugs, the present study adopted network pharmacology and molecular-docking methods in order to predict which PXDZD main bioactive compounds play a role, the potential targets of the compounds, and the signaling pathways involved. The research sequence of this study is depicted in the comprehensive flow chart presented in Figure 1.

MAIN POINTS

- *The possible active components of PXDZD are kaempferol, quercetin, β-sitosterol, stigmasterol, and luteolin.*
- *The action mechanism of PXDZD in the treatment of core symptoms and mitigation of adverse effects caused by antipsychotic treatment for schizophrenia regulates various pathways, including the mitogen-activated protein kinase (MAPK) cascade, the cellular response to cadmium ions, GABAergic synapse and neurotransmitter receptor activity, the PI3K-Akt signaling pathway, the MAPK signaling pathway, and the TNF signaling pathway.*
- *Kaempferol, β-sitosterol, and stigmasterol exhibited a robust binding potential to TNF-α. We postulate that PXDZD exerts its biological effects through this pathway.*

Identification of Active Compounds and Their Corresponding Targets in Pingxin Dingzhi Decoction

The active components and targets of *Cyperi Rhizoma*, *Citri Reticulatae Pericarpium*, *Rhizoma Pinelliae*, *Citrus aurantium*, *aquilariae lignum*, *Phellodendri Chinensis Cortex*, *Tulipa gesneriana L.*, fried *jujube* kernel, and *Scutellaria baicalensis* root in PXDZD were obtained from the TCMSP (https://tcmsp-e.com/) database. The active ingredients of*Bambusae Caulis In Taenias* and *Bambusae Concretio Silicea* resin were obtained from the Symmap (http://www.symmap.org/) and HERB (http://herb.ac.cn/) databases, and the Simplified Molecular Input Line Entry System (SMILES) number for the active ingredients of the compounds was obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). The SMILES number was later utilized in the SwissTargetPrediction database (http://swisstargetprediction.ch/) for the purpose of forecasting its prospective targets. *Arisaema Cum Bile* was obtained through the fermentation and processing of Zhongtian South Star and the bile of pigs, cattle, and sheep. The TCMSP database was utilized to acquire the active ingredients and targets of *Arisaematis rhizoma*. The active components of pig, cattle, and sheep bile were obtained from CNKI (https://www.cnki.net/), and their corresponding targets were downloaded from SwissTargetPrediction. The screening criteria for pharmacokinetic data were defined as having an oral bioavailability (OB) of at least 30%, and a drug similarity (DL) value of not less than 0.18.

Active Ingredient-Target Network

The components and targets of PXDZD were imported into Cytoscape 3.8.2 (https://cytoscape.org/), and a network depicting the interactions among PXDZD components and targets was constructed. The active ingredients with the top 5 scores were selected to continue the follow-up study.

The Compilation of Targets Related to Schizophrenia Disease

The disease targets for SCZ were obtained by conducting a comprehensive search in the OMIM database (https://omim.org/) and the DisGeNET database (https://www.disgenet.org/). The disease targets extracted from both databases were consolidated. The elimination of duplicates resulted in a refined compilation of targets associated with SCZ, for subsequent investigation.

The Establishment of the Protein–Protein Interaction Network

The present study involved conducting an intersection analysis between the collected drug targets and disease targets. The overlapping targets were submitted to the STRING database (https://string-db.org/) to obtain information about the protein-interaction network. The protein-protein interaction (PPI) data were entered into Cytoscape 3.8.2 for the generation of informative PPI-network visualizations. The 5 most significant targets were chosen for subsequent investigation.

Gene Ontology and Kyoto Encyclopedia of Genes and Genomes Analyses

The DAVID database (https://david.ncifcrf.gov) was used to gather Gene Ontology Consortium (GO, http://geneontology.org/) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG, https://www.kegg.jp) data for investigating the biological functions of potential

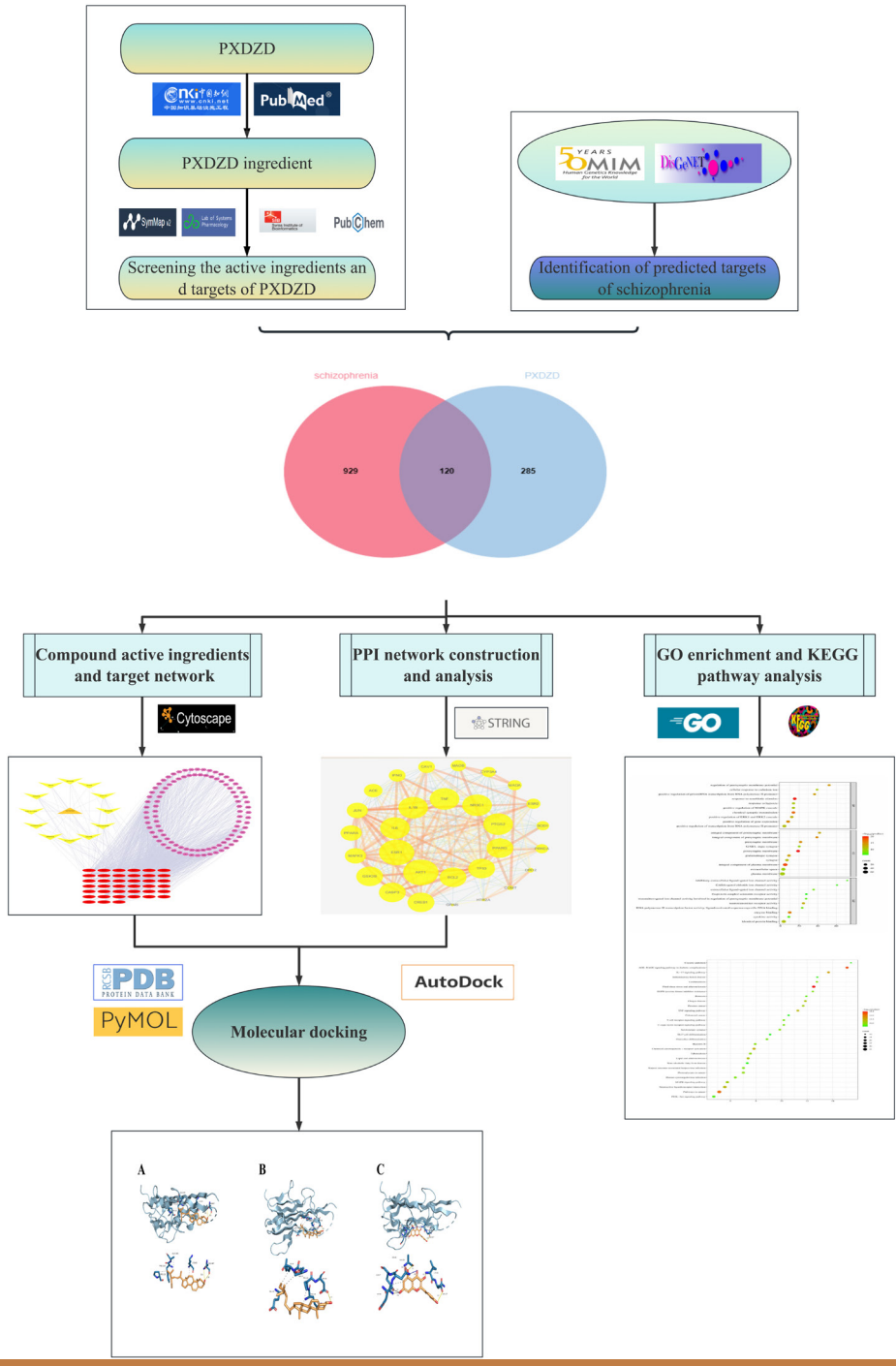

Figure 1. Flowchart of the study. The figure was generated using Adobe Illustrator 2021 software (version 25.3.1).

targets in SCZ. The GO analysis was used for the screening of biological processes (BP), cellular components (CC), and molecular functions (MF). Kyoto Encyclopedia of Genes and Genomes enrichment analysis allowed for the identification of pivotal signaling pathways implicated in biological.

Molecular Docking

Based on the outcomes from network pharmacology screening, the three-dimensional structure of the target protein was extracted from the Worldwide Protein Data Bank archive database (wwPDB, http://www.wwpdb.org/). Subsequently, water molecules and ligands were eliminated using PyMOL (Schrödinger, Inc., New York, USA; https://pymol.org/), and the resulting files were saved as PDB format files. The PDB file for the receptor was then imported into AutoDock 4.0 software (https://ccsb.scripps.edu) for processes such as hydrogenation and charge calculation. This file was saved in Protein Data Bank, Partial Charge (Q), & Atom Type (T) (PDBQT) format with the protein set as the receptor. Similarly, based on network pharmacology screening results, the desired small molecule target was retrieved either from the TCMSP or the PubChem database (https://pubchem.

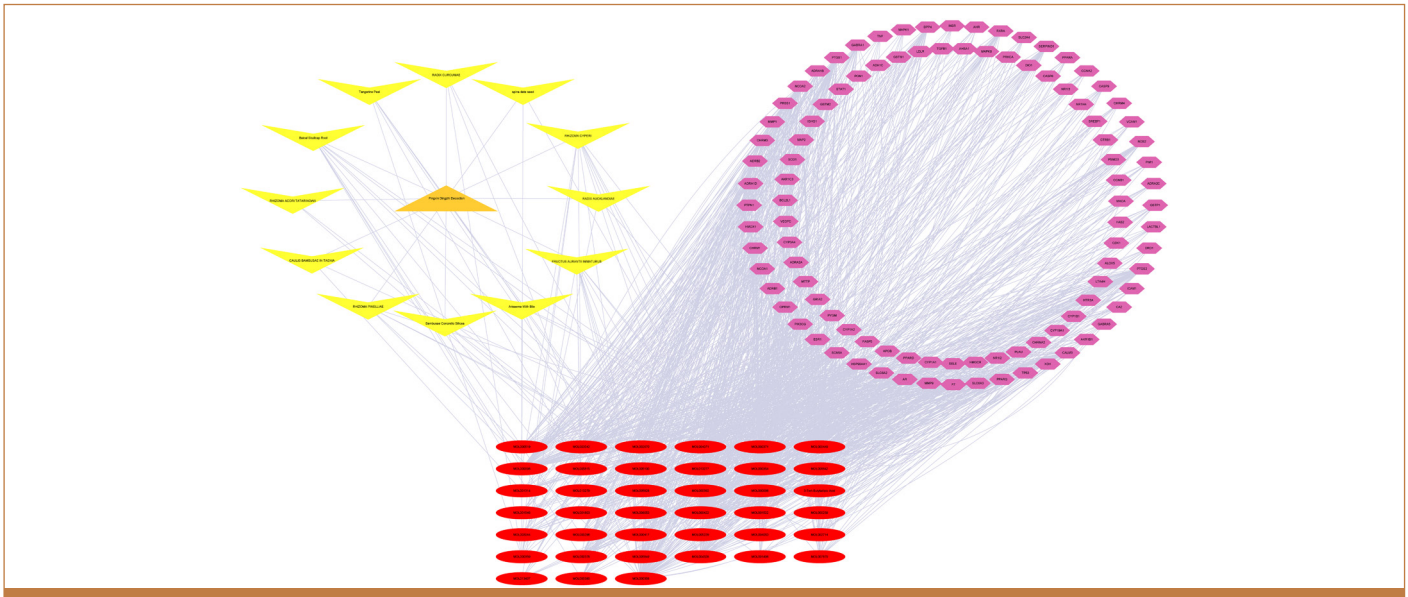

Figure 2. Herb-compound-target network of PXDZD. The light yellow represents the herbal components of PXDZD, the red signifies its active constituent, and the purple denotes the relevant target of PXDZD. PXDZD, Pingxin Dingzhi Decoction.

ncbi.nlm.nih.gov/) along with its corresponding structure file. Open Babel 3.1.1 software (https://openbabel.org/) facilitated the conversion of these downloaded small-molecule-structure files into mol2 format files. The mol2 file that represented the small molecule ligand was subsequently imported into AutoDock 4.0 software for procedures like hydrogenation, torsion bond detection, and charge addition; this resulted in a PDBQT-formatted file in which the small molecule served as a ligand. The respective PDBQT files of both the receptor and ligand were imported into AutoDock 4.0 software, followed by the setup of a docking box, using the Grid Box feature, to encompass all protein receptors within it. For conducting molecular-docking simulations, a Lamarckian genetic algorithm (LGA) was selected, which further utilized Autogrid and AutoDock programs accordingly. Finally, Open Babel 3.1.1 software was used to convert the PDBQT files for the molecular-docking results back to their original form, i.e., PDB files. These outcomes were visualized using PyMol alongside the PLIP tool (Technische Universität Dresden, Dresden, Germany, https://plip-tool.biotec.tu-resden.de/plip-web/).

Results

Compound Active Ingredients and Target Network

Pingxin Dingzhi Decoction contains 12 TCM components, 131 active components, and 1982 predicted targets. The name of the target was unified as a gene symbol. The network of compound-active ingredient-target is shown in Figure 2.

Screening was performed according to degree, OB, and DL; the top active substances are seen in Table 1. The top active substances were ranked according to their degree value, with quercetin exhibiting the highest OB and stigmasterol displaying the highest DL.

Protein-Protein Interaction Network Construction and Analysis

The active component targets of PXDZD were gathered, and any duplicate targets were eliminated. The overlap was identified between the collected targets, obtained by removing duplicate entries, and the targets extracted from the disease database. Based on the analysis using a Venn diagram, we found that there were 120 shared targets of PXDZD and SCZ (see Supplementary Figure 1). Cytoscape software (Free Software Foundation, Inc., Boston, USA) was used to construct the PPI network for essential targets (see Supplementary Figure 2). The primary objectives of the top 5 targets are presented in Table 2.

The findings from the analysis of GO and KEGG enrichment are illustrated in Figures 3 and 4. The GO enrichment analysis revealed that hub genes primarily participate in the facilitation of the MAPK cascade, the cellular reaction to cadmium ion, GABA-ergic synapse function, and neurotransmitter receptor activity, etc. The KEGG enrichment analysis indicated a significant enrichment of the hub genes in crucial signaling pathways such as the PI3K-Akt pathway, the MAPK pathway, the TNF pathway, and other associated pathways.

Table 1. The Information Regarding the Top Five Active Components of the Pingxin Dingzhi Decoction

Name	Degree	Betweenness	Closeness	OB	DL
Kaempferol	186	34828.715	0.380	41.88	0.24
Quercetin	153	75683.595	0.433	46.43	0.28
Beta-sitosterol	151	23784.750	0.346	36.91	0.75
Stigmasterol	123	17764.070	0.345	43.83	0.76
Luteolin	116	25674.030	0.373	36.16	0.25

DL, drug-likeness; OB, oral bioavailability.

Table 2. The Information Pertaining to the Most Significant Five Targets of the Protein-Protein Interaction Network

Name	Betweenness Centrality	Closeness Centrality	Degree
AKT1	0.029	0.933	26
ESR1	0.031	0.933	26
IL6	0.010	0.875	24
IL-1β	0.010	0.875	24
TNF-α	0.010	0.875	24

Molecular Docking

Based on the findings from network pharmacology analysis, 5 active substances and 5 core target proteins were screened. The active substances and target proteins were subjected to separate molecular-docking analyses. The energy requirements needed for the compounds to bind to the core targets are presented in Table 3. The outcomes of the molecular-docking analysis for the main receptors and ligands are illustrated in Figure 5. The results demonstrated an interaction between TNF-α and stigmasterol, β-sitosterol, and kaempferol. Stigmasterol establishes 2 hydrogen bonds with GLN-67 in TNF-α (Figure 5A). A hydrogen bond forms between β-sitosterol and ASP-140 in TNF-α (Figure 5B). Kaempferol forms 2 hydrogen bonds with ASP-45 and ALA-134, as well as one hydrogen bond with ILE-136 and ASN-137 in TNF-α (Figure 5C).

Discussion

In 2007, Hopkins first proposed the idea of network pharmacology.[12] The emergence of network pharmacology disrupted the conventional model of drug research and development, which previously focused on a "one drug, one target, one disease" approach. Instead, it introduced a novel method that explores intricate network relationships between multiple targets and diseases. Traditional Chinese Medicine compounds contain intricate combinations of components, making it challenging to determine their specific therapeutic effects. However, with the advent of network pharmacology, it became possible to predict the active ingredients in TCM compounds through database analysis and computer simulations.

The present study investigated the underlying action mechanism of PXDZD against SCZ. The study found that PXDZD contains 131 active ingredients, the main ones being kaempferol, quercetin, β-sitosterol, stigmasterol, and luteolin. Kaempferol and quercetin have sedative and anxiolytic effects.[13] The identification of β-sitosterol as a potential solution for cognitive disorders such as Alzheimer's disease (AD) has significant implications in the scientific field.[14] The cerebral cortex and hippocampus exhibit high levels of β-sitosterol, leading to a reduction in the activity of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE).[14] Luteolin can reduce or prevent neurological abnormalities induced by maternal immune activation.[15]

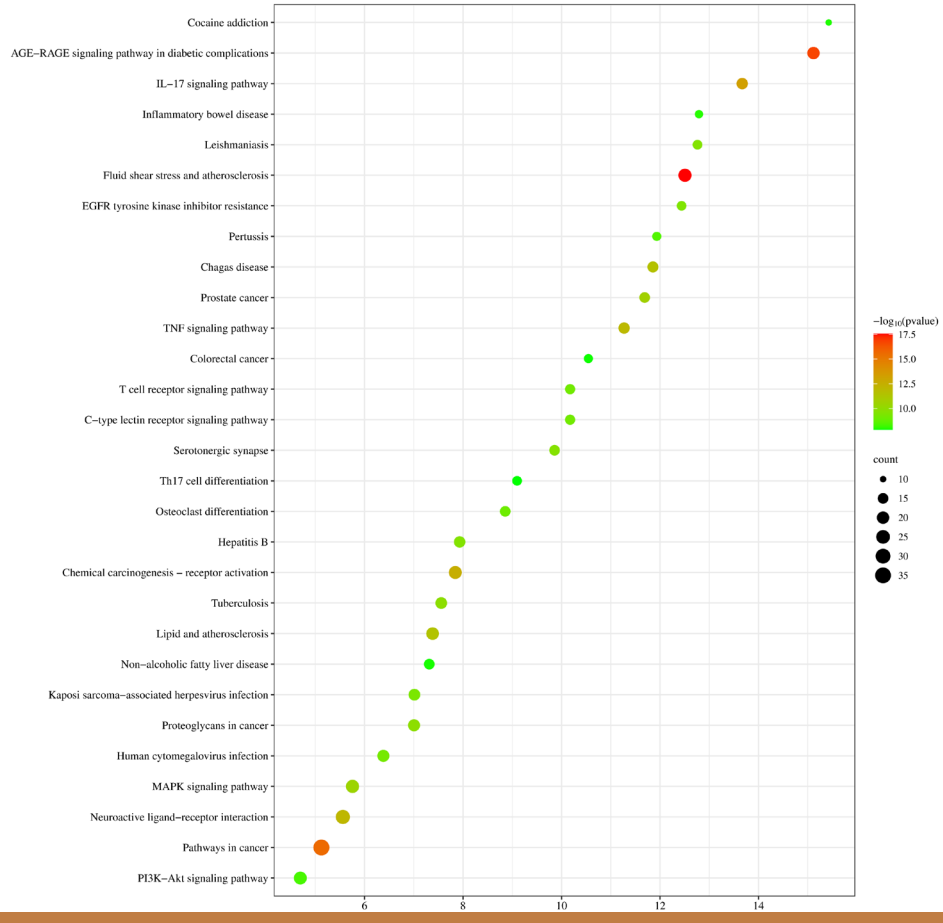

Figure 3. The top 10 GO terms associated with hub genes. The X-axis represents the gene ratios, and the Y-axis depicts the GO terms. Log10 P-values are indicated using colors. Node sizes correspond to the number of genes enriched in GO pathways. GO, Gene Ontology.

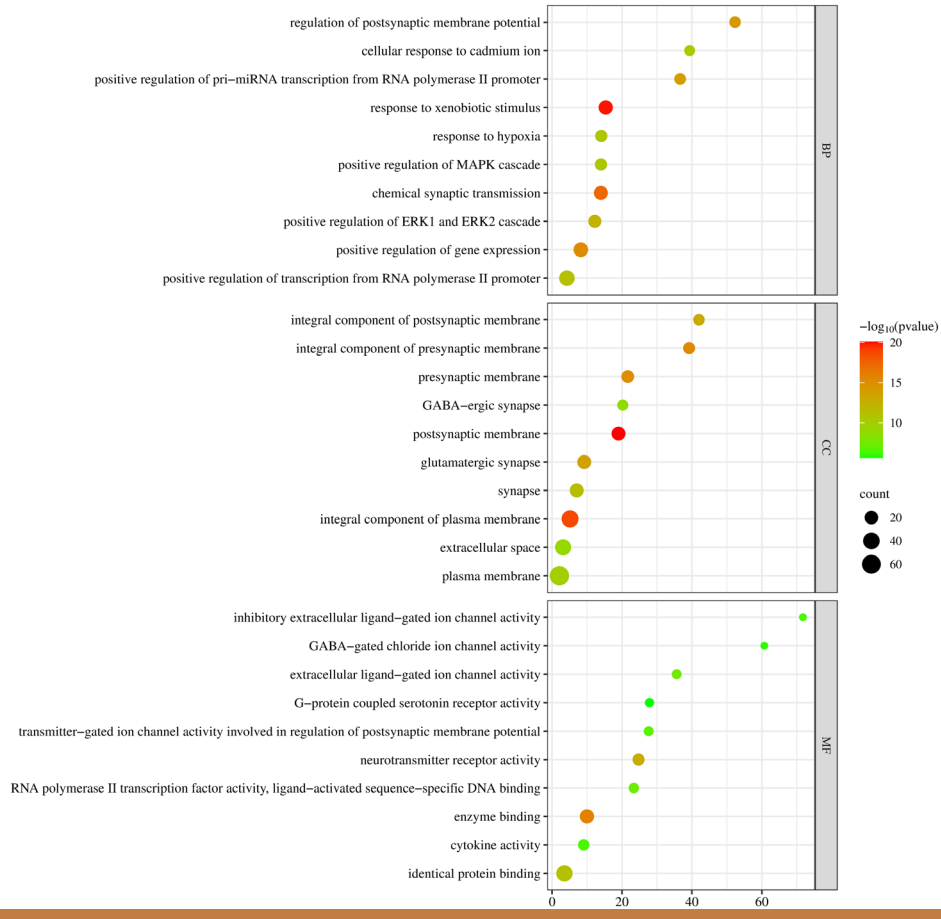

Figure 4. The top 30 KEGG pathways associated with hub genes. The X-axis represents the gene ratios, and the Y-axis represents the KEGG terms. Log10 *P*-values are indicated using colors. Node sizes correspond to the number of genes enriched in KEGG pathways. KEGG, Kyoto Encyclopedia of Genes and Genomes.

These components have potential value in the treatment of SCZ and deserve further exploration.

The present research resulted in identifying a combined count of 120 common targets. The analysis of the PPI network indicated that AKT1, ESR1, IL6, IL1B, and TNF-α were identified as crucial targets. The aforementioned genes are also associated with psychiatric disorders.

The peripheral blood of individuals diagnosed with bipolar disorder exhibits increased levels of AKT1 expression, which are specifically associated with manic symptoms.[16] Variations in the AKT1 gene (located at 14q32.32) have been linked to an increased susceptibility to SCZ as well as changes in the structure and function of the prefrontal cortex and hippocampus in humans.[17] The AKT/GSK-3β signaling pathways are involved in the cellular response to dopamine,

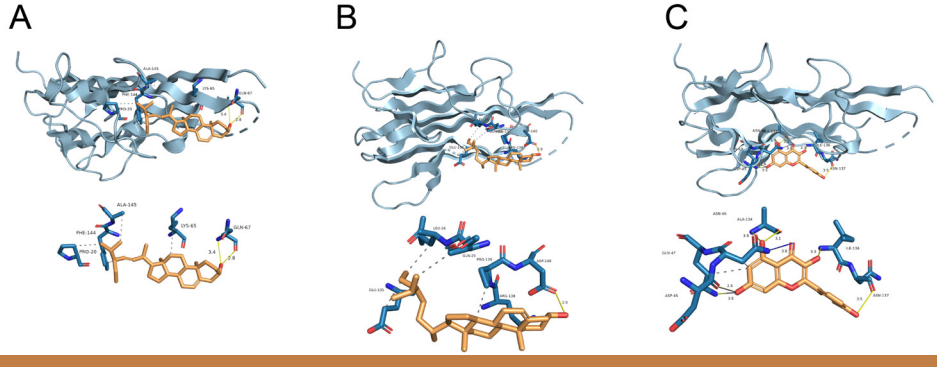

Figure 5. Molecular docking results of drug active ingredients and targets. (A) TNF-α/stigmasterol; (B) TNF-α/β-sitosterol; (C) TNF-α/kaempferol. The hydrogen bonding is depicted by a continuous line; the hydrophobic interaction is represented by a dashed gray line. TNF, tumor necrosis factor.

Table 3. The Binding Energy of Compound and Core Targets (kcal/mol)

Target	PDB ID	Compound	Affinity (kcal/mol)
AKT1	1unp	kaempferol	−3.69
		quercetin	−2.89
		β-sitosterol	−4.12
		stigmasterol	−3.98
		luteolin	−3.75
ESR1	6sbo	kaempferol	−4.17
		quercetin	−3.5
		β-sitosterol	−4.31
		stigmasterol	−4.64
		luteolin	−3.37
IL6	1alu	kaempferol	−3.82
		quercetin	−3.68
		β-sitosterol	−3.77
		stigmasterol	−3.58
		luteolin	−3.83
TNF-α	5uui	kaempferol	−4.68
		quercetin	−3.62
		β-sitosterol	−4.63
		stigmasterol	−5.99
		luteolin	−4.18
NR3C1	1nhz	kaempferol	−3.38
		quercetin	−2.67
		β-sitosterol	−3.96
		stigmasterol	−4.08
		luteolin	−3.83

PDB ID, Protein Data Bank Identification Code.

affecting cognitive deficits in schizophrenia and influencing brain activity as well as the response to antipsychotic drugs.[18] The genetic variation ESR1 SNP rs2144025-C>T has the potential to affect the expression of ESR1 and is associated with behavioral characteristics observed in individuals with SCZ and bipolar disorder.[19] The findings of various studies have consistently demonstrated that patients with SCZ exhibit significantly higher serum levels of IL6, IL1B, and TNF-α than do healthy individuals. However, it has been observed that after drug treatment, there is a notable reduction in the levels of IL6, IL1B, and TNF-α.[20,21] The KEGG enrichment analysis revealed that the hub genes were predominantly enriched in pathways such as the PI3K-Akt signaling pathway, the MAPK signaling pathway, the TNF signaling pathway, and several others. The signaling pathways exhibit intricate interconnections with the initiation and advancement of mental health disorders.[22-24] Pingxin Dingzhi Decoction can be used to alleviate the clinical symptoms of SCZ through these pathways. Brain-derived neurotrophic factor (BDNF) is prominent in the brain and plays a pivotal role in the differentiation, regeneration, and plasticity of glutamatergic and GABAergic synapses.[25] The interaction between BDNF and its high-affinity receptor TrkB triggers the phosphorylation of TrkB, leading to the activation of 3 essential intracellular signaling pathways in neural cells. These include the PI3K/PKB pathway, which involves phosphatidylinositol 3-kinase and protein kinase B; the PLCγ pathway, involving phospholipase C-γ; and the MAPK/ERK pathway, which encompasses mitogen-activated protein kinase and extracellular signal-related kinase.[26] The potential impact of pro-inflammatory cytokines on the processes of gliogenesis and neurogenesis may contribute to difficulties in cognitive functions such as learning and memory.[27] The development of schizophrenia is closely associated with glutamate metabolic disorders as well as the expression of TNF-α and IL-1β.[28-30]

Molecular docking is a widely used technique in pharmaceutical research for investigating the interaction and compatibility between receptors and their corresponding ligands. This computational approach serves as a valuable tool for drug design, facilitating the analysis of molecular interactions at the atomic level and providing predictions about binding patterns and strengths. This theoretical simulation technique is used to explore intermolecular interactions, as well as to forecast their binding modes and affinity. The present study used molecular-docking techniques to investigate the potential interaction between the core proteins and PXDZD core components identified through network pharmacology. In the present study, molecular-docking techniques were used to examine the interaction between the PXDZD core components, as pinpointed by network pharmacology, and the key proteins in order to determine if they can bind together. The selection of 5 bioactive components (kaempferol, quercetin, β-sitosterol, stigmasterol, and luteolin) and 5 core targets (AKT1, ESR1, IL6, IL-1β, and TNF-α) was based on network pharmacology research. Molecular-docking technology was used to investigate the binding mode and affinity. The molecular-docking results revealed a strong binding affinity of ESR1 with β-sitosterol as well as with stigmasterol. The stability of the interaction between TNF-α and kaempferol, β-sitosterol, and stigmasterol was observed, with stigmasterol exhibiting the highest affinity towards TNF-α, implying a significantly favorable binding.

Conclusion

The aim of this study was to investigate the mechanism of action of PXDZD in treating the core symptoms of SCZ and in mitigating the adverse effects caused by antipsychotics, by using network pharmacology and molecular-docking techniques. However, the computational methods of network pharmacology analysis may produce certain crucial problems, such as error propagation and the potential for false positives. In addition, the database is constantly updated, and its accuracy and timeliness have yet to be verified. Although the present study has identified certain compounds as important active constituents of PXDZD for treating SCZ, the effect of the treatment should be further validated through animal and cell experiments.

Pingxin Dingzhi Decoction produces its therapeutic influence by modulating several pivotal genes, such as AKT1, ESR1, and IL6. It has the potential to treat SCZ by engaging a variety of biological mechanisms, including inflammatory responses and apoptotic processes, and by working through diverse signaling pathways, such as the PI3K-Akt, MAPK, TNF signaling pathways, among others. Findings from the molecular-docking investigation revealed that kaempferol, β-sitosterol, and stigmasterol demonstrated a pronounced affinity for binding to TNF-α. Moreover, this study serves as a valuable reference for subsequent theoretical inquiries into the therapeutic application of PXDZD in the treatment of SCZ.

Data Availability Statement: The data supporting the results of this study are available on reasonable request from the corresponding author (hekuanjun666@126.com).

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Informed Consent: *N/A.*

Peer-review: *Externally peer-reviewed.*

Author Contributions: *Concept – K.H.; Design – K.H.; Supervision – K.H., C.Q.; Data Collection and/or Processing – X.J., C.Q., Y.B.; Analysis and/or Interpretation – Y.B., P.L.; Writing – X.J., Y.B., K.H.; Critical Review – C.Q., P.L., K.H.*

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