Exploring the Possible Mechanism of Combination of *Polygonati rhizoma* and *Angelicae sinensis* in Preventing Alzheimer's Disease

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Abstract: Alzheimer's disease (AD) is a neurodegenerative disorder, mainly manifested by cognitive and behavioral impairments, a serious threat to life, and without specific therapeutic drugs. The combination of *Polygonati rhizoma* and *Angelicae sinensis* (CPA) conforms to the principle of Chinese medicine formulation and has good effect on the prevention and treatment of AD, but there is a lack of discussion on its target effect and clinical mechanism. In this study, the core targets and key pathways between CPA and AD were analyzed by network pharmacology and molecular docking methods. The results showed that active compounds in CPA (such as baicalin, glycyrrhizin, stigmasterol, diosgenin, cis-ligustilide, etc.) played anti-inflammatory and anti-apoptotic effects by regulating key targets (such as APP, EGFR, ESR1, PTGS2, etc.) in related signaling pathways (such as Alzheimer's disease pathway, NF-kB signaling pathways, etc.) to reduce the aggregation of β amyloid protein and inhibit Tau hyperphosphorylation, and the core components of CPA had good molecular docking activity with the core targets of the pathway. These results indicated that CPA played a role in preventing and improving Alzheimer's disease by regulating multi-target and multi-channel. These results will provide effective treatment basis for future clinical research.

Keywords: Combination of *Polygonati rhizoma* and *Angelicae sinensis* (CPA); Alzheimer's Disease (AD); Network Pharmacology; Baicalein; APP; Alzheimer Disease Pathway

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1 Introduction

Alzheimer's disease (AD) is a chronic neurodegenerative brain disease, and the exact cause of the disease is not fully understood. It is generally believed that as age increases, genetic or environmental factors accumulate, oxidative stress, intracellular calcium imbalance, and mitochondrial dysfunction can all lead to cell damage and accumulation of β amyloid protein (Aβ). The accumulation of elderly plaques and neuronal fiber tangles [1] ultimately leads to brain cell death. According to data from the World Health Organization, the global number of AD patients was approximately 55 million in 2019, and it is expected to increase to 152 million by 2050 [2]. The propor-

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tion of the elderly population in China is on the rise, accounting for 14.2% in 2021. The impact of the COVID-19 in the past three years has increased the negative emotions of the elderly, such as anxiety and loneliness, leading to an increased risk of AD among the elderly. The incidence rate of AD brings huge economic burden to individuals, families, and society. At present, there is no specific therapeutic drug for AD, and the drugs commonly used in clinical treatment of AD are acetylcholinesterase inhibitors (such as donepezil, kabalatine, galanthamine, etc.) and N-methyl-D-aspartic acid receptor antagonists (such as memantine, etc.). These drugs can improve and alleviate symptoms, but they cannot prevent and cure AD, and often accompany side effects. Oral administration of donepezil and cabaratinin may cause vomiting, diarrhea, bronchospasm, as well as adverse reactions such as mental disorders, heart rate abnormalities, and liver function damage [3]. Galantamine has few adverse reactions and good tolerance but has not been able to achieve mass production [4]. Memantine is the mainstream drug for treating AD at present, which can block the overexcitation of N-methyl-D-aspartic acid receptor caused by glutamic acid, prevent cell apoptosis caused by high intracellular calcium concentration [5], thus improving the cognitive status of AD patients. Memantine has no obvious adverse liver reactions, but has side effects such as nausea, insomnia, and decreased appetite [6]. Other drugs such as aspirin, huperzine A, and teprenone, although studies have shown that they can improve cognitive abilities in AD patients, still lack effective support from clinical controlled trials [7, 8].

Many scholars have focused on the use of Chinese herbal medicines for the prevention and treatment of AD. Zhu et al. [9] found that *Coptis chinensis franch* polysaccharide had an inhibitory effect on Aβ peptide aggregation. Uddin et al. [10] found that genistein flavonoids could cause autophagy activation, eliminate hyperphosphorylated Tau protein in the brain, promote the degradation of Aβ in the brain, and protect neural cells. Li et al. [11] found that resveratrol could slow down the deposition of Aβ, inhibit neuronal apoptosis, and significantly reduce the expression of Caspase-3 protein, suggesting that resveratrol has anti-AD potential. Zhou et al. [12] used CSI-MS technology to explore the interaction between ginsenosides and Aβ at the molecular level, pointing out that ginsenoside Rg1 is expected to be a new drug for the treatment of AD.

Polygonati rhizoma is a herbaceous plant of the *Polygonatum* genus, with a medicinal history of nearly 2,000 years in China. It was first recorded in the "Famous Medical Records" as having the effects of tonifying the kidneys, strengthening the spleen, nourishing qi and yin, and moistening the lungs and generating fluids. Modern pharmacological studies have shown that it has various effects such as antibacterial, antioxidant, hypoglycaemic and immune regulation, etc. It is widely used in the prevention and treatment of cough, hypertension, diabetes, cardiovascular, AD and other diseases [13]. Some studies have shown that the combination of *Polygonati rhizoma* as the main herb and with other herbs can significantly enhance its anti-AD advantages, such as combination of *Polygonati rhizoma* - *Lycii fructus* [14] and combination of *Polygonati rhizoma* – *Pheretima* [15], etc. *Angelicae sinensis*, the root of perennial herbaceous plants in the Umbelliferae family, has the effects of regulating menstruation, relieving pain, promoting blood circulation and removing blood stasis. It has various effects such as antioxidant, anti-tumour, and the prevention and treatment on AD [16]. Studies have shown [17, 18] that the combination of *Polygonati rhizoma* and *Angelicae sinensis* (CPA) can alleviate oxidative stress, improve learning and memory, reduce hippocampal Aβ levels, repair damaged brain nerve function, and effectively improve the symptoms in mild to moderate AD patients. The combination of the two kinds of herbs not only conforms to the prescription principles of traditional Chinese medicine (TCM), but also conforms to the theory of the same origin of medicine and food in TCM, to achieve the effect of anti-aging, health care and health maintenance, and realize the overall treatment [19].

Although CPA has clinical medical practice in anti-AD, there is a lack of exploration on its target and mechanism of action. Therefore, this study used network pharmacology technology [20] to study the potential active compounds and targets of CPA in the prevention and treatment of AD. The "CPA - compounds- targets- pathways- AD" interaction network was constructed, and the binding sites of key compounds were identified through molecular docking technology. The molecular basis of CPA in the prevention and treatment of AD was analyzed at the molecular level. The technical roadmap of this study is shown in Figure 1.

Figure 1 Technology roadmap

2 Method

2.1 Screening of Potential Active Compounds and Targets of CPA

In the Traditional Chinese Medicine Systematic Pharmacology Database (TCMSP, https://old.tcmsp-e.com/tcmsp.php), "*Polygonati rhizoma*" and "*Angelicae sinensis*" were used as keywords, respectively, and oral bioavailability (OB≥30%), drug likeness (DL) and blood-brain barrier permeability (BBB≥-0.3) were used as screening parameters. The active compounds were screened for *Polygonati rhizoma* (DL≥0.18) and *Angelicae sinensis* (DL≥0.05).

The SMILES formulae of the active compounds of CPA were obtained using the PubChem database. The relevant targets of CPA were collected by Swiss target prediction and Herb database, and the gene symbols of the targets were retrieved through Uniprot database.

2.2 Collecting of Relevant Targets for AD

Using "Alzheimer's disease" as the keyword, the target genes of AD were obtained by searching and deleting duplicate target genes in DisGeNET, Genecard and Omim database. Their protein names were corrected to the offi-

cial names through Uniprot database, and the species was identified as human (Homo sapiens).

2.3 Obtaining Intersection Targets for CPA-AD and Constructing Protein-protein Interaction (PPI) Networks

Venn diagrams (https://online.visual-paradigm.com/) were drawn between the targets of CPA and the disease targets of AD, and the intersection targets were obtained between CPA and disease.

The intersection targets were inputted into the String11.5 database, the PPI network data (TSV file) was obtained under the conditions of human species (Homo sapiens), confidence level > 0.4, hidden free nodes and other settings as default. PPI map was visualized using Cytoscape 3.9.1 software. The core targets were to determine from degree values through topology analysis.

2.4 Constructing of GO and KEGG Enrichment

The intersection targets between CPA and AD were inputted into the Metascape database and the species selected as "Homo sapiens" for enrichment analysis. For GO analysis, three modules of biological process (BP), cellular component (CC) and molecular function (MF) were selected. For KEGG analysis, the KEGG pathways module was selected. The enriched bubble maps of GO and KEGG were created by downloading relevant data and using visualization tools on Microbiology website (http://www.bioinformatics.com.cn).

2.5 Constructing of "CPA- Compounds- Targets -Pathways- AD" Network

Data of the active compounds, intersection targets and major KEGG pathways were imported into Cytoscape 3.9.1 software to build a "CPA - compounds- targets -pathways- AD" network and to perform network topology analysis.

The KEGG pathway that was ranked top and related to AD disease was draw by Adobe Illustrator 2023 to analyze the action mechanism of CPA on AD.

2.6 Molecular Docking

The SDF format of the molecular structure of key compounds was downloaded from PubChem database, and the SDF format was converted to MOL2 format through Open babel. The best protein structure of the core target was obtained through RCSB PDB database and downloaded in PDB format. The molecular docking between the key compound and the core target was performed using Autodock 1.5.6 software, and then the docking results were visualized using Pymol software.

3 Result

3.1 Screening Results of Active Compounds in CPA

38 compounds of *Polygonati rhizoma* and 125 compounds of *Angelicae sinensis* were collected from TCMSP database. The potential active compounds of *Polygonati rhizoma* and *Angelicae sinensis* that met the OB, DL and BBB requirements were 8 and 19 respectively, as well as one common compound. The results were shown in Table 1.

Table 1 Basic information on the active compounds of CPA

Mol id	Molecule name	OB	DL	BBB	Source
MOL001792	liquiritigenin	32.76	0.18	-0.29	*Polygonati rhizoma*
MOL002714	baicalein	33.52	0.21	-0.05	
MOL002959	3'-methoxydaidzein	48.57	0.24	-0.32	
MOL000359	sitosterol	36.91	0.75	0.87	
MOL009763	acanthoside B	43.35	0.77	-1.83	
MOL000546	diosgenin	80.88	0.81	0.27	
MOL006331	4',5-dihydroxyflavone	48.55	0.19	-0.03	
MOL004941	(2R)-7-hydroxy-2-(4-hydroxyphenyl)chroman-4-one	71.12	0.18	-0.25	
MOL000125	(-)-alpha-pinene	46.25	0.05	2.30	*Angelicae sinensis*
MOL001273	(+)-verbenone	50.63	0.06	1.59	
MOL000162	beta-chamigrene	31.99	0.08	2.07	
MOL002029	( )-cuparene	38.26	0.07	2.15	
MOL002033	cis-thujopsene	56.4 3	0.12	2.24	

Mol id	Molecule name	OB	DL	BBB	Source
MOL002111	butylidenephthalide	42.44	0.07	1.27	
MOL002098	Senkyunolide-B	62.68	0.08	0.90	
MOL002143	senkyunolide-C	46.8	0.08	0.50	
MOL002144	senkyunolide-D	79.13	0.1	-0.07	
MOL002145	senkyunolide-E	34.4	0.08	0.06	
MOL002201	cis-ligustilide	51.3	0.07	1.24	
MOL003587	acoradiene	36.73	0.07	2.11	
MOL000389	ferulic acid (CIS)	54.97	0.06	0.36	
MOL000449	stigmasterol	43.83	0.76	1.00	
MOL008251	sedanolide	62.46	0.07	1.40	
MOL008265	2-valerylbenzoic acid	78.26	0.06	0.45	
MOL008277	7,10-pentadecadiynoic acid	41.5	0.09	0.57	
MOL008286	( )-camphoric acid	99.13	0.07	0.13	
MOL008287	(3E)-3-butylidene-7-hydroxy-2-benzofuran-1-one	42.17	0.08	0.94	
MOL000358	beta-sitosterol	36.91	0.75	0.99	Common

3.2 Intersecting Targets and PPI Network of CPA Against AD

After extracting and deleting duplicate targets from Swiss targets prediction and Herb database, 275 and 419 targets were obtained from *Polygonati rhizoma* and *Angelicae sinensis*, respectively. After obtaining and removing duplicate targets from DisGeNET, Genecard and Omim, a total of 717 targets related to AD were obtained. A total of 83 intersection targets between CPA and AD were obtained through the Venn diagram (Figure 2).

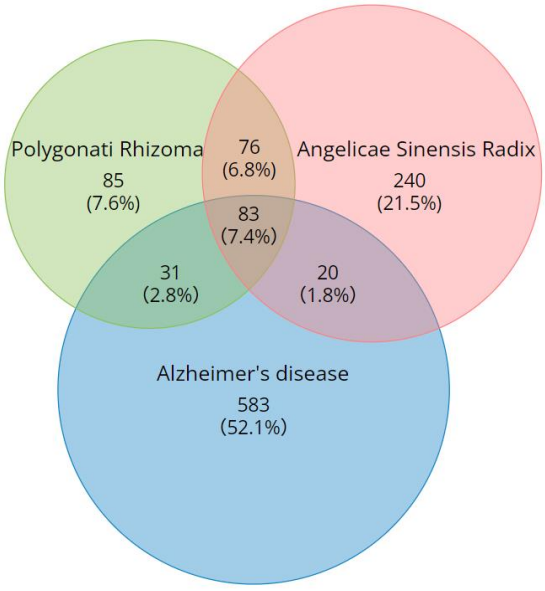


Figure 2 Intersecting targets between CPA and AD

These 83 intersection targets were: ABCB1, ACHE, ADORA1, ALDH2, ALOX5, ALPL, APEX1, APP, AR, BACE1, BCHE, BCL2L1, BRAF, CA2, CHRM2, CSNK2A1, CTSB, CYP17A1, CYP19A1, CYP1A1, DAO, EDNRA, EGFR, ESR1, ESR2, F2, F3, FGFR1, FLT3, FYN, G6PD, GLO1, GLRA1, GRK2, GRM2, GRM5, GSK3B, HMGCR, HNF4A, HTR2A, HTR2C, IGF1R, IKBKB, MAOA, MAOB, MAPK14, MAPK3, MAPK8, MDM2, MET, MIF, MMP12, MMP13, MMP2, MMP3, MMP9, NOS2, NR1H3, NR1H4, NR3C1, OPRM1, PARP1, PIK3CA, PIK3CB, PLA2G1B, PLA2G2A, PLA2G4A, PPARA, PPARG, PTGS1, PTGS2, PTPN1, PYGL, SHBG, SIGMAR1, SLC6A2, SLC6A4, TLR9, TTR, TYR, VCP, VDR, VEGFA. The PPI network was shown in Figure 3.

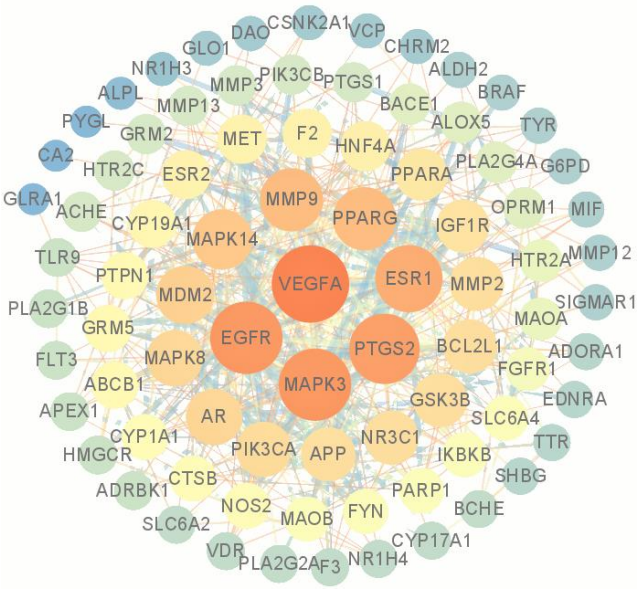


Figure 3 PPI network of intersecting targets of CPA with AD

Notes: The larger the circle, the more important the target is; The wider the line, the stronger the interaction force between two protein targets.

Topological analysis in Figure 3 showed that the aver-

age degree value of the targets was 13.2, with 547 edges between the targets. The target proteins above average degree values were vascular endothelial growth factor A (VEGFA, degree value 45), epidermal growth factor receptor (EGFR, 40), mitogen-activated protein kinase 3 (MAPK3, 40), prostaglandin G/H synthase 2 (PTGS2, 38), estrogen receptor (ESR1, 35), peroxisome proliferator-activated receptor γ (PPARG, 31), matrix metalloproteinase-9 (MMP9, 31). It is suggested that these targets may be important potential targets for CPA against AD.

3.3 GO Classification Enrichment Results

GO enrichment analysis was performed on 83 intersectional targets related to CPA - AD, and a total of 3843 items of BP, 344 items of CC and 657 items of MF were obtained. The top 10 items sorted by -log10(P) value were selected and the bubble enrichment map was obtained as shown in Figure 4.

BP was significantly enriched such as cellular responses to nitrogen compound, responses to peptide, cellular responses to organic cyclic compound, responses to inorganic substance, responses to amyloid beta, regulation of secretion by cell, regulation of MAPK cascade, positive regulation of response to external stimulus, regulation of cellular response to stress, regulation of defense response, etc. CC was significantly enriched in membrane raft, receptor complex, postsynapse, vesicle lumen, extracellular matrix, perinuclear region of cytoplasm, nuclear envelope, nuclear envelope lumen, glial cell projection, organelle outer membrane, etc. MF was significantly enriched in molecular functions such as nuclear receptor activity, protein kinase activity, oxidoreductase activity, serine hydrolase activity, carboxylate hydrolase activity, and neurotransmitter receptor activity, etc.

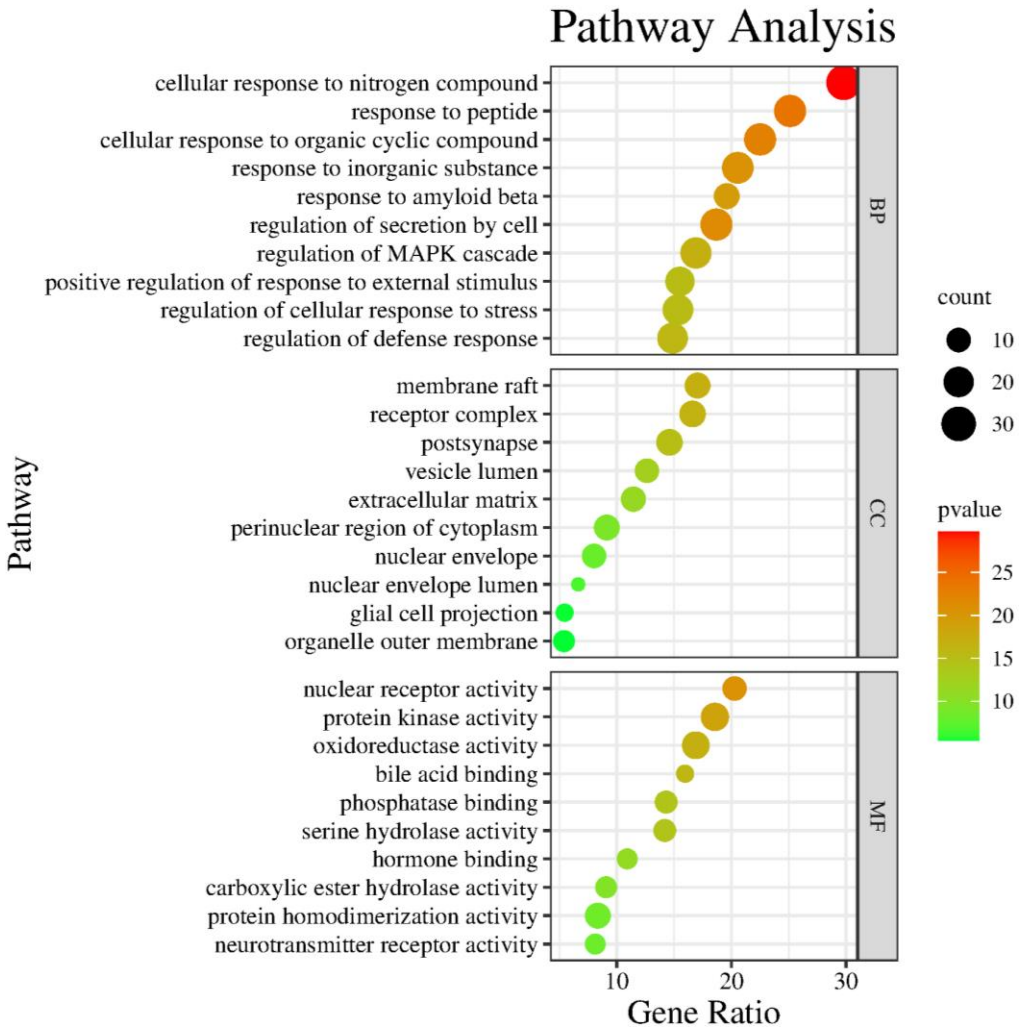


Figure 4 GO bubble diagram of intersecting targets of CPA-AD

3.4 KEGG Pathway Enrichment Results

KEGG enrichment analysis was performed on 83 intersectional targets related to CPA- AD, and a total of 236 pathways were obtained. The KEGG pathways with the top 20 *p* values were screened, a total of 61 intersectional targets were enriched, accounting for 70.11% of the 83 intersectional targets. See Figure 5 and Table 2.

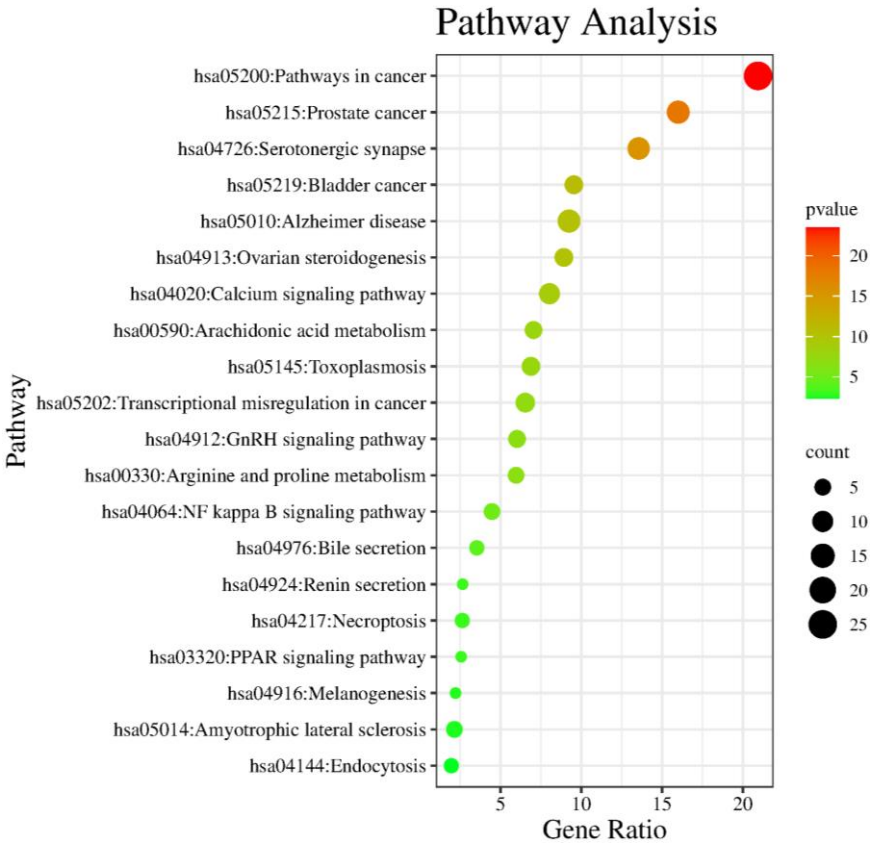


Figure 5 KEGG bubble diagram involved in intersecting targets of CPA-AD

Table 2 Classification of the top 20 ranked KEGG pathways

Types of pathways	Pathway name	Enrichment targets in pathway	Percentage / %
Cancer Related	hsa05200: Pathways in cancer	GSK3B / FLT3 / PIK3CB / PTGS2 / EGFR / IGF1R / IKBKB / MAPK8 / EDNRA / MAPK3 / NOS2 / MMP2 / BRAF / F2 / MMP9 / ESR1 / ESR2 / VEGFA / AR / PIK3CA / MDM2 / PPARG / MET / FGFR1 / BCL2L1	35.33
	hsa05215: Prostate cancer	GSK3B / MMP3 / BRAF / PIK3CB / MMP9 / EGFR / IGF1R / IKBKB / AR / PIK3CA / MDM2 / FGFR1 / MAPK3	
	hsa05202: Transcriptional misregulation in cancer	FLT3 / MMP3 / MDM2 / PPARG / MET / MMP9 / BCL2L1 / IGF1R	
	hsa05219: Bladder cancer	MMP2 / MDM2 / BRAF / MMP9 / EGFR / MAPK3 / VEGFA	
Signaling Pathways	hsa04020: Calcium signaling pathway	CHRM2 / GRM5 / EDNRA / NOS2 / HTR2C / HTR2A / MET / EGFR / FGFR1 / VEGFA	16
	hsa03320: PPAR signaling pathway	PPARA / PPARG / NR1H3	
	hsa04064: NF kappa B signaling pathway	IKBKB / CSNK2A1 / PARP1 / PTGS2 / BCL2L1	
	hsa04912: GnRH signaling pathway	MAPK8 / MMP2 / PLA2G4A / MAPK14 / EGFR / MAPK3	
Metabolism related	hsa00590: Arachidonic acid metabolism	PLA2G1B / ALOX5 / PLA2G2A / LA2G4A / PTGS2 / PTGS1	15.33
	hsa04144: Endocytosis	GRK2 / EGFR / IGF1R / MDM2	
	hsa04976: Bile secretion	ABCB1 / CA2 / NR1H4 / HMGCR	

Types of pathways	Pathway name	Enrichment targets in pathway	Percentage / %
	hsa04217: Necroptosis	PARP1 / PLA2G4A / MAPK8 / PYGL	
	hsa00330: Arginine and proline metabolism	DAO / MAOB / ALDH2 / NOS2 / MAOA	
Neural activity related	hsa05010: Alzheimer disease	GSK3B / APP / NOS2 / CSNK2A1 / BRAF / PIK3CB / PTGS2 / BACE1 / IKBKB / GRM5 / MAPK8 / PIK3CA / MAPK3	14
	hsa05014: Amyotrophic lateral sclerosis	BCL2L1 / MAPK14 / NOS2 / VCP / SIGMAR1	
	hsa04924: Renin secretion	ADORA1 / CTSB / EDNRA	
Synapse related	hsa04726: Serotonergic synapse	APP / MAOB / MAOA / ALOX5 / PLA2G4A / HTR2C / BRAF / HTR2A / PTGS2 / SLC6A4 / MAPK3 / PTGS1	8
Synthesis related	hsa04916: Melanogenesis	GSK3B / MAPK3 / TYR	6.67
	hsa04913: Ovarian steroidogenesis	ALOX5 / CYP1A1 / PLA2G4A / PTGS2 / CYP19A1 / CYP17A1 / IGF1R	
Immunity related	hsa05145: Toxoplasmosis	IKBKB / MAPK8 / NOS2 / ALOX5 / MAPK14 / BCL2L1 / MAPK3	4.67

KEGG enrichment results showed more intersecting targets were enriched for pathways in cancer (25), prostate cancer (13), alzheimer's disease (13), serotonergic synapse (12), calcium signaling pathway (10), NF-κB signaling pathway (5), etc.

3.5 "CPA-Compounds-Targets-Pathways-AD" Network Diagram

The 28 active compounds, 83 intersecting targets and top 20 KEGG pathways of CPA against AD were jointly analyzed, and a network diagram of "CPA - compounds - targets - pathways - AD" was constructed by Cytoscape (Figure 6).

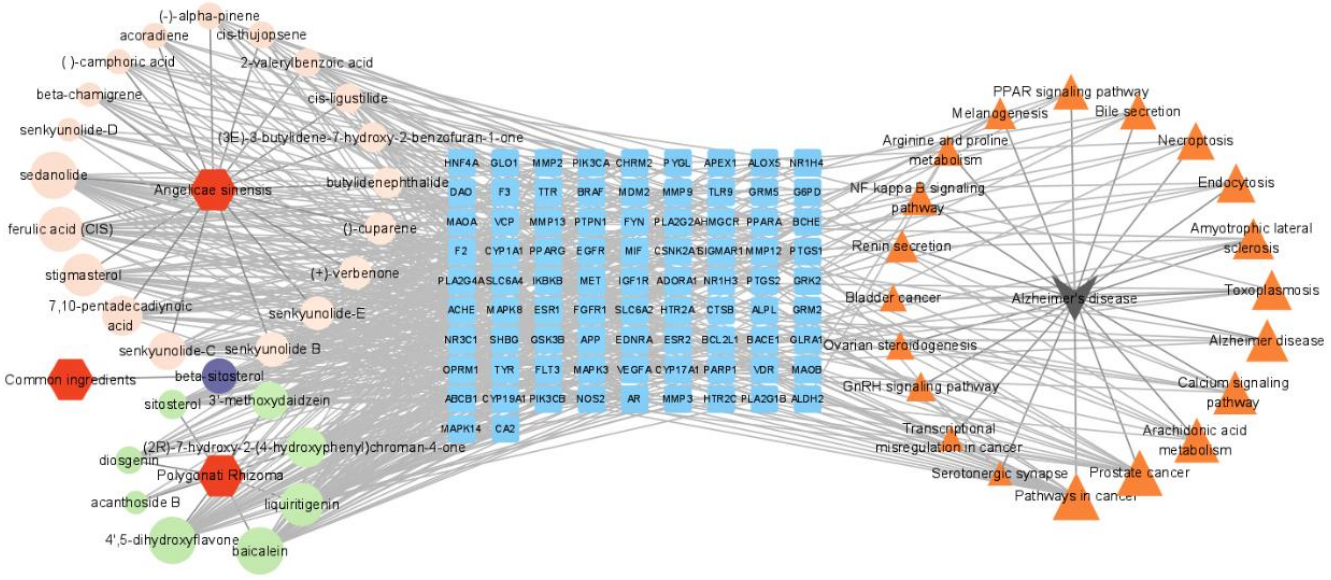


Figure 6 "CPA - compounds - targets - pathways - AD" network

Note: Red hexagon represents *Polygonati rhizoma* and *Angelicae sinensis*; green circle represents 8 active compounds of *Polygonati rhizoma*, while pink circle represents 19 active compounds of *Angelicae sinensis*, purple circle represents 1 common compound of both herbs; blue square represents 83 intersection targets between CPA and AD; yellow triangle represents 20 KEGG pathways. The lines indicate their interrelationship. Compounds and pathways are arranged in clockwise descending order of degree values.

Topological analysis showed that the top 5 compounds in terms of degree value were 4',5-dihydroxyflavone (36), baicalein (36), liquiritigenin (34), sedanolide (28), and ferulic acid (26). Modern pharmacological studies have shown that diosgenin (13) is the most important active compound in *Polygonati rhizome* [21], stigmasterol (21) is the high content ingredient and cis-ligustilide (12), which accounts for 45% of the volatile oil, is the main compound in *Angelicae sinensis* [22]. The top 10 targets in terms of degree value were VEGFA (45), EGFR (40), MAPK3 (40), PTGS2 (38),

ESR1 (35), PPARG (31), MMP9 (31), MAPK14 (28), MDM2 (26) and MAPK8 (24). In addition, the target protein APP (22) is a precursor protein of Aβ, which is the occurrence of AD is closely related. Inhibition of Aβ production by suppressing APP protein expression has been investigated as a direction for treating AD [23]. The top 5 pathways in terms of degree value were: pathways in cancer (22), prostate cancer (13), calcium signaling pathway (8), arachidonic acid metabolism (8), and Alzheimer's disease (7).

4 Molecular Docking Results

4,5-dihydroxyflavone, baicalein, liquiritigenin, stigmasterol, diosgenin and cis-ligustilide were key compounds of the CPA. These compounds were involved in signaling pathways such as Ca^{2+} and AD through key targets such as EGFR, PTGS2, ESR1, PPARG, MMP9 and APP, directly or indirectly regulating alzheimer's disease. Relevant studies have shown that donepezil can improve or regulate AD symptoms by acting on target proteins APP [24], EGFR [25], ESR1 [26], MMP9 [27], etc., so donepezil was selected as a positive control. Molecular docking of small molecules to target proteins was performed by Autodock, and the results were shown in Table 3.

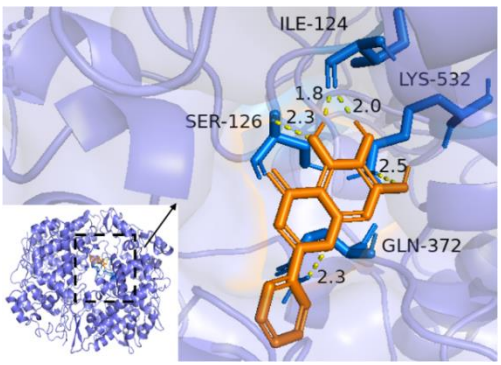
Table 3 Binding energy of active compounds and their associated target proteins (Kcal/mol).

Protein (PDB ID) **Compounds**	**EGFR (5xwd)**	**PTGS2 (5f19)**	**ESR1 (2ocf)**	**PPARG (1i7i)**	**MMP9 (1l6j)**	**APP (4pqd)**
4',5-dihydroxyflavone	-5.57	-7.17	-6.02	-7.29	-7.40	-5.46
Baicalein	-5.03	-7.02	-6.87	-5.79	-6.03	-5.50
Liquiritigenin	-5.13	-6.54	-7.28	-7.33	-7.15	-5.71
Stigmasterol	-5.73	-9.04	-7.16	-6.83	-6.34	-7.87
Diosgenin	-8.03	-7.88	-9.36	-9.4	-8.11	-7.93
Cis-ligustilide	-6.57	-5.97	-6.38	-4.64	-6.76	-5.41
Donepezil hydrochloride	-5.43	-6.67	-7.26	-6.77	-8.00	-5.39

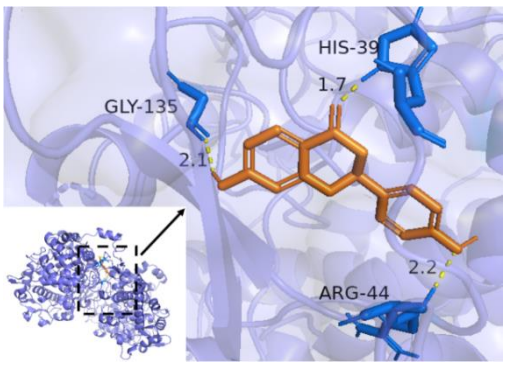
The results in Table 3 showed that the binding energy of 4',5-dihydroxyflavone, baicalein, liquiritigenin, stigmasterol, diosgenin and cis-ligustilide with EGFR, PTGS2, ESR1, MMP9 and APP were all less than -5 Kcal/mol, indicating that these compounds of CPA could stably bind the protein receptors encoded by the core target genes and exert their effects in the treatment of AD.

One-way ANOVA of docking energy was performed using SPAA24.0 software. Compared with the positive control donepezil hydrochloride, the *p*-values of 4',5-dihydroxyflavone (p=0.859), baicalein (p=0.323), liquiritigenin (p=0.913), stigmasterol (p=0.387), diosgenin (p=0.055) and cis-ligustilide (p=0.263) were all greater than 0.05, indicating that the binding energy between key compounds and core targets was not significantly different from the positive control.

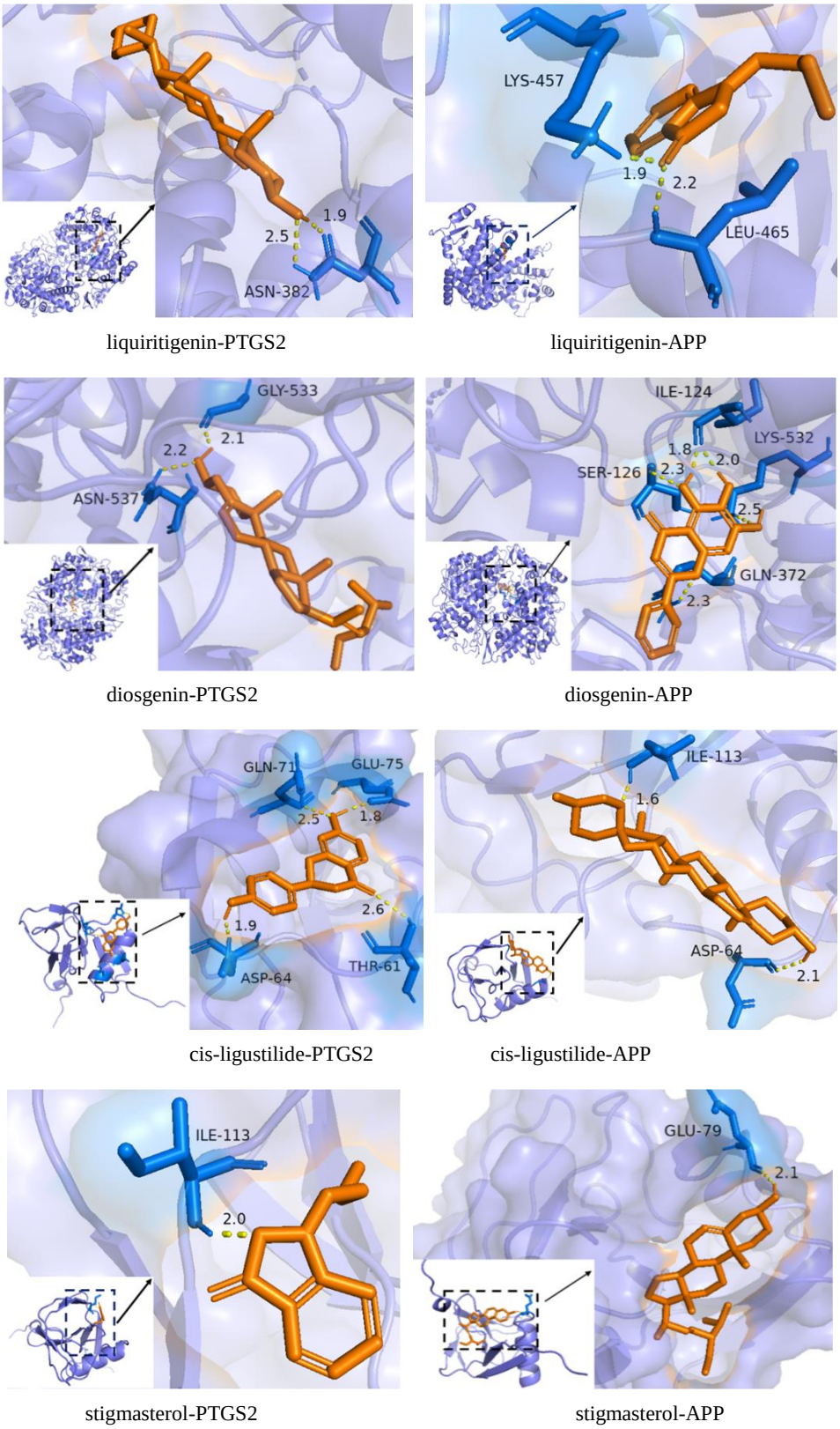
The docking diagrams of baicalein, liquiritigenin, stigmasterol, diosgenin and cis-ligustilide with PTGS2 and APP targets, respectively, were shown in Figure 7.



baicalein-PTGS2



baicalein-APP



liquiritigenin-PTGS2 liquiritigenin-APP

diosgenin-PTGS2 diosgenin-APP

cis-ligustilide-PTGS2 cis-ligustilide-APP

stigmasterol-PTGS2 stigmasterol-APP

Figure 7 Visualization diagram of molecular docking

Note: The purple represents the core protein, the orange represents the key compound, the blue represents the amino acid residues at binding site, and the yellow represents the hydrogen bond.

Analysis of the interactions showed that the binding between the active compound and the target protein was mainly hydrogen bonding and hydrophobic interactions. As shown in Figure 7, the compound-protein binding modes of baicalein, liquiritigenin, stigmasterol, diosgenin and cis-ligustilide with PTGS2 and baicalein with APP were dominated by hydrogen bonding and supplemented by hydrophobic interactions; the compound-protein binding modes of liquiritigenin, stigmasterol, diosgenin and cis-ligustilide with APP were dominated by hydrophobic interactions and supplemented by hydrogen bonding.

5 Discussion

The development of AD is a continuous pathological process that goes through the stages of pre-mild cognitive impairment, mild cognitive impairment, and dementia. Existing studies on AD mostly focus on the dementia stage, but many neurons have died in this stage, and the pathological process is difficult to reverse, and the effect of drugs developed for this stage is not ideal. Studying the early prevention and pathological delay of Alzheimer's disease through traditional Chinese medicine has important fundamental and clinical significance.

In this paper, a "CPA- compounds- targets- pathways-AD" network for CPA anti AD was constructed based on network pharmacology methods. 28 active compounds were screened from CPA, acting on 83 intersecting targets, and participating in regulating 236 pathways. Among them, 4',5-dihydroxyflavone (degree value 36) had the most associated targets, followed by baicalein (36), liquiritigenin (34), sedanolide (28), and ferulic acid (26), stigmasterol (21), diosgenin (13), and ligustilide (12). Meanwhile, the targets most closely related to CPA-AD included VEGFA (degree value 45), EGFR (40), MAPK3 (40), PTGS2 (38), ESR1 (35), PPARG (31), MMP9 (31), MAPK14 (28), MDM2 (26), MAPK8 (24), APP (22), etc. The involved pathways mainly included cancer pathway (degree value 22), prostate cancer (13), calcium signaling pathway (8), arachidonic acid metabolism (8) and Alzheimer's disease (7) etc.

Liquiritigenin in *Polygonati rhizoma* could increase the activity of intracellular antioxidant enzymes, achieving protection of the liver from oxidative damage and reducing the risk of local ischemia in the heart [28]. Molecular docking results showed that liquiritigenin had good binding ability with APP. It was shown that liquiritigenin down-regulated APP, inhibited its binding to secreted enzymes and suppressed the accumulation of Aβ in rat hippocampal neurons [29]. Liquiritigenin could also inhibit the increase of acetylcholinesterase, improve cognitive dysfunction, and play an effective role in learning and memory ability of mice [1]. Baicalin could reduce the production of amyloid peptide by promoting the production of Beclin1 and microtubule-binding protein 1 light chain 3 molecules (LC3) to protect neurons [30]. Molecular docking results showed that baicalein and PTGS2 had good binding ability. Baicalin inhibited the development or deterioration of AD by downregulating the expression of PTGS2 [31]. In a variety of disease models and clinical experiments, stigmasterol has been proven to have better anti-inflammatory, antioxidant, anti-cancer, and liver protective [32] effects. It has been reported that stigmasterol can improve the cognitive ability of AD model animals. By reducing the internalization of BACE1 from the plasma membrane to the endosomes, stigmasterol directly inhibits the activity of β- secretase and reduces the cleavage of APP by β-secretase, thereby significantly reducing the level of Aβ [33]. Molecular docking shows that stigmasterol has a good binding with ESR1 and can act as an agonist to activate ESR1, thus releasing estrogen to regulate neurotransmitter turnover and improve neuronal cells. In addition, stigmasterol can also bind to EGFR, which is significantly up-regulated in astrocytes, inhibit the over activation of cortex and hippocampus in microglia and astrocyte, regulate NF-kB signaling pathway, thereby reducing the secretion of pro-inflammatory factors, inhibiting inflammation [34], and preventing brain damage and cognitive impairment. In preclinical research on cancer and metabolic diseases, diosgenin has shown its beneficial effects [35]. In the central nervous system, diosgenin can alleviate cognitive impairment, reduce synaptic loss, effectively repair axonal degeneration caused by Aβ, and improve memory in mouse model of AD [36]. Diosgenin inhibits the NF-κB signaling pathway by down-regulating the expression of PTGS2 in the body, thereby alleviating movement disorders, inflammatory reactions, and oxidative stress in diseases [37]. Cis-ligustilide is the main active substance in *Angelicae sinensis*, which has pharmacological effects in protecting nerves, relaxing blood vessels, alleviating pain and inflammation [38]. Cis-ligustilide inhibits NF-κB stimulation by tumor necrosis factor, effectively prevents activation and release of NF-κB into the nucleus, thereby inhibiting cell apoptosis

and inflammation, and playing a protective role on neurons [39]. Cis-ligustilide also acts on APP protein, inhibits the activation and proliferation of astrocytes, especially in the neuronal damage areas of parietal cortex and hippocampal CA1, down-regulates related enzymes, reduces the level of inflammatory factors, improves oxidative stress, and increases the SOD content in the brain hippocampus, thus delaying the development of AD [40]. EGFR is an epidermal growth factor receptor, which is expressed in both the central and peripheral nervous systems. The expression of EGFR is significantly up regulated in astrocytes of the brain of AD patients. The use of EGFR inhibitors can improve the pathological and behavioral performance of AD, and its mechanism of action is to play a therapeutic role by inducing autophagy and the attenuation of reactive astrocyte [41]. PTGS2 is a prostaglandin synthase, which is mainly distributed in the cerebral cortex, hippocampus, hypothalamus, thalamus, cerebellum, and other parts [42], affecting brain function and nerve excitability. PTGS2 is also frequently found in inflammatory responses, and over expression of PTGS2 may threaten the survival of brain neurons. Studies have found that activation of NF-kB pathway has been observed in a variety of neurodegenerative diseases, and the loss of PTGS2 can block downstream NF-kB signaling pathway. Thus, down regulating of PTGS2 expression can inhibit cell apoptosis and inflammation, and enhance cell survival [43]. The main estrogen receptor ESR1 regulates gene expression and estrogen response in brain regions associated with major depression disorder. The fluctuation and low levels of estrogen are associated with depressed mood, and it has been proven that estrogen can protect and nourish nerves by increasing non-amyloidogenic processing of amyloid precursor proteins and upregulating expression of apolipoprotein E (APOE) [44]. The variations of Pvull and Xbal in the ESR1 gene may affect the expression of estrogen receptors to regulate the risk of AD. ESR1 is regulated by APOE, which is the primary genetic risk factor for delayed onset AD [45]. APP is an amyloid precursor protein that is cleaved by β-secretase and γ-secretase to produce Aβ. Aβ is a major component of elderly plaques, which ultimately cause neuronal death. And Aβ affects the homeostasis of Ca^{2+}. Aβ oligomers can promote the permeability of Ca^{2+}, allowing extracellular Ca^{2+} to flow into mitochondria through the calcium signaling pathway. The overloading of Ca^{2+} in mitochondria will cause autophagy of mitochondria, disrupt mitochondrial cycle, and eventually cause neuronal apoptosis.

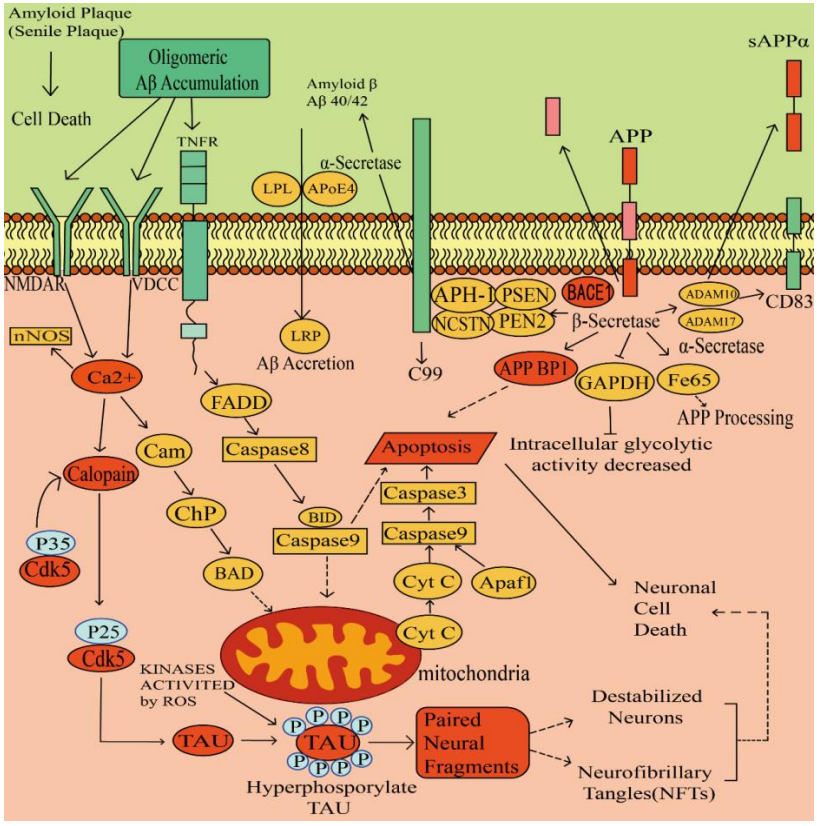


Figure 8 Modulation of the Alzheimer's pathway by the key compounds of CPA

The enrichment analysis of GO and KEGG pathways revealed that the main pathways of CPA for the prevention and treatment of AD were related to the response to Aβ, cellular secretion regulation, protein kinase activity, oxidoreductase activity, neurotransmitter receptor activity, etc. The enriched signaling pathways were mainly pathways in cancer, prostate cancer, serotonergic synapse, Alzheimer disease, calcium signaling pathway and NF-κB signaling pathway associated with inflammation. Taking the Alzheimer's pathway as an example, 13 intersectional targets were enriched in the pathway, and the regulation of the targets by the key compounds of CPA was shown in Figure 8.

The occurrence of AD is related to plaque deposits and nerve fiber tangles formed between brain cells. Aβ is the main component of age-related plaques. The transmembrane protein APP is cleaved by β-secretase and γ-secretase into Aβ fragments that degrade to form Aβ 40 and Aβ 42 peptides. Aβ 40 is the predominant form and Aβ 42 is more fibrillated. These protein fragments lead to oligomerization of β-amyloid and phagocytic plaque formation, leading to neurotoxicity, blocking ion channels, decreaseing homeostasis of Ca^{2+}, causing oxidative stress in mitochondria, affecting energy metabolism, abnormal glucose regulation, and ultimately leading to neuronal cell death [46].

Liquiritigenin, baicalin, cis-ligustilide and ferulic acid in CPA can all act on APP target in the figure to reduce Aβ aggregation and inhibit plaque deposition. Tau protein plays a role in carrying nutrients and other essential substances within brain cells. Cdk5 and other kinases are activated by reactive oxygen species produced in cells, leading to excessive phosphorylation of Tau, leading to destabilization and oligomerization of microtubules of Tau proteins in cells, leading to entanglement of nerve fibers and neuronal apoptosis, ultimately causing damage to cells [47]. Baicalin, 4',5-dihydroxyflavone and senkyunolide-E in CPA can all act on the CDK5 gene, thereby inhibiting hyperphosphorylation of Tau and preventing entanglement of nerve fiber.

6 Conclusions

In summary, the possible mechanisms of action of combination of *Polygonati rhizoma* and *Angelicae sinensis (*CPA*)*, which have the homology of medicine and food, may be that 28 active compounds such as baicalein, liquiritigenin, stigmasterol, diosgenin and cis-ligustilide act on 83 potential intersecting targets such as APP, PTGS2, ESR1 and EGFR, in the KEGG pathways such as Alzheimer's pathway, calcium signaling pathway and NF-kB signaling pathway etc., inhibiting generation of Aβ and hyperphosphorylation of Tau, slowing down oxidative stress, inflammatory response and neuronal damage. It is shown that the CPA can improve the pathological characteristics of AD to varying degrees through multi-component, multi-target and multi-pathway. The research results point out the direction for further experimental studies and provide a theoretical basis for the clinical application of CPA against AD.

Data Availability

All data generated or analysed during this study are included in this article. Further enquiries can be directed to the corresponding author.

Ethics Consent

This article does not contain any experimental studies with human or animal subjects.

Author Contributions

Liping Liu, Shengdong Wang designed the study and drafted the manuscript. Xu Xu performed and analysed the data of network pharmacology and molecular docking. All authors have read and approved the final manuscript.

Conflicts of Interest

The authors declare that they have no competing interests with respect to the research, authorship, and/or publication of this article.

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