# Mechanism of action of Fangji Dihuang Tang in the treatment of Alzheimer's disease based on network pharmacology and molecular docking studies

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Abstract: Alzheimer's disease (AD) is a neurodegenerative disorder characterized by cognitive decline, memory loss, and worsening condition over time, primarily affecting individuals over 65 years old. Fangji Dihuang Decoction, a traditional Chinese Medicine Compound, has been historically used to treat symptoms similar to those of AD. This study utilized network pharmacology and molecular docking to investigate the potential synergistic effects of Fangji Dihuang Decoction in treating AD. Through a series of analyses, forty key targets were identified between AD and Fangji Dihuang Decoction. The study revealed that the effective components of Fangji Dihuang Decoction, such as quercetin, glycyrrhizin, beta-sitosterol, and wogonin, act through multiple pathways including heme binding and antioxidant activity to maintain nervous system health. These findings provide theoretical and experimental support for the pharmacological effects of Fangji Dihuang Decoction in treating AD.

*Keywords:* Fangji Dihuang Decoction, Chinese Medicine Compound, Network Pharmacology, Molecular Docking

## 1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disease with a gradual onset and slow progression[1], characterized by cognitive decline, memory loss, reduced communication ability, and worsening condition over time, mainly occurring in people over 65 years of age. AD is closely related to changes in central nervous system functions. Its occurrence is mainly related to the formation of extracellular  $\beta$ -amyloid protein, abnormal hyperphosphorylation of tau protein forming neurofibrillary tangles (NFTs), neuronal loss, and malnutrition, but the specific pathogenesis is not yet clear[3]. Existing anti-AD drugs can only delay the worsening of clinical symptoms and cannot stop or reverse the disease process[2-4].

Fangji Dihuang Decoction comes from "Synopsis of the Golden Chamber: Chapter Five on the Pulse Conditions and Treatment of Wind-Stroke and Joint Diseases", which states: "Fangji Dihuang Decoction treats diseases like madness, reckless behavior, incessant talking, without chills or fever, with a floating pulse..."[5]. The classic indications of Fangji Dihuang Decoction include symptoms such as restlessness, mania, reckless behavior, incessant talking, and a floating pulse...[6-7], which are highly similar to the descriptions of Alzheimer's disease.

Network pharmacology explores the molecular associations between drugs and diseases from a systematic level and the perspective of biological networks, aligning with the holistic theoretical ideas of traditional Chinese medicine, providing new approaches for Chinese medicine research, and offering new methods for clinical drug recognition and application[8]. Molecular docking technology is an auxiliary technique and tool for drug molecular design, which has significant theoretical significance for elucidating the mechanism of drug action. Considering the complexity of AD pathogenesis and the characteristics of molecular and cellular process changes in AD patients, this study delves into the potential synergistic effects and advantages of related target genes between Fangji Dihuang Decoction and AD pathogenesis. Hence, this paper, based on network pharmacology, mines the target genes related to Fangji Dihuang Decoction and AD, further exploring the mechanism of Fangji Dihuang Decoction in treating Alzheimer's disease.

## 2. Data and Methods

#### 2.1. Screening of Effective Components of Fangji Dihuang Decoction

Chemical components of Rehmannia, Stephania tetrandra, Saposhnikovia divaricata, Cinnamomum cassia, and Glycyrrhiza uralensis were searched through TCMSP (http://tcmspw.com/index.php)[9]. The chemical components were screened with oral bioavailability (OB)  $\geq$ 30%[10], drug-likeness (DL)  $\geq$ 0.18[11], and blood-brain barrier permeability (BBB)  $\geq$ -0.3[12] as the screening thresholds to select the main effective components.

#### 2.2. Prediction of Targets of Effective Components

**TCMSP** The effective components' corresponding targets were searched in (http://tcmspw.com/index.php). The collected targets were converted using STRING (https://string-db.org) [13] to convert the collected target protein names to gene names.

#### 2.3. Collection of Disease Targets

With "Alzheimer's disease" as the keyword, key genes related to AD were selected in DisGeNET (https://www.disgenet.org/) [14] with a Score gda  $\geq 0.1$ .

## 2.4. Prediction of Key Targets for Drug Treatment of Disease

The component target genes of Rehmannia, Stephania tetrandra, Saposhnikovia divaricata, Cinnamomum cassia, and Glycyrrhiza uralensis converted using the UniProt protein database(https://www.uniprot.org/)[15] were intersected with disease targets using Venny (https://bioinfogp.cnb.csic.es/tools/venny/) to obtain a Venn diagram. Eventually, forty key targets were identified between AD and Fangji Dihuang Decoction.

#### 2.5. Drawing the "Drug Component-Common Target-Disease" Network Relationship Diagram

The common targets and the corresponding active drug components obtained above were imported into Cytoscape 3.9.0 software[16] to draw the "Drug Component-Common Target-Disease" network relationship diagram using Cytoscape 3.9.0 software.

## 2.6. Building Protein-Protein Interaction (PPI) Network and Screening Core of PPI Network

The STRING database (https://cn.string-db.org/) was used to build the PPI network of target proteins. The gene intersection obtained from the Venn diagram for AD and Fangji Dihuang Decoction was entered into the STRING database, selecting "homo sapiens" as the species and medium confidence = 0.4 as the threshold to obtain STRING data.

The STRING data was downloaded and imported into Cytoscape 3.9.0 software for further analysis, and R was used to find the number of connections for each gene and sort them. The top 30 genes were visualized using a bar chart.

#### 2.7. GO Enrichment Analysis

The BiocManager software package[17] was installed in R4.3.1 software, followed by the org.Hs.eg.db package. Using "org.Hs.eg.db," the drug's action targets on the disease were converted to entrezID. Using the BiocManager software package installed in R software, additional packages such as stringi, colorspace, and enrichplot were installed. With the converted entrezID, gene ontology (GO) enrichment analysis was performed with Q<0.05, P<0.05, and a bar chart was drawn. The most significant pathways included heme binding, tetrapyrrole binding, RNA polymerase II-specific DNA-binding transcription factor binding, DNA-binding transcription factor binding, antioxidant activity, cysteine-type endopeptidase activity involved in apoptotic process, oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, insulin-like growth factor receptor binding, protease binding, amyloid-beta binding.

GO analysis was performed using the BiNGO plugin in Cytoscape 3.9.0 software. The common genes were imported into the BiNGO plugin, the species was set to Homo sapiens, and "GO\_Full" was

selected for the overall analysis with the selection criterion FDR<0.01, and the enriched genes were as shown in the figure.

## 2.8. KEGG Enrichment Analysis

The pathview package installed in R software was used to perform Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis with the converted entrezID, with Q<0.05, P<0.05, and bar charts and pathway diagrams of the enriched pathways were obtained. The most significant pathways included Lipid and atherosclerosis, Non-alcoholic fatty liver disease, IL-17 signaling pathway, Fluid shear stress and atherosclerosis, Human cytomegalovirus infection, Alzheimer disease, Pathways of neurodegeneration - multiple diseases, Tuberculosis, Legionellosis, Measles.

## 2.9. Selection of HUB Genes

The common genes were imported into Cytoscape 3.9.0 software[18] and the cytoHubba plugin was used with the MCC algorithm to score and select the top ten key genes.

#### 2.10. Molecular Docking

The pharmacophore molecular structure files of the core components (mol2 format) were searched in PubChem (https://pubchem.ncbi.nlm.nih.gov/) and converted into 3D structure files using ChemBio3D Ultra. The core protein structure files were obtained from the PDB database. After converting the ligand structure files and protein structure files to pdbqt format, the molecular docking simulation software AutoDock[19] was used to simulate the docking of molecules and targets, and the docking results were visualized using PyMOL software.

#### 3. Results

## 3.1. Selection of Effective Components of Fangji Dihuang Decoction

Chemical components of Rehmannia, Stephania tetrandra, Saposhnikovia divaricata, Cinnamomum cassia were searched through TCMSP (http://tcmspw.com/index.php). The screened chemical components had 402 targets for Stephania tetrandra with 3 active components; 1931 targets for Saposhnikovia divaricata with 13 active components; 2402 targets for Cinnamomum cassia with 3 active components; 2506 targets for Glycyrrhiza uralensis with 69 active components. The effective components of Rehmannia were obtained through literature search[20-21]. In total, there were 9 kinds: stigmasterol, catalpol, stachyose, acteoside, rehmaglutin D, rehmaglutin B, rehmaglutin A, FERULIC ACID METHYL ESTER,  $\beta$ -sitosterol.

#### 3.2. Prediction of Targets of Effective Components

The corresponding targets of the effective components of Rehmannia, Stephania tetrandra, Saposhnikovia divaricata, Cinnamomum cassia, and Glycyrrhiza uralensis were searched in TCMSP (http://tcmspw.com/index.php). The collected targets were converted using STRING (https://string-db.org) to convert the collected target protein names to gene names. A total of 266 target genes were obtained.

## 3.3. Collection of Disease Targets

With "Alzheimer's disease" as the keyword, key genes related to AD were selected in DisGeNET (https://www.disgenet.org/) with a Score gda  $\geq 0.1$ . After selection, a total of 180 key genes related to AD were obtained.

#### 3.4. Prediction of Key Targets for Drug Treatment of Disease

The component target genes of Rehmannia, Stephania tetrandra, Saposhnikovia divaricata, Cinnamomum cassia, and Glycyrrhiza uralensis converted using the UniProt protein database were intersected with disease targets using Venny (https://bioinfogp.cnb.csic.es/tools/venny/) to obtain a Venn diagram. Eventually, forty key targets were identified between AD and Fangji Dihuang Decoction

(See Figure 1).

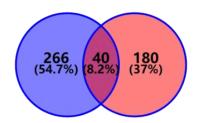


Figure 1: Venny diagram of the intersection of component targets and disease targets (blue represents component targets, red represents disease targets).

## 3.5. Drawing the "Drug Component-Common Target-Disease" Network Relationship Diagram

The common targets and the corresponding active drug components obtained above were imported into Cytoscape 3.9.0 software to draw the "Drug Component-Common Target-Disease" network relationship diagram using Cytoscape 3.9.0 software (See Figure 2).

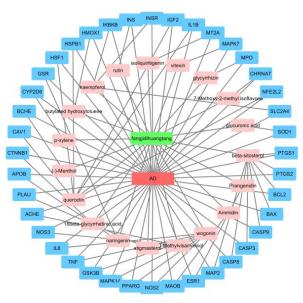


Figure 2: "Drug Ingredient-Common Target-Disease" Network Relationship Diagram.

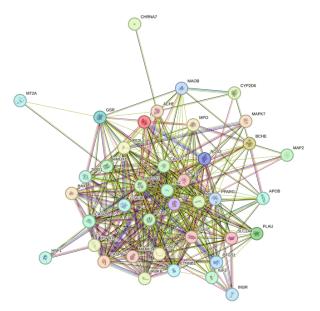


Figure 3: STRING data.

#### 3.6. Building Protein-Protein Interaction (PPI) Network and Screening Core of PPI Network

The STRING database (https://cn.string-db.org/) was used to build the PPI network of target proteins. The gene intersection obtained from the Venn diagram for AD and Fangji Dihuang Decoction was entered into the STRING database, selecting "homo sapiens" as the species and medium confidence = 0.4 as the threshold to obtain STRING data (See Figure 3).

The STRING data was downloaded and imported into Cytoscape 3.9.0 software for further analysis, and R was used to find the number of connections for each gene and sort them. The top 30 genes were visualized using a bar chart (See Figure 4).

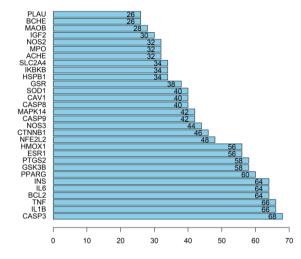


Figure 4: The number of nodes in the top 30 gene junctions is sorted

#### 3.7. GO Enrichment Analysis

The BiocManager software package was installed in R4.3.1 software, followed by the org.Hs.eg.db package. Using "org.Hs.eg.db," the drug's action targets on the disease were converted to entrezID. Using the BiocManager software package installed in R software, additional packages such as stringi, colorspace, and enrichplot were installed. With the converted entrezID, gene ontology (GO) enrichment analysis was performed with Q<0.05, P<0.05, and a bar chart was drawn. The most significant pathways included heme binding, tetrapyrrole binding, RNA polymerase II-specific DNA-binding transcription factor binding, DNA-binding transcription factor binding, antioxidant activity, cysteine-type endopeptidase activity involved in apoptotic process, oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, insulin-like growth factor receptor binding, protease binding, amyloid-beta binding (See Figure 5 and Figure 6).

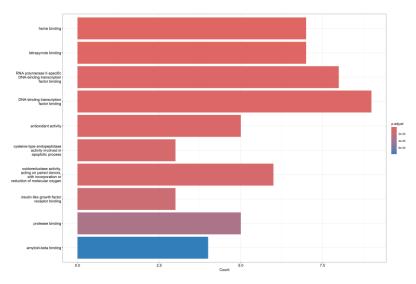


Figure 5: GO enrichment analysis histogram.

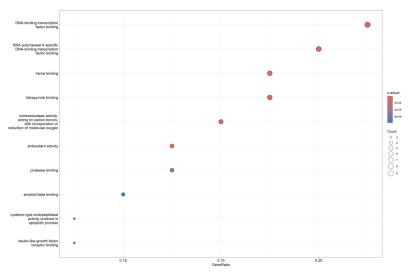


Figure 6: GO enrichment analysis dot plot.

GO analysis was performed using the BiNGO plugin in Cytoscape 3.9.0 software. The common genes were imported into the BiNGO plugin, the species was set to Homo sapiens, and "GO\_Full" was selected for the overall analysis with the selection criterion FDR<0.01, and the enriched genes were as shown in the Figure 7.

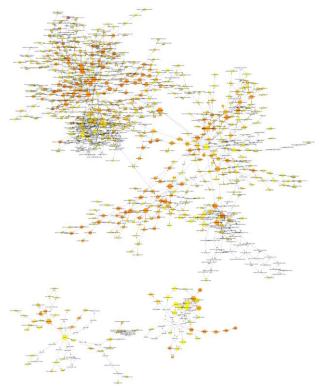


Figure 7: Enrichment genogram using the BiNGO plug-in.

## 3.8. KEGG Enrichment Analysis

The pathview package installed in R software was used to perform Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis with the converted entrezID, with Q<0.05, P<0.05, and bar charts and pathway diagrams of the enriched pathways were obtained (See Figure 8 and See Figure 9). The most significant pathways included Lipid and atherosclerosis, Non-alcoholic fatty liver disease, IL-17 signaling pathway, Fluid shear stress and atherosclerosis, Human cytomegalovirus infection, Alzheimer disease, Pathways of neurodegeneration - multiple diseases, Tuberculosis, Legionellosis, Measles (See Figure 10).

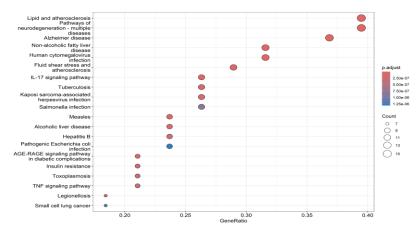


Figure 8: Dot plot of the enrichment pathway.

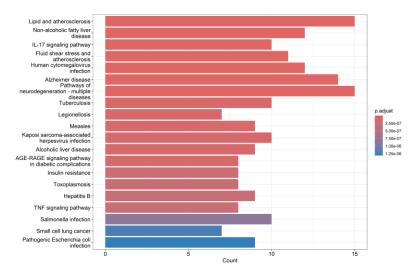


Figure 9: Histogram of the enrichment pathway.

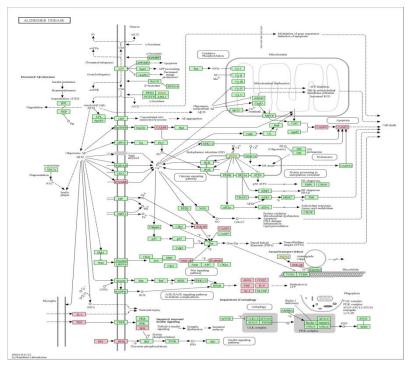


Figure 10: The signaling pathway diagram of drug-disease common targets in Alzheimer's disease.

## 3.9. Selection of HUB Genes

The common genes were imported into Cytoscape 3.9.0 software and the cytoHubba plugin was used with the MCC algorithm to score and select the top ten key genes (See Figure 11).

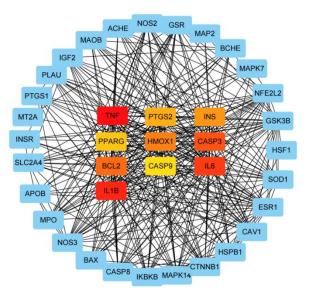


Figure 11: Hub analysis results (the items marked by warm colors are the top 10 genes)

## 3.10. Molecular Docking

The pharmacodynamic molecular structure files of the core components (mol2 format) were searched in PubChem (https://pubchem.ncbi.nlm.nih.gov/) and converted into 3D structure files using ChemBio3D Ultra. The core protein structure files were obtained from the PDB database (Table 1). After converting the drug structure files and protein structure files to pdbqt format, the molecular docking simulation software AutoDock was used to simulate the docking of molecules and targets, and the docking results were visualized using PyMOL software.

active ingredient	IL1B	BCL2	INS	TNF	CASP3	IL6
quercetin	-7.9	-7.9	-6.5	-7.6	-7.8	-7.0
wogonin	-7.5	-7.4	-7.2	-6.5	-8.1	-6.4
Beta-sitosterol	-7.2	-6.9	-6.2	-6.8	-8.4	-6.1
glycyrrhizin	-7.6	-9.5	-6.6	-7.6	-8.0	-7.2

Table 1: Binding energy between key active ingredients and core target molecules(kcal/mol)

## 4. Conclusions

This study used network pharmacology to explore the material basis and molecular mechanisms of Fangji Dihuang Decoction in treating Alzheimer's disease, finding that it acts through multiple pathways such as heme binding, tetrapyrrole binding, RNA polymerase II-specific DNA-binding transcription factor binding, DNA-binding transcription factor binding, antioxidant activity, to maintain the health of the nervous system.

The effective components of Fangji Dihuang Decoction in treating Alzheimer's disease include quercetin, glycyrrhizin, beta-sitosterol, and wogonin. Quercetin is a polyhydroxy flavonoid compound, chemically known as 3,3',4',5,7-pentahydroxyflavone. Its molecular structure contains a double bond between positions 2 and 3 and two hydroxyl groups, so it functions as a metal chelator or a free radical acceptor produced during oxidation processes such as lipids, i.e., it has antioxidant and free radical scavenging effects. It has antioxidant and free radical scavenging effects. It not only has antioxidant and free radical scavenging effects. It not only has antioxidant and free radical scavenging effects. [22]. Glycyrrhizin can reduce the activity of glycogen synthase kinase  $3\beta$  (GSK- $3\beta$ )through phosphorylation, enhance the expression of nuclear factor erythroid-2-related factor 2 (NRF2), reduce NF- $\kappa$ B response, inhibit neuroinflammation, and protect against cognitive

impairment and neuronal damage[23]. Glycyrrhizin mainly enhances memory and can be used to treat learning and memory disorders. Glycyrrhizic acid derivatives are acetylcholinesterase inhibitors with significant inhibitory activity, which can increase the level and duration of acetylcholine action, thereby alleviating the symptoms of Alzheimer's disease[24]. Beta-sitosterol is a tetracyclic triterpenoid compound with a fully hydrogenated phenanthrene as its core structure. Its molecular structure is similar to cholesterol, with the main differences being the structures at C-17 and C-24, leading to different physiological activities. Beta-sitosterol on the plasma membrane can affect the estrogen receptor to recruit PI3K in lipid rafts, thereby activating the PI3K signaling pathway, increasing the activity of PI3K, the expression of p-GSK3β, inhibiting oxidative stress induced by glucose oxidase, lipid peroxidation reaction, improving Alzheimer's disease caused by brain lipid peroxidation[25]. Beta-sitosterol can also bind to the mitochondrial membrane, increasing mitochondrial membrane fluidity without affecting the outer mitochondrial membrane, increasing mitochondrial membrane potential and ATP content, playing a role in alleviating neurodegenerative diseases. Wogonin has biological activities such as antiviral, antitumor, and neuroprotection. It can inhibit the proliferation and differentiation of lung cancer cells and promote apoptosis[26]. Studies have shown that wogonin can significantly reduce cell invasion and infiltration ability[27]. In summary, Fangji Dihuang Decoction can achieve the purpose of treating Alzheimer's disease through multiple synergistic active components, exerting anti-inflammatory, antioxidant, anti-anxiety effects, and improving brain and nerve functions.

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