Exploring the Mechanisms Underlying the Therapeutic Effect of Xixin Decoction on Alzheimer's Disease Based on Network Pharmacology and Molecular Docking

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Abstract

Background and objective: Alzheimer's disease (AD) has been a worldwide problem, not only the treatment but also the prevention. As a commonly used Chinese Herbal Formula, Xixin Decoction (XXD) has significant therapeutic effect on AD but without clear mechanism. This study was aimed to predict the main active compounds and targets of XXD in the treatment of AD and to explore the potential mechanism by using network pharmacology and molecular docking.

Methods: The compounds of XXD were searched in the TCMSP and the TCMID database, and the active compounds were screened based on the ADME model and SwissADME platform. SwissTargetPrediction platform was used to search for the primary candidate targets of XXD. The common targets related to AD obtained by two databases (GeneCards and DisGeNET) were determined as candidate proteins involved in AD. To acquire the related targets of XXD in the treatment of AD, the target proteins related to AD were intersected with the predicted targets of XXD. Then these overlapping targets were imported into the STRING database to build PPI network including hub targets; Cytoscape 3.7.2 software was used to construct the topology analysis for the herb-compound-target network diagram while one of it's plug-in called CytoNCA was used to calculate degree value to screen the main active compounds of XXD. GO and KEGG pathway enrichment analyses were conducted to explore the core mechanism of action and biological pathways associated with the decoction via Metascape platform. We used AutoDock Vina and PyMOL 2.4.0 softwares for molecular docking of hub targets and main compounds.

Results: We determined 114 active compounds which meet the conditions of ADME screening, 973 drug targets, and 973 disease targets. However, intersection analysis screened out 208 shared targets. PPI network identified 9 hub targets, including TP53, PIK3CA, MAPK1, MAPK3, STAT3, AKT1, etc. The 10 main active compounds play a major role in treatment of AD by XXD. Hub targets were found to be enriched in 10 KEGG pathways, involving the Pathways in cancer, AGE-RAGE signaling pathway in diabetic complications, Alzheimer's disease, Neuroactive ligand-receptor interaction, Dopaminergic synapse, Serotonergic synapse and MAPK signaling pathway. The docking results indicated that the 8 hub targets exhibit good binding activity with the 9 main active compounds of XXD.

Conclusions: We found the advantages of multi-compounds-multi-targets-multi-pathways regulation to reveal the mechanism of XXD for treating AD based on network pharmacology and molecular docking. Our study provided a theorical basis for further clinical application and experimental research of XXD for anti-AD in the future.

Introduction

Alzheimer's disease (AD) is a neurodegenerative disease, which is the commonest cause of dementia. The primary clinical features of dementia are deficiency of activity of daily living, abnormal behavior and cognitive impairment. The disease's growing high incidence rate and its pathogenesis which has not yet been fully elucidated are global public health concerns for not only individuals and caregivers but also the whole society. Current surveys estimate that there are over 50 million people living with dementia worldwide at present[1]. The number of new AD cases all around the word is estimated to reach 100 million by 2050[2].Therefore, the prevention and treatment of AD are a worldwide problem to be solved. The pathological features of AD are neurodegeneration, neuron damage and apoptosis, formation of senile plaques involving the amyloid-β(Aβ) accumulation, neurofibrillary tangles involving hyperphosphorylation of the tau protein, as well as neuroinflammation, abnormal insulin signaling pathway and brain insulin resistance[3]. However, studies that target on Aβ and the tau protein to treat AD didn't show satisfactory results. Current drugs, such as Donepezil and Memantine, are not effective enough to meet clinical needs. Therefore, it is necessary to study the treatment of AD from the perspective of multi-target therapy.

Traditional Chinese medicine (TCM) has been used in treating AD which can be traced back to the traditional Chinese medicine book "TSO Chuan" for thousands of years and previous dynasties Chinese medicine physicians have

accumulated a tremendous amount of significant clinical evidences. The advantages of TCM in the treatment of AD are as follows:(1)TCM treatment improves patients' cognitive function and make it last longer; (2)TCM aims to some physical symptoms that are difficult to solve with western medicine, such as behavioral and psychological symptoms of dementia(BPSD),loss of appetite, insomnia, constipation;(3)TCM has less side effects than western medicine on liver, kidney, stomach and other organs. (4) Multiple targets of TCM act on diseases through various pathways.

XXD comes from "Syndrome Differentiation Record" written in Qing Dynasty. It has the effect of opening depression, expelling phlegm and invigorating the stomach, consisting of Renshen(Panax ginseng), Fushen(Poria cum Radix Pini), Suanzaoren(Ziziphus jujuba Mill), Banxia(Pinellia ternate), tangerine peel(Citrus reticulata Blanco), Medicated Leaven (Massa Medicata Fermentata), licorice(Glycyrrhiza uralensis Fisch), aconite(Aconitum carmichaeli Debx), Acorus calamus(Acorus tatarinowii). The formula has been mainly used to treat dementia. A large number of experiments have found that XXD can prevent SAD(sporadic Alzheimer's disease) pathological progress by inhibiting hyperphosphorylation at the key sites of tau proteins[4], enhancing O-GlcNAc glycosylation of tau proteins[5], improving the expressions of synaptic functional proteins and receptors[6], repairing mitochondrial function and increasing the survival rate of $A\beta_{1-42}$ induced cells[7]. No report has systematically analyzed the constituents of XXD and its potential mechanism of action in treating dementia so far.

As an interdisciplinary product of traditional pharmacology, molecular biology, bioinformatics and other disciplines, network pharmacology has broken through the traditional concept of "single target-single pathway" drug research. Its holistic and systematic characteristics coincide with TCM holistic view, the principle of syndrome differentiation and treatment, and the compatibility of prescriptions. It provides a new idea and method for elucidating the specific mechanism of XXD in the treatment of AD. The purpose of this study is to explore the main material basis, core target and potential mechanism of XXD's action in treating AD using the network pharmacology method and molecular docking. The study design flowchart was shown in Fig. 1, the active compounds and intersection genes for AD were collected at first. Then, the protein-protein interaction (PPI) and Pathway-target-compound network were constructed. In addition, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis were performed. Furtherly, the potential main active compounds and core targets were validated by the molecular docking simulation.

Materials And Methods

Screening of the active compounds of XXD

Traditional Chinese Medicine System Pharmacology Database (TCMSP) is a systems pharmacology platform of Chinese herbal medicines that captures the relationships between drugs, targets and diseases[8]. It includes chemicals, targets and drug-target networks, and associated drug-target-disease networks, as well as pharmacokinetic properties for natural compounds involving oral bioavailability, drug-likeness, intestinal epithelial permeability, blood-brain-barrier, aqueous solubility and etc. Traditional Chinese Medicine Integrated Database (TCMID) is a comprehensive database to provide information and bridge the gap between traditional Chinese medicine and modern life sciences, having collected information on all respects of TCM including formulae, herbs and herbal ingredients[9]. It has also contained information for drugs, diseases which are deeply studied by modern pharmacology and biomedical sciences. We searched TCMSP and TCMID to collect the pharmacologically active compounds of XXD. ADME (absorption, distribution, metabolism, and excretion) screening model is an effective model to screen the main active ingredients of herbs in the TCMSP database by using pharmacokinetic parameters such as oral bioavailability (OB), druglikeness (DL) and blood brain barrier (BBB). OB is defined as the rate of active ingredient being absorbed from a drug product and becoming available at the site of action and DL is a qualitative concept describe the optimize pharmacokinetics and molecular properties[10]. In this study, active compounds were screened according to ADME features with OB $\geq$ 30%, DL

≥ 0.18 and BBB≥-0.3. 113 compounds were obtained after removing the overlapping compounds among the 9 herbs of XXD and they were used to the subsequent network pharmacology study.

Prediction Of Targets Of Active Compounds

SwissTargetPrediction database can predict compound targets according to 2D and 3D structures of known compounds[11]. The targets of XXD's active compounds were predicted from this database and the probability value ≥ 0 served as the target screening standard. "Common name" in the information table refers to gene symbol.

Collection Of Targets For Ad

We screened AD-related genes by using DisGeNET[12] and GeneCards[13]. DisGeNET is a comprehensive discovery platform containing publicly available collections of genes and variants associated to human diseases. GeneCards is a searchable, integrative database that provides predicted human genes and user-friendly information, including genomic, transcriptomic, proteomic, genetic, clinical and functional information. We searched AD-related targets from these two databases using "Alzheimer's Disease" as keyword. In GeneCards database, the higher the score is, the closer the target is related to the disease. As a rule of thumb, if there are too many targets, the targets with score greater than the median score is set as the potential target of AD. After combining the two databases' targets and deleting the repeated targets, we got the AD target.

Target Mapping

To acquire the candidate targets responsible for AD therapy, the target proteins related to AD were intersected with the predicted targets of XXD. For overlapping targets, numbers were visualized with a Venn diagram plotted by http://www.bioinformatics.com.cn, an online platform for data analysis and visualization.

Protein-protein Interaction (Ppi) Analysis

The overlapping targets were then imported into STRING[14], a database that predict interactions including direct (physical) and indirect (functional) associations, to build protein-protein interaction(PPI) network which would be used for the subsequent topological properties analysis to identify hub targets in treatment of AD, and the targets were selected with the highest confidence score (low: < 0.4; medium:0.4 to 0.7; high: > 0.7; highest :> 0.9).

Go And Kegg Pathway Enrichment Analysis

Metascape[15] is a web-based portal designed to provide a comprehensive gene list annotation and analysis resource for experimental biologists. It combines functional enrichment, interactome analysis, gene annotation, and membership search and facilitates comparative analyses of datasets across multiple independent and orthogonal experiments. The candidate targets' gene symbols of XXD responsible for AD therapy were imported into Metascape platform, setting species as "Homo sapiens", and then the Gene Ontology (GO)biological process enrichment and Kyoto Encyclopedia of Genes and Genomes(KEGG) enrichment were analyzed and enriched[16].The smaller modified fisher exact p-value, the more enriched.

Network Construction

Cytoscape 3.7.2 software was used to construct the following networks and topology analyses: (1) The herb-compound-target network of XXD; (2) The network of shared targets between XXD's active compounds targets and AD targets; (3) The network of shared targets and their corresponding compounds;(4) The pathway- target-compound network of XXD for treating AD. A plug-in of Cytoscape called CytoNCA was used to calculate parameter to screen the main active compounds of XXD and the hub targets of shared targets. Degree[17], betweenness centrality(BC),closeness centrality(CC) and Local average connectivity-based method(LAC)[18] are the common topology parameters for evaluating the central properties of nodes in a network. The main active compounds were screened through the degree value size sorting. The screening parameter settings of hub targets of the XXD-AD target interaction network are as follow: The first screening threshold was Degree ≥ twofold median, the second threshold was Degree ≥ median, BC ≥ median, CC ≥ median and LAC ≥ median. The higher the values are, the more significant the nodes are. The screened hub target genes are used for further molecular docking study.

Molecular Docking Technology

We investigated the possible interaction activity between hub genes and main active compounds of XXD with molecular docking. Meanwhlie, the hub targets and main active compounds were further screened by the docking affinity values or reported by AutoDock Vina. There were 72 pairs delivered into the docking simulation (Table 4). The scoring function of AutoDock and AutoDock Vina stipulates that when the docking affinity values < 0, the ligand and the receptor molecule can bind stably, and the greater the absolute value, the stronger the binding stability between the compounds and the active site of the hub targets. According to the docking results, most binding complexes possessed high binding affinity values with an average of − 9 kcal/mol. Besides, there is at least one hydrogen bond formation between ligand of every target and residues of active compounds. And most hydrogen bond interactions were considered as strong interaction with the average distance of 3.4, also recorded in Table 4. 9 pairs docking models with hydrogen bond and their affinity are shown in Fig. 9. Over all, molecular docking indicated that all XXD's active compounds could easily enter and bind the active pocket of the hub targets well.

Table 4
Information for molecular docking of

Results

Active compounds of XXD

XXD compounds were retrieved from the TCMSP and TCMID database and after the screening with OB ≥ 30%, DL ≥ 0.18 and BBB≥-0.3, 114 active compounds were obtained after deleting repetitions. The details besides common active compounds of different herbs are shown in the Supplementary file, Table S1.

Potential Targets

We gained a total of 973 XXD target genes from SwissTargetPrediction and 973 AD targets from DisGeNET and GeneCards, respectively showed in Table S2 and Table S3. All the targets' names were put into UniProtKB to be converted to gene symbol. Then, we obtained 208 shared targets by further integrating the potential XXD-target genes and AD-related genes(Fig. 2and Table S4).

Ppi Analysis Of Shared Targets

The PPI network of shared targets containing 174 nodes constructed by Cytoscape 3.7.2 software, after selected with the highest confidence score 0.9 in the STRING database, depicts the interactions between them identified, as shown in Fig. 3A. The hub targets were screened by plug-in CytoNCA through calculating four topological features by two steps for each node in the network. The first screening threshold was Degree ≥ 14 median, which resulted in 28 nodes and 161 edges. Then these 28 nodes were screened with the second threshold of Degree ≥ 22.5, BC ≥ 0.04, CC ≥ 0.44 and LAC ≥ 5.7, ultimately remained nine hub targets including TP53, PIK3CA, MAPK1, MAPK3, STAT3, AKT1, NFKB1, EGFR and HSP90AA1(Table 1 and Fig. 3B).

Table 1
Information of 9 hub targets

Uniprot ID	Gene Symbol	Description	Degree
P04637	TP53	Tumor Protein P53	39
P42336	PIK3CA	Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha	37
P28482	MAPK1	Mitogen-Activated Protein Kinase 1	37
P27361	MAPK3	Mitogen-Activated Protein Kinase 3	33
P40763	STAT3	Signal Transducer And Activator Of Transcription 3	31
P31749	AKT1	AKT Serine/Threonine Kinase 1	29
P07900	HSP90AA1	Heat Shock Protein 90 Alpha Family Class A Member 1	29
P00533	EGFR	Epidermal Growth Factor Receptor	25
P19838	NFKB1	Nuclear Factor Kappa B Subunit 1	24

Networks Construction

The information of 9 herbs,114 active compounds and 970 XXD targets were imported into Cytoscape3.7.2 software and the herb-compound-target network of XXD was constructed(Fig. 4); The network of 208 shared targets, their corresponding compounds and herbs are shown in Fig. 5 with details in Table S5, while using Network Analyzer to calculate degree and sorting 10 main active compounds shown in Table 2 and Fig. 6;

Table 2
Information of 10 main active compounds in XXD

Molecular Name	Mol ID	Label	Degree	Source Herb
licochalcone a	MOL000497	GC49	43	Gancao
Gomisin B	MOL005357	RS9	43	Renshen
7-Methoxy-2-methyl isoflavone	MOL003896	GC8	42	Gancao
baicalein	MOL002714	BX4	40	Banxia
Glypallichalcone	MOL004835	GC20	40	Gancao
zizyphusine	MOL001546	SZR6	40	Suanzaoren
7-Acetoxy-2-methylisoflavone	MOL004991	GC56	39	Gancao
Karanjin	MOL002398	FZ4	38	Fuzi
Girinimbin	MOL005356	RS8	38	Renshen
6-prenylated eriodictyol	MOL004989	GC55	37	Gancao

Go Analysis

The gene ontology (GO) enrichment analysis was consisted of BP (biological process), CC (cellular component), and MF (molecular function). We used GO to further elucidate the significance of overlapping genes targets. As shown in the results of the enrichment, the top 10 GO items are selected based on counts of hit genes and P values (Fig. 7A). For biological processes, the targets were mainly enriched in cellular response to nitrogen compound (GO:1901699, GO:0010942), positive regulation of kinase activity (GO:0033674, GO:0051347, GO:0043408), cellular response to organonitrogen compound (GO:0071417) and transmembrane receptor protein tyrosine kinase sign (GO:0007169). Additionally, the rest functional annotations are associated with response to inorganic substance (GO:0010035) and behavior (GO:0099537, GO:0099536). XXD effected AD by regulating three principal CC, namely, dendrite, membrane raft and postsynapse, which might reflect that most ingredients of XXD were targeted to neural cells. Protein kinase activity, kinase binding, endopeptidase activity and protein domain specific binding were ranked as top four MF.

Kegg Pathway Enrichment Analysis

We conducted KEGG pathway enrichment of 208 shared targets to determine the potential therapeutic mechanism of the XXD for AD. Based on the log10(P value), the bubble plot of the most significant 20 KEGG pathways was shown in Fig. 7B. We sorted top 10 pathways including Pathways in cancer (hsa05200), AGE-RAGE signaling pathway in diabetic complications (hsa04933), Alzheimer's disease (hsa05010), Toxoplasmosis (hsa05145), Neuroactive ligand-receptor interaction (hsa04080), Dopaminergic synapse (hsa04728), Serotonergic synapse (hsa04726), MAPK signaling pathway (hsa04010), Viral carcinogenesis (hsa05203) and Transcriptional misregulation in cancer(hsa05202) with details in Table 3. The pathway maps of the Alzheimer's disease and MAPK signaling pathway were illustrated in Fig. 7(C, D). Then we constructed a pathway- target-compound network based on the shared targets enriched in each pathway (Fig. 8).

Table 3
Information for top 10 KEGG pathways

GO	Description	Count	Corresponding hub genes	P Value
hsa05200	Pathways in cancer	45	AKT1,EGFR,HSP90AA1,NFKB1,PIK3CA,MAPK1,MAPK3,STAT3, TP53	2.0893E-37
hsa04933	AGE-RAGE signaling pathway in diabetic complications	25	AKT1,NFKB1,PIK3CA,MAPK1,MAPK3,STAT3	4.57088E-30
hsa05010	Alzheimer's disease	28	MAPK1,MAPK3	6.76083E-28
hsa05145	Toxoplasmosis	21	AKT1,NFKB1,MAPK1,MAPK3,STAT3	2.29087E-22
hsa04080	Neuroactive ligand-receptor interaction	25	no	1.65959E-18
hsa04728	Dopaminergic synapse	17	AKT1	1.23027E-15
hsa04726	Serotonergic synapse	16	MAPK1,MAPK3	2.39883E-15
hsa04010	MAPK signaling pathway	21	AKT1,EGFR,NFKB1,MAPK1,MAPK3, TP53	6.60693E-15
hsa05203	Viral carcinogenesis	18	NFKB1,PIK3CA,MAPK1,MAPK3,TP53	1.44544E-13
hsa05202	Transcriptional misregulation in cancer	17	NFKB1,TP53	2.88403E-13

9 hub genes binding to main active compounds

Hub gene	PDB ID	Ligand	Main active compound	Docking affinity (kcal/mol)	hydrogen bond distance
TP53	6GGB	EXQ	Gomisin B	-5.8	
			licochalcone a	-7.3	
			7-Methoxy-2-methyl isoflavone	-7.2	
			zizyphusine	-6.2	
			Glypallichalcone	-6.7	
			baicalein	-7.9	3.3
			7-Acetoxy-2-methylisoflavone	-7.3	
			Girinimbin	-7.8	
			Karanjin	-7.6	
PIK3CA	4L23	X6K	Gomisin B	-5.6	
			licochalcone a	-7.5	
			7-Methoxy-2-methyl isoflavone	-6.9	
			zizyphusine	-6.8	
			Glypallichalcone	-7.3	3.5and3.0
			baicalein	-7.9	
			7-Acetoxy-2-methylisoflavone	-7.3	
			Girinimbin	-9	
			Karanjin	-8.2	3.2
MAPK1	5BUE	4V8	Gomisin B	-6.3	3.3
			licochalcone a	-8.2	
			7-Methoxy-2-methyl isoflavone	-8.1	
			zizyphusine	-8.2	
			Glypallichalcone	-7.4	
			baicalein	-8.1	
			7-Acetoxy-2-methylisoflavone	-8.3	
			Girinimbin	-9.2	
			Karanjin	-8.5	
MAPK3	4QTB	38Z	Gomisin B	-5.2	

Hub gene	PDB ID	Ligand	Main active compound	Docking affinity (kcal/mol)	hydrogen bond distance
			licochalcone a	-8.6	3.4
			7-Methoxy-2-methyl isoflavone	-9.6	
			zizyphusine	-9.6	
			Glypallichalcone	-8.5	
			baicalein	-9.5	
			7-Acetoxy-2-methylisoflavone	-8.8	
			Girinimbin	-10	
			Karanjin	-9.8	
STAT3	5AX3	5ID	Gomisin B	-5.8	
			licochalcone a	-7.2	
			7-Methoxy-2-methyl isoflavone	-7.3	
			zizyphusine	-7.5	
			Glypallichalcone	-6.7	
			baicalein	-7.7	2.9and2.2
			7-Acetoxy-2-methylisoflavone	-7.4	
			Girinimbin	-8.3	
			Karanjin	-7.6	
AKT1	6HHH	G4Q	Gomisin B	-5.9	
			licochalcone a	-8.9	
			7-Methoxy-2-methyl isoflavone	-9.5	
			zizyphusine	-9.2	
			Glypallichalcone	-8.6	
			baicalein	-9.7	3
			7-Acetoxy-2-methylisoflavone	-10	
			Girinimbin	-10.5	
			Karanjin	-10.4	
HSP90AA1	2YKJ	YKJ	Gomisin B	-8	
			licochalcone a	-9.9	

Hub gene	PDB ID	Ligand	Main active compound	Docking affinity (kcal/mol)	hydrogen bond distance
			7-Methoxy-2-methyl isoflavone	-10.1	
			zizyphusine	-8.8	
			Glypallichalcone	-8.6	
			baicalein	-9.7	
			7-Acetoxy-2-methylisoflavone	-10	
			Girinimbin	-11	
			Karanjin	-9.8	3.4
EGFR	5X2C	7XR	Gomisin B	-5.5	
			licochalcone a	-8	
			7-Methoxy-2-methyl isoflavone	-7.7	
			zizyphusine	-8.8	
			Glypallichalcone	-7.4	
			baicalein	-8.5	
			7-Acetoxy-2-methylisoflavone	-8.4	3.5
			Girinimbin	-9.4	
			Karanjin	-9	
NFKB1		no			

Discussion

AD is a complicated neurodegenerative disease characterized by a progressive loss of cognitive function resulted from many etiological factors that causes and pathogenesis are not clear. So, combination treatments rather than monotherapy are warranted for the development of effective approaches in treating AD. The unique advantages of TCM such as naturally sourced, multitargeted, individual mode and a holistic concept are obvious in treating complicated diseases especially those with a poor efficacy to western medicine alone. In the theory of TCM, AD is mainly caused by old and weak with deficiency of healthy Qi as a result of endogenesis of turbid phlegm, blinding the clear orifices. The basic pathogenesis is deficiency of origin and excess of sign. XXD is a classical formula which is used to resolve phlegm, induce resuscitation and nourish the heart to calm the mind. XXD has been used to treat dementia clinically for a long history from hundreds of years ago in China. But it is undeniable that the complexity of compounds and compatibility in the use of TCM, as well as the dynamic process of the development of chronic and complex diseases, bring great challenges to the research of TCM. With the continuous development of bioinformatics, TCM network pharmacology has become a topic on hot spots to reveal above mentioned complex biological processes from the perspective of integrated multi-component networks[22].There are few studies on the mechanism of XXD in the

prevention and treatment of AD. To reveal the beneficial effects of XXD on AD, we used network pharmacology approach to explore the putative active compounds and potential mechanisms.

In our study, we constructed the following network to reveal the potential targets and pathways of XXD in AD treatment: compound-target network, PPI network of shared targets-corresponding compounds and compound-hub target -pathway network. After integrating and consolidating information from diverse sources of available databases, 10 active compounds of XXD acted on 208 different targets associated with AD. According to the shared targets-corresponding compounds network,10 compounds and 208 shared targets are highly connected and they can be defined as vital compounds in XXD. In XXD, according to the TCM theory in formula prescribing, the Jun or “monarch herb” is Pinellia ternate (Banxia), exerting the effects of resolving phlegm. The Chen or “minister herbs” consist of Panax ginseng (Renshen) and Aconitum carmichaeli Debx(Fuzi), with the function of tonifying Qi and Warming and activating Yang Qi, respectively. In addition, Glycyrrhiza uralensis Fisch (Gancao), playing the role as Zuoshi or “adjuvant and guide herb”, helps tonifying Qi and harmonizing various herbs. In our study, three of main active compounds including Gomisin B, baicalein and Karanjin, were identified from the “monarch herbs”, the “minister herbs” and the“adjuvant and guide herb”, respectively. This prescription feature might provide the pharmacological evidences for XXD to be used as multi-target regulation in treating AD.

Moreover, according to the pathway- target-compound network, components in XXD such as licochalcone a, Gomisin B,7-Methoxy-2-methyl isoflavone, baicalein, Glypallichalcone, zizyphusine,7-Acetoxy-2-methylisoflavone, Karanjin, Girinimbin and 6-prenylated eriodictyol interacted with a large number of targets, indicating the important roles in the anti-AD system. Some of them have been reported to show biological activities against AD in the past. For example, schisantherin B(Gomisin B) identified in Renshen was able to protect against learning and memory impairments and remit the toxicity caused by excessive activation of hyperphosphorylated tau in the dementia mouse model induced by $A\beta_{1-42}$ through regulating glial glutamate transporter type 1 (GLT-1), which has an effect on the level of p-tau protein[23]. Baicalein in Banxia was proved to show therapeutic potential for AD through reducing oxidative stress, anti-inflammatory properties, inhibiting aggregation of amyloid proteins, stimulating neurogenesis and differentiation action and anti-apoptosis effects[24].A research showed that karanjin in Fuzi possesses learning and memory improvement [25].

According to GO enrichment analysis, BP terms enriched by target genes were mainly concentrated in neurodegeneration-related progressions such as positive regulation of kinase activity, positive regulation of cell death and synaptic signaling. Through KEGG enrichment analysis, we found that the mechanisms of XXD in treatment of AD mainly include Alzheimer's disease pathway, neuroactive ligand-receptor interaction pathway, serotonergic synapse and MAPK signaling pathway.

The Alzheimer's disease pathway with a high degree, containing insulin signaling pathway with hub targets such as PIK3CA and AKT, is thought to be tightly associated with the effect of XXD on AD. Previous research indicated that insulin signaling pathway depicts a strong correlation of energy utilization, mitochondrial function, oxidative stress, synaptic plasticity and cognitive function and the occurrence and development of AD is partially attributed to the impairment of insulin signaling pathway[26].

Some receptor genes control the neuroactive ligand-receptor interaction pathway, which regulates learning and memory ability through enhancing cholinergic function[27], and may participate in cognitive and emotional regulation[28]. The abnormal function of $GABA_A$ (γ-aminobutyric acid A)receptor, one of the important receptors of neuroactive ligand-receptor interaction pathway, is closely associated with AD[29].

Serotonergic synapse is one of the primary pathological factors in neuropsychiatric symptoms[30, 31]. Previous researches provided sufficient evidence that serotonergic synapse pathway are likely to play a role in memory

dysfunction or AD[32, 33].

Mitogen-Activated Protein Kinase (MAPK) pathways, including the Extracellular Signal-Regulated Kinase (ERK), c-Jun N-terminal kinase (JNK) and p38 pathways, play the critical roles in regulating cellular functions such as cell apoptosis, synapse plasticity, neural cell survival and neuroinflammation. All the MAPKs are activated in related neurons in patients with AD, suggesting that MAPK pathways are involved in AD pathophysiology and pathogenesis[34]. Furthermore, inhibition of these pathways depressed multiple pathological features including Aβ production, tau hyperphosphorylation and synaptic loss, and also made the degeneration of cognitive function in AD animal models[35, 36]. Among the potential active ingredients, baicalien was demonstrated to alleviate neurotoxicity in $A\beta_{25-35}$-induced PC12 cells via the increased expression of MAPK pathway, suggesting its neuroprotective effect [37].

We performed molecular docking simulation between 8 hub target genes and 9 main active compounds to verify the importance of hub targets. The results showed that all of the target-compound pairs possess high scores of docking affinity, especially, TP53, PIK3CA, AKT1. Previous studies have shown that these genes regulate multiple functions contributing to AD progression. As a downstream effector of DAPK-1 and a Ca^{2+}/calmodulin (CaM)-dependent serine/threonine protein kinase pathways ,TP53 plays an important role in cell apoptosis involving transcriptional induction of proapoptotic genes which consequently trigger mitochondrial pathways.TP53 has been suggested as potential therapeutic targets of AD because of its correlation with AD-related neurodegeneration in the brain[38]. Akt1, a cell survival kinase, mediate Akt1 signaling through its oxidative modification, occurring in the APP/PS1 transgenic mouse model of AD. The loss of Akt1 kinase lead to potential synaptic dysfunction in neurodegeneration including AD[39]. PIK3CA, a catalytic subunit of PI3K, plays a critical role in PI3K/AKT signaling pathways and affects the regulation of the pathogenesis of AD via inhibition of tyrosine phosphatases to protective against Aβ toxicity[40].

Conclusion

AD treatments have been an intractable problem up till now, but TCM may be a potential alternative treatment to a certain extent. XXD has been reported to play a protective effect through various mechanisms in AD in the past. For the first time, this study systematically illustrated the mechanism of action of XXD anti-AD using network pharmacology. Besides, this potential mechanism is verified and visualized through molecular docking. In the present study, 10 main compounds of XXD anti-AD are identified. Moreover, TP53, PIK3CA, MAPK1, MAPK3, STAT3, AKT1, etc., are the key genes enriched to pathways (e.g apoptosis, synapse plasticity and neuroinflammation) for XXD to exert its anti-apoptosis and synapse protect effect on AD. Our research not only contributed to deepening the understanding of multi-compounds-multi-targets-multi-pathways regulation of XXD against AD, but also provided a theorical basis for further experiments to unravel the above possibility.

Declarations

Authors' contributions

Jing Shi responsible for conception and design; Zhuo Zhang and Jiang-lin Xu were responsible for development of the methodology and collected and analyzed the data; Ming-qing Wei, Jing-nian Ni and Ting Li provided useful suggestions on the methodology; Zhuo Zhang wrote the paper; Zhuo Zhang and Jiang-lin Xu contributed equally to this work, and they should be regarded as co-first author; All authors read and approved the final version of the manuscript.

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Availability of data and materials

All data are available in the manuscript and the they are showed in tables, figures, and supplemental files.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

There is no conflict of interest declared.

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Figures

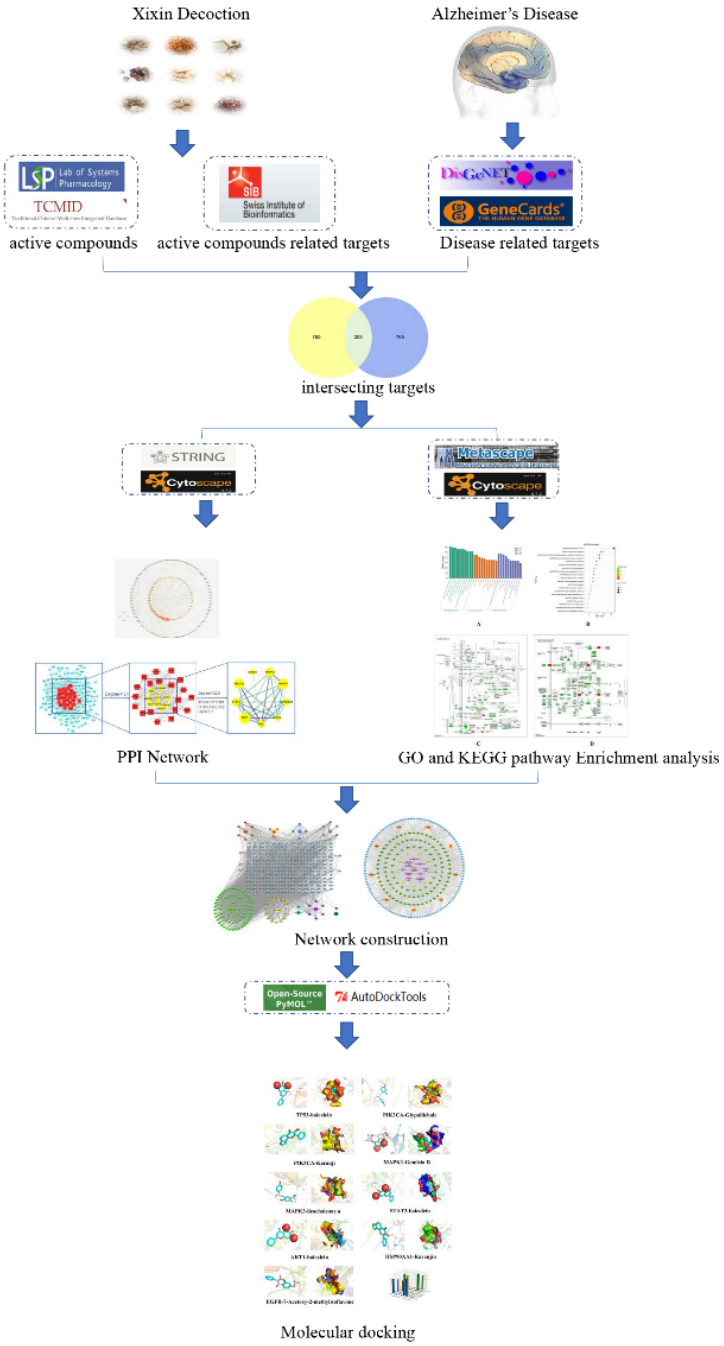

Figure 1

The study design flowchart

Figure 2

Venn diagram of intersecting targets of XXD and AD. The yellow part represents 765 potential targets of XXD. The blue part represents 765 AD-related targets. The light green part represents overlapped targets between XXD potential targets and AD targets.

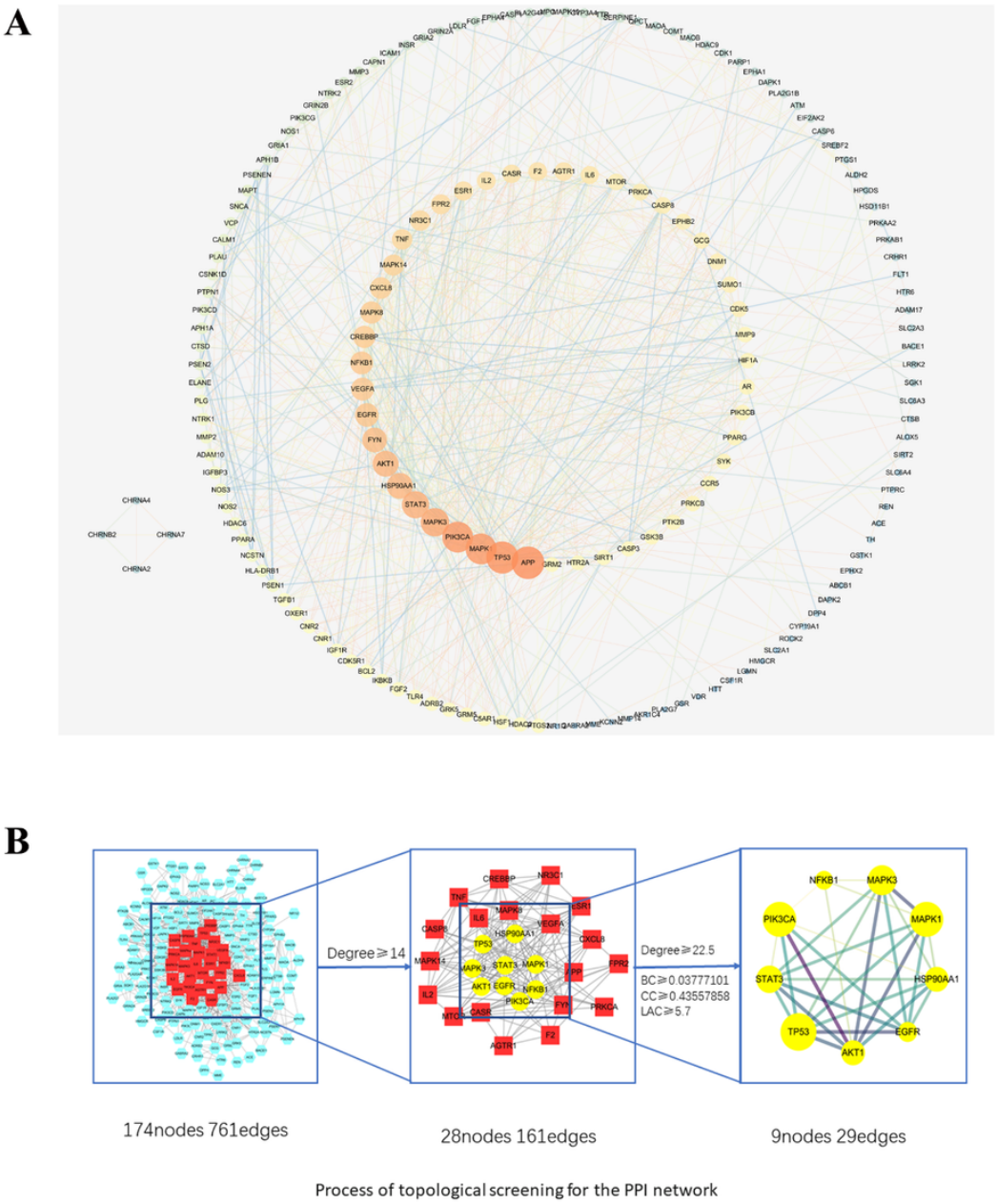

Figure 3

XXD potential target-AD target network. (A) PPI Network of shared targets. The sizes and colors of the nodes are illustrated from big to small and blue to orange in descending order of degree values. (B) The whole screening process for the PPI network through a topological method. In the third image, the bigger size represents higher degree value.

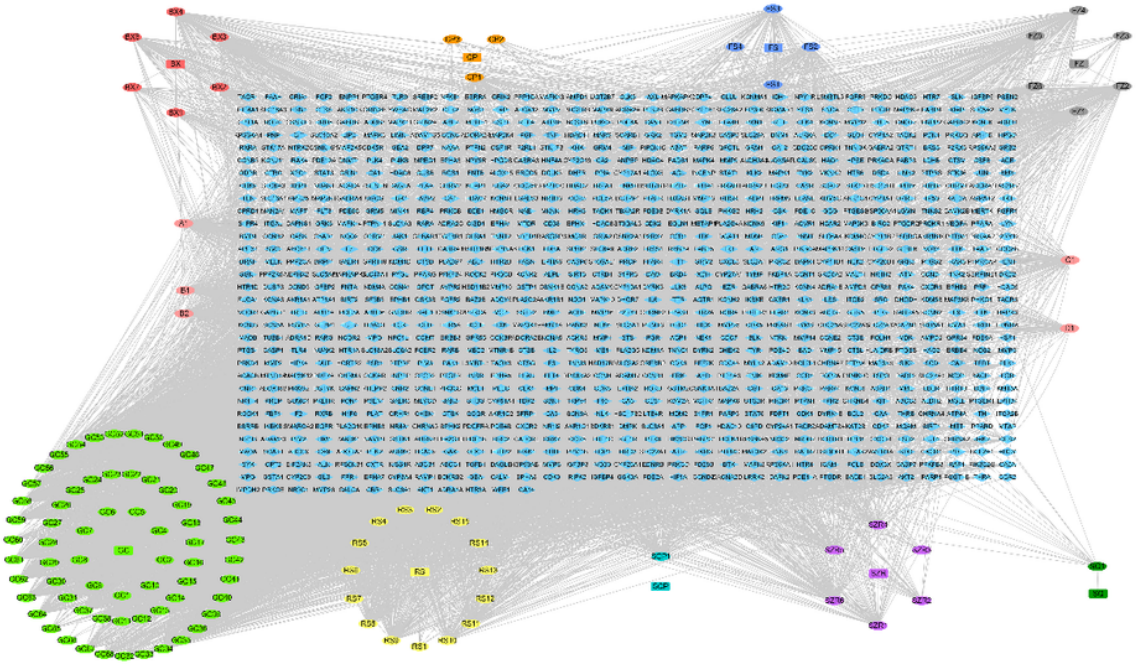

Figure 4

Herb-compound-target network of XXD. The diamond-shaped blue nodes with rectangular distribution in the middle represent the potential targets of XXD. The elliptical nodes distributed in concentric circles above and below in different colors represent the active compounds of XXD and rectangular center of the 9 concentric circles represent 9 herbs of XXD. The five independent elliptical nodes represent the common active compounds of different herbs.

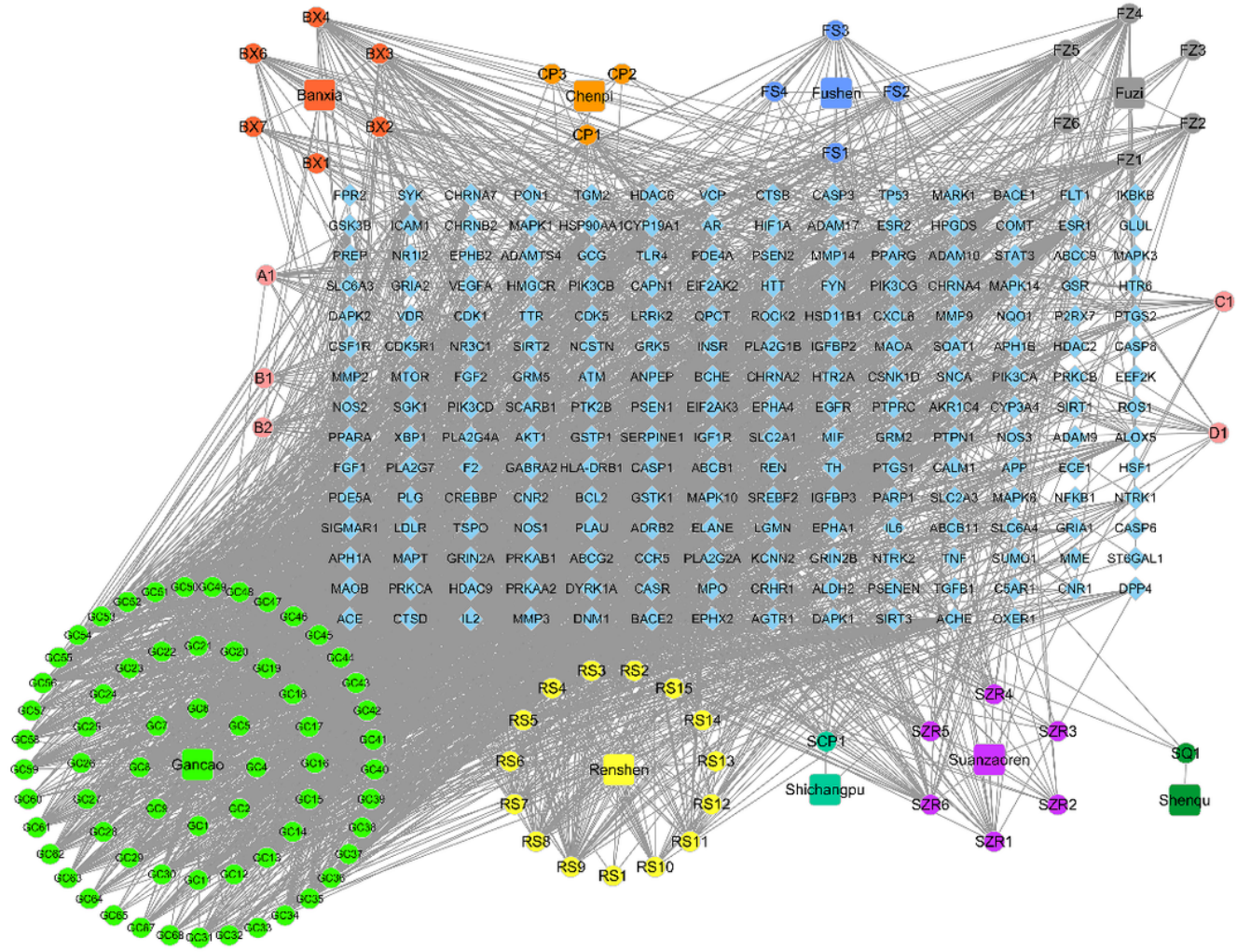

Figure 5

Herb-compound-target network of AD in treatment of XXD. The diamond-shaped blue nodes with rectangular distribution in the middle represent the potential targets of AD in treatment of XXD. The elliptical nodes distributed in concentric circles above and below in different colors represent the targets' corresponding compounds and herbs. The five independent elliptical nodes represent the common active compounds of different herbs.

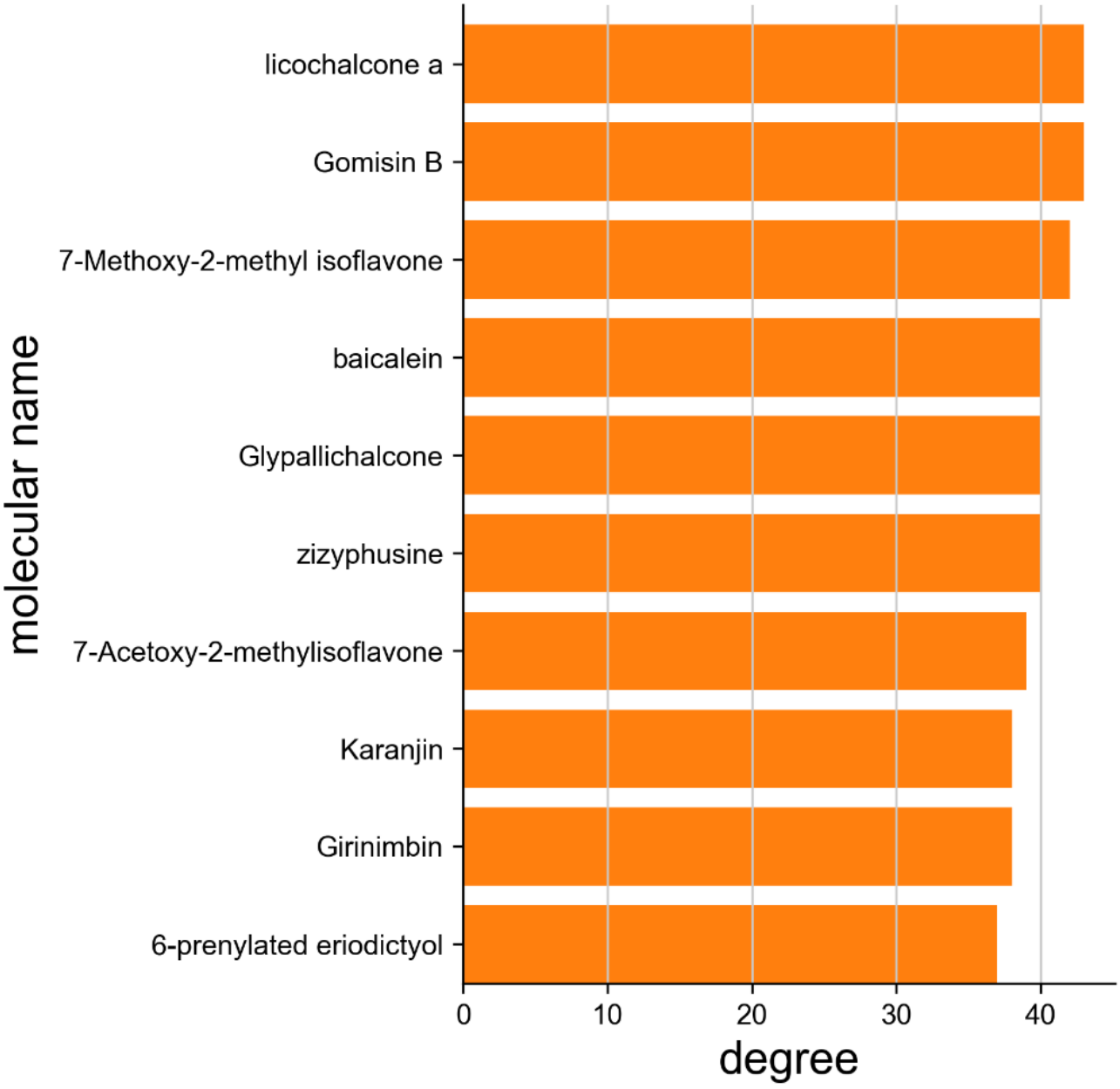

Figure 6

Bar chart of 10 main active compounds of XXD treating AD. The length of the bar graph represents its degree values.

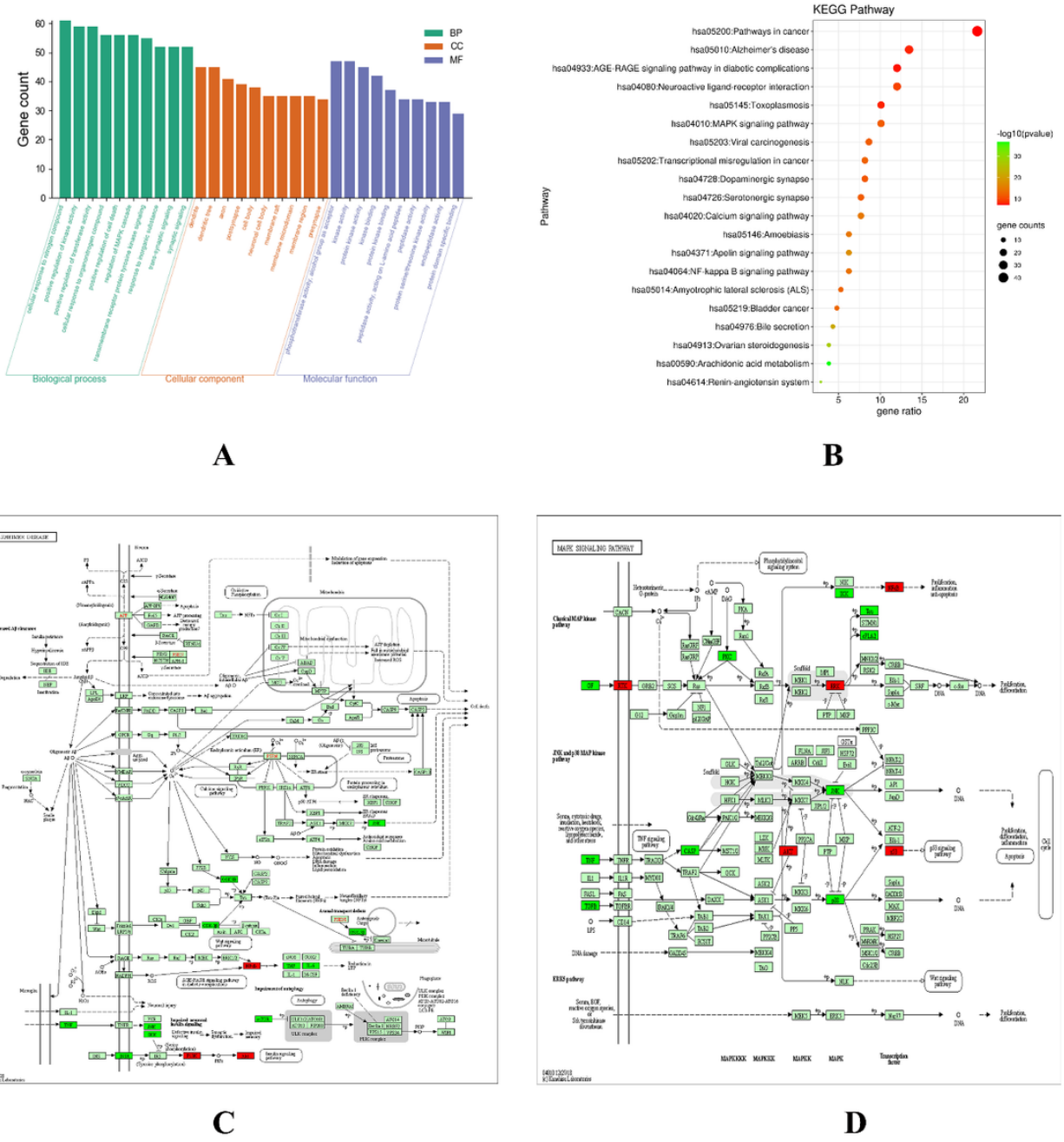

Figure 7

Enrichment analysis of shared targets. (A) GO enrichment analysis. The top 10 terms of each part are shown. BP: biological processes, CC: cell component, MF: molecular function. (B) KEGG pathway analysis. Gene ratio refers to the ratio of enriched genes to all of the shared target genes. Counts refer to the number of the enriched genes. The sizes of the bubbles are illustrated from big to small in descending order of the number of the potential targets involved in the pathways, the colors of the bubbles are illustrated from red to green in descending order of P values. (C) Pathway map of Alzheimer's disease as one of the most significant enriched pathways. The red nodes represent hub targets of SP and TAO targets, the bright green nodes represent other targets in Alzheimer's disease pathway. (D) Pathway map of MAPK signaling pathway as one of the most significant enriched pathways. The red nodes represent hub targets of SP and TAO targets, the bright green nodes represent other targets in MAPK signaling pathway.

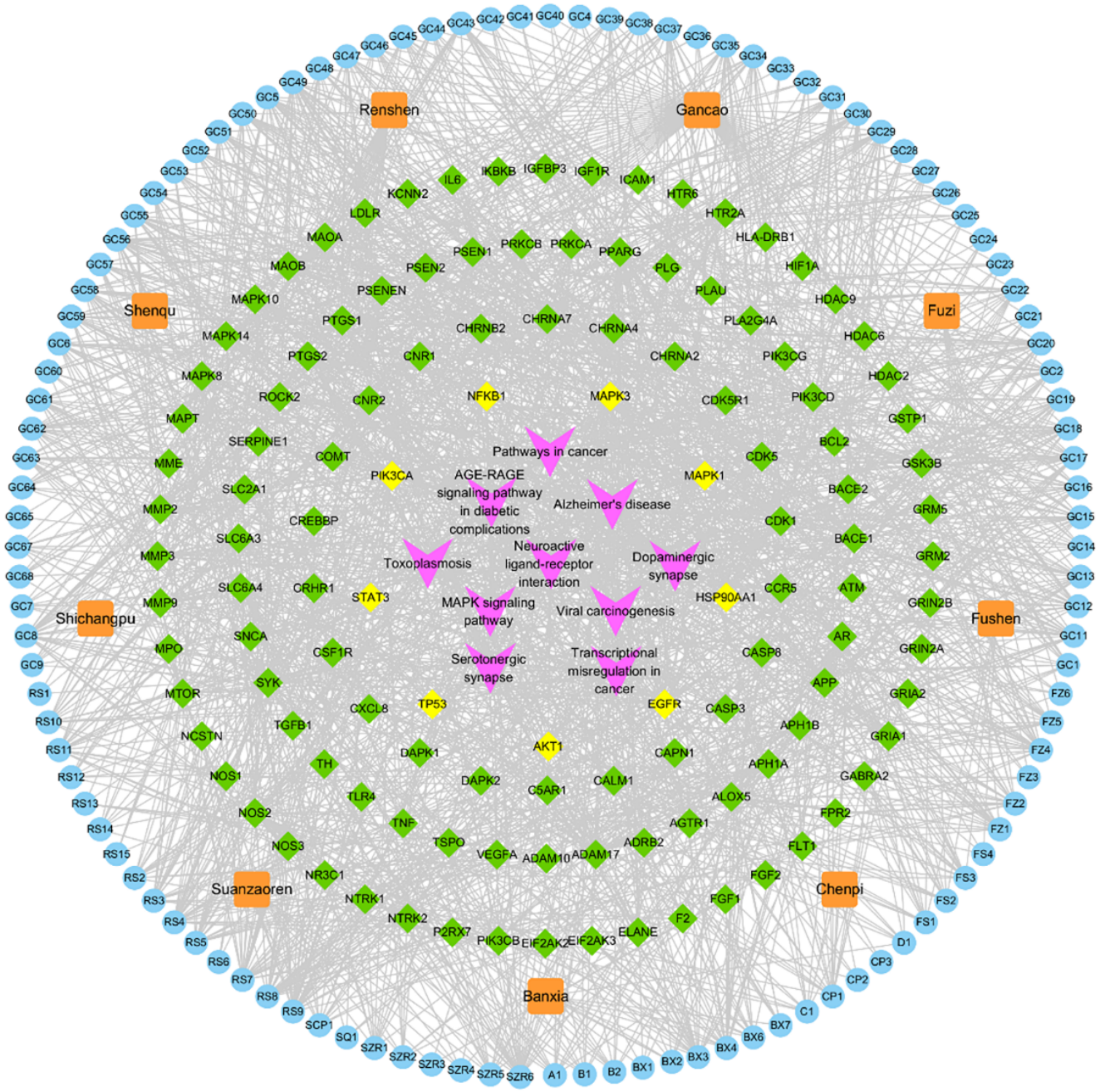

Figure 8

Pathway-target-compound network. Purple V-shaped nodes represent the most significant 10 potential pathways, yellow diamond nodes represent the 9 hub targets ,the other green diamond nodes represent the other corresponding targets , blue circular nodes represent corresponding active compounds of XXD and orange round rectangle nodes represent source herbs.

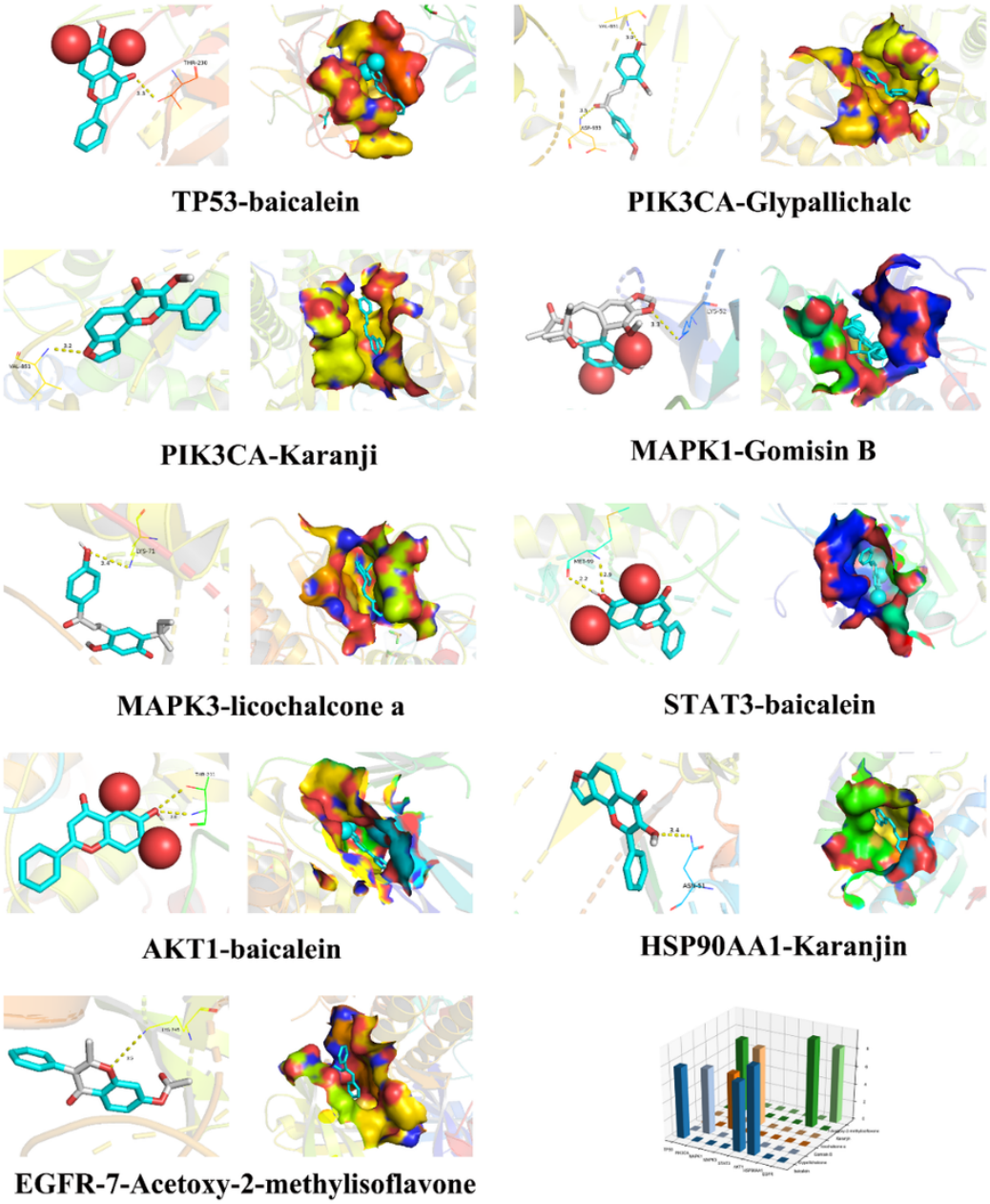

Figure 9

Molecular docking models of main active compounds binding to hub targets. The 9 pairs of molecular docking simulation with hydrogen bond are shown. Schematics (3D) on the left represent that molecular model of the compound is in the binding pocket of the protein. The compounds are shown as stick model with cyan colored. The amino acid residues surrounding are represented by surface. Schematics (3D) on the right show the interactions between compounds and surrounding residues. The yellow dashed lines represent hydrogen bonds and the interaction distances are indicated beside to the bonds. 3D column diagram shows the affinity of 9 pairs docking models. X-axis: protein names, Y-axis: main active compounds, Z-axis: the absolute value of the docking affinity.

Supplementary Files

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