Novel Combination of HuanglianJiedu Decoction in Behavioral and Psychological Symptoms of Dementia in Alzheimer's Disease

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Research Article

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Abstract

Alzheimer's disease (AD) is characterized by progressive cognitive decline. Besides cognitive deficit, AD is also characterized by behavioral and psychological symptoms in dementia (BPSD). However, therapeutic management of BPSD remains challenging. HuanglianJiedu decoction (HLJDD), a traditional Chinese prescription, consisting of four herbs, is applied to treat AD, especially AD with BPSD. Though HLJDD, has the traditional combination with the principal herb *Coptidis rhizoma* (Huang-lian), it might, however, not be suitable for treating BPSD. Elucidating the mechanism underlying each herb is critical to the disease-matched combination of HLJDD. In this study, network pharmacology was used to determine the targets and biological processes regulated by HLJDD in the treatment of BPSD. Moreover, molecular docking was utilized to evaluate the binding activity between the herbs' main active ingredients and neurotransmitter receptors. The results showed that *Scutellariae radix* (Huang-qin) and *Phellodendri chinrnsis cortex* (Huang-bai) exhibited better anti-BPSD effects when compared to *Coptidis rhizoma* and *Gardeniae fructus* (Zhi-zi). *Scutellariae radi*x exhibited superior anti-neuroinflammation functions, with better blood vessel regulation effects. *Phellodendri chinrnsis cortex* showed a higher binding affinity to the dopamine D2 receptor (DRD2) and 5-hydroxytryptamine receptor 2A (HTR2A). *Coptidis rhizoma* and *Gardeniae fructus* were better in neuronal signaling. In conclusion, for treating BPSD, *Scutellariae radix* and *Phellodendri chinrnsis cortex* are the principal herbs while *Coptidis rhizoma* and *Gardeniae fructus* are the ancillary herbs.

1. Introduction

In traditional Chinese medicine (TCM), the combination of herbs into a prescription to enhance their overall therapeutic effects or eliminate the adverse effects[1], is referred to as TCM combination. Although prescriptions have conventional combinations, it is essential to consider novel combinations for different diseases. In this study, we propose an innovative method that is based on molecular mechanism for determining herbs combination. We investigated the novel combination of HLJDD in BPSD.

The prevalence of BPSD, including anxiety, agitation, aggression, irritation, depression, apathy, disinhibition, delusions, or hallucinations in AD patients is more than 90%[2]. Molecules involved in the pathogenesis of BPSD have been widely studied. Neuroinflammation, a response that involves neurons and microglia, has been reported to characterize several neurodegenerative diseases and neuropsychiatric conditions, resulting in the elevated production of pro-inflammatory cytokines, like IL-6, TNF-α, IL-8, and IL-4. Notably, microglia activation is the first sign of neuroinflammation. Activated microglia can release various oxidants such as reactive oxygen species and activate several genes and proteins, such as inducible nitric oxide synthase[3]. Neurotransmitters and their receptors are also considered to play a crucial role in BPSD[4]. Evaluation of genetic risk factors provide a powerful approach for elucidating the mechanisms underlying BPSD. APOE epsilon4, the most common genetic risk factor for the late-onset of AD, elevates the risk of BPSD[5]. However, the efficacy of antipsychotics for the treatment of BPSD is scanty. Antipsychotic drugs used in the clinical management of BPSD have extrapyramidal severe side effects (EPS). Memantine, an NMDA receptor antagonist for treating moderate-to-severe AD, inhibits antipsychotic-induced EPS[6], but is controversial for treating BPSD. The current primary therapeutic options for AD, acetylcholinesterase inhibitors, are not effective for treating BPSD[7]. Several researchers turn their attention to natural products as an alternative or complementary method to BPSD for their clinical efficacy with minimal side effects.

BPSD has different TCM syndrome differentiation. “Toxin damaging brain collateral” an essential BPSD differentiation, was proposed by Yongyan Wang. For this syndrome differentiation, brain collateral is injured by fire toxins. For the treatment, purging fire for removing toxins is commonly used. HLJDD, which is composed of *Scutellariae radix*, *Coptidis rhizoma*, *Phellodendri chinrnsis cortex*, and *Gardeniae Fructus*, is a classic prescription for heat-clearance and detoxification. Studies have documented that HLJDD is frequently used in AD management[8], especially with BPSD[9]. While the TCM physicians seldom treat BPSD with whole herbs in HLJDD or with HLJDD only. They prefer to choose two or more principal herbs in HLJDD with other syndrome-match herbs. According to HLJDD’s traditional combination, the TCM physicians now prefer to choose *Coptidis rhizoma* as the primary herb to treat BPSD. However, in this research, *Scutellariae radix* and *Phellodendri chinrnsis cortex* are the remedying herbs in BPSD. So we put forward that the traditional combination of prescription need refers to the novel combination with modern technology. To elucidate HLJDD’s combination in treating BPSD, we used network pharmacology and molecular docking to provide useful drugs for BPSD therapeutics by emphasizing their molecular activities (Figure 1).

2. Material And Methods

2.1. Determination of active ingredients and their matched potential target proteins. A natural plant contains various chemical compounds. We used the TCM systems pharmacology database and analysis platform (TCMSP, https://tcmspw.com/index.php) to determine the main active ingredients in the herbs. Compounds were filtered by oral bioavailability (OB) ≥ 30%, drug-likeness (DL) ≥ 0.18, and drug half-life (HL) ≥ 4 h. The potential molecular targets for the active compounds were predicted using the search tool for interactions of chemicals database (STITCH, http://stitch.embl.de/), with the species limited to "Homo sapiens."

2.2. Potential targets prediction for BPSD. BPSD-associated protein targets were identified using the GeneCards database (http://www.genecards.org/) [10]. These target proteins exhibited a higher rank score, and a higher correlation with BPSD. The keywords used in the search were "behavioral and psychological symptoms of dementia in Alzheimer's disease." The top 50% of the predicted targets were selected as potential BPSD targets.

2.3. Network construction and analysis. We constructed the "compounds-targets-BPSD" networks by Cytoscape 3.7.2 software [11]. The .csv format files whose data combined target compounds and BPSD targets were imported into Cytoscape. The node size was based on the target proteins score values

as provided by the GeneCards database. Overlapping targets between active compounds and BPSD were the herbs' putative targets, and set with a red rectangle node. Memantine was used to identify the potential neurotransmitter receptors.

2.4. Enrichment analysis of target proteins. We performed Gene Ontology (GO) functional enrichment analysis using the ClueGO plugin [12], with the species limited to "Homo sapiens" and $p < 0.05$.

2.5. Molecular docking for active compounds with DRD2 and HTR2A. Memantine was selected as the active control medicine. The molecular mechanism of memantine on BPSD was determined by network pharmacology, same as the HLJDD herbs. The results showed that DRD2 and HTR2A were the targets of neurotransmitter receptors. Then molecular docking studies were conducted using AutoDock software [13] to evaluate the affinity of the active compounds bind with DRD2 and HTR2A neurotransmitter receptors. Crystal structures of the proteins were downloaded from the RCSB Protein Data Bank (http://www.rcsb.org/). PDB ID "6CM4" and "6A93", whose ligand is Risperdal, were selected as DRD2 and HTR2A's crystal structure. Memantine, Risperdal, and HLJDD's active compounds were then docked with DRD2 and HTR2A.

3. Results

3.1. HLJDD's main active compounds and potential target proteins. After searching the TCMSP database, we identified 80 compounds in HLJDD. Scutellariae radix, Coptidis rhizoma, Phellodendri chinrnsis cortex, and Gardeniae fructus have 32, 10, 27, and 11 ingredients, respectively. Then, these compounds were imported into the STITCH database to achieve the potential target proteins (Table 1).

TABLE 1: HLJDD's active compounds and potential molecular target proteins

Herbs	Active ingredients	Potential target proteins
Scutellariae radix	acacetin	IL13␣CYP1A2␣VEGFA␣JUN␣STAT1␣NR1I2␣SELE␣CYP1A1␣IL5␣CYP1B1
Scutellariae radix	wogonin	MMP9␣MYC␣HMGB1␣MCL1␣PTGS2␣CDK9␣GATA1␣KCNK10␣PLSCR1␣CCL2
Scutellariae radix	baicalein	MAPK1␣ALOX15␣CDK4␣MMP2␣PLAU␣CYP1A2␣AKT1␣CYP3A4␣ALOX12␣MMP9
Scutellariae radix	oroxylin a	MAPK3␣BDNF␣HK2␣SIRT3␣CASP8␣PPIF␣IL6␣SOD2␣PARP1␣NOS1
Scutellariae radix	Panicolin␣skullcapflavone I␣	CASP3
Scutellariae radix	beta-sitosterol	DHCR24␣CASP3␣ABCG5␣SREBF2␣ICAM1␣ABCG8␣CYP7A1␣SREBF1␣APOE␣ABCB11
Scutellariae radix	Stigmasterol	TNF␣IL8␣ABCA1␣ABCG8␣NR1H2␣SREBF2␣ABCG5␣NR1H3␣IL10␣SLCO1B1
Scutellariae radix	coptisine	PAWR␣XPO1␣TNFSF11␣ESF1
Coptidis Rhizoma	quercetin	MCL1␣ATP5B␣CYP1A1␣HIBCH␣HCK␣STK17B␣SLC2A2␣CYP1B1␣CYP2C8␣PIM1
Coptidis Rhizoma	berberine	CCND1␣CASP3␣DPP4␣TP53␣AKT1␣MAPK1␣HMOX1␣LDLR␣PCSK9␣ATP5G2
Coptidis Rhizoma	coptisine	PAWR␣TNFSF11␣ESF1␣XPO1
Phellodendri chinrnsis cortex	berberine	PCSK9␣ATP5G2␣HMOX1␣MAPK1␣TP53␣CASP3␣AKT1␣DPP4␣LDLR␣CCND1
Phellodendri chinrnsis cortex	coptisine	PAWR␣XPO1␣ESF1␣TNFSF11
Phellodendri chinrnsis cortex	rutaecarpine	CYP1A1␣TNF␣CYP1A2
Phellodendri chinrnsis cortex	Chelerythrine	GAPDH␣PRKCE␣PLA2G1B␣F2␣CFTR␣CHI3L2␣SELP␣PLA2G2A␣CORT␣ABCB11
Phellodendri chinrnsis cortex	Stigmasterol	IL10␣TNF␣SREBF2␣IL8␣SLCO1B1␣ABCG8␣ABCG5␣NR1H2␣NR1H3␣ABCA1
Phellodendri chinrnsis cortex	beta-sitosterol	ABCG5␣SREBF2␣DHCR24␣CYP7A1␣ABCB11␣CASP3␣ICAM1␣SREBF1␣ABCG8␣APOE
Phellodendri chinrnsis cortex	Fumarine␣protopine␣	HRH1␣F2␣KIAA0101
Phellodendri chinrnsis cortex	quercetin	CYP1B1␣STK17B␣PIM1␣SLC2A2␣CYP1A1␣HCK␣ATP5B␣HIBCH␣CYP2C8␣MCL1
Phellodendri chinrnsis cortex	poriferast-5-en-3beta-ol␣beta-sitosterol␣	CASP9␣NR1H2␣PARP1␣CASP3␣ABCG8␣ABCG5␣ICAM1
Phellodendri chinrnsis cortex	campesterol	HSD3B2␣ABCG8␣ABCG5␣CYP7A1␣DHCR24␣CSN1S1␣NR1H2
Gardeniae fructus	Sudan III	CELA1␣CELA3B
Gardeniae fructus	quercetin	CYP1B1␣SLC2A2␣STK17B␣CYP2C8␣HCK␣CYP1A1␣MCL1␣PIM1␣HIBCH␣ATP5B␣ATP5F1B
Gardeniae fructus	beta-sitosterol	SREBF1␣CYP7A1␣ABCB11␣CASP3␣ABCG5␣ APOE␣ABCG8␣SREBF2␣DHCR24␣ICAM1
Gardeniae fructus	kaempferol	UGT1A9␣UGT1A8␣UGT3A1␣UGT1A7␣CYP1B1␣CDK1␣AHR␣UGT1A3␣NR1I2␣RPS6KA3
Gardeniae fructus	Stigmasterol	ABCG8␣IL8␣TNF␣NR1H2␣NR1H3␣SREBF2␣SLCO1B1␣ABCG5␣ABCA1␣IL10

3.2. *"Compounds-targets-BPSD" network and GO biological process analysis.* In this network, the red rectangular nodes represent the key protein targets of the herbs. The targets for *Scutellariae radix* were the highest (24), followed by *Phellodendri chinrnsis cortex* (15). We used the GO biological process analysis to further study the herbs' key protein targets. Figure 2 b and Table 2 show the biological processes of the key target proteins of *Scutellariae radix*, including positive regulation of smooth muscle cell proliferation, lipopolysaccharide-mediated signaling pathway, and regulation of neuroinflammatory responses, *etc*. Figure 3 b, c, d, and Table 3 show the biological processes of the key target proteins of *Coptidis rhizoma*. Its biological processes include regulation of cellular responses to cadmium ions, responses to nicotine, and negative regulation of macroautophagy. Figure 4 b, c, d, and Table 4 show the biological processes of the key target proteins for *Phellodendri chinrnsis cortex*. These processes include regulating responses to nicotine, glial cell apoptotic process, plasma lipoprotein particle, *etc*. Figure 5 b, c, d, and Table 5 show the biological processes of the key target proteins of *Gardeniae fructus*, which are mainly associated with the regulation of amyloid-beta formation, regulation of membrane protein ectodomain proteolysis and regulation of nitric oxide biosynthetic process.

TABLE 2: Biological functions of *Scutellariae radix* in BPSD

Function	Groups	Group Genes
positive regulation of smooth muscle cell proliferation	Group15	AKT1|APOE|BDNF|CASP3|CCL2|HMGB1|ICAM1|IL10|IL13|IL6|JUN|MAPK1|MAPK3|MMP9|MYC|PTGS2|SOD2|STAT1|TNF|TNFSF11|VEGFA
lipopolysaccharide-mediated signaling pathway	Group14	AKT1|CCL2|ICAM1|IL10|IL13|IL6|JUN|MAPK1|MAPK3|MMP9|MYC|PTGS2|SOD2|TNF|TNFSF11
regulation of neuroinflammatory response	Group13	AKT1|CASP3|HMGB1|IL10|IL6|MAPK1|MMP9|PTGS2|SOD2|TNF|VEGFA
regulation of nitric oxide biosynthetic process	Group12	AKT1|HMGB1|ICAM1|IL10|IL13|MAPK1|MAPK3|PTGS2|TNF|VEGFA
positive regulation of endothelial cell proliferation	Group11	AKT1|CASP3|HMGB1|ICAM1|IL10|IL6|JUN|MMP9|PTGS2|VEGFA
regulation of smooth muscle cell proliferation	Group10	AKT1|IL10|IL13|IL6|JUN|MMP9|MYC|PTGS2|SOD2|STAT1|TNF
positive regulation of nucleotide biosynthetic process	Group09	MYC|NOS1|PTGS2
positive chemotaxis	Group08	AKT1|CASP3|HMGB1|IL6|PTGS2|TNF|VEGFA
negative regulation of epithelial cell differentiation	Group07	ICAM1|IL13|MMP9|MYC|STAT1|VEGFA
regulation of endothelial cell proliferation	Group06	AKT1|APOE|CCL2|HMGB1|IL10|JUN|PTGS2|STAT1|TNF|VEGFA
negative regulation of cysteine-type endopeptidase activity involved in apoptotic process	Group05	AKT1|HMGB1|IL6|MMP9|PTGS2|TNF|VEGFA
positive regulation of blood vessel endothelial cell migration	Group04	AKT1|CASP3|HMGB1|IL6|PTGS2|VEGFA
cellular response to interleukin-6	Group03	ICAM1|IL6|STAT1
vasodilation	Group02	APOE|NOS1|SOD2
regulation of DNA-templated transcription, initiation	Group01	CCL2|HMGB1|JUN
regulation of interferon-alpha production	Group00	HMGB1|IL10|STAT1

TABLE 3: Biological functions of *Coptidis rhizoma* on BPSD

Function	Groups	Group Genes
cellular response to cadmium ion	Group2	AKT1|HMOX1|MAPK1
response to nicotine	Group1	CASP3|HMOX1|MAPK1
negative regulation of macroautophagy	Group0	AKT1|HMOX1|TP53

TABLE 4: Biological functions of *Phellodendri chinrnsis cortex* in BPSD

Function	Groups	Group Genes
response to nicotine	Group4	AKT1|APOE|CASP3|CASP9|HMOX1|ICAM1|IL10|MAPK1|SELP|TNF|TP53
glial cell apoptotic process	Group3	AKT1|APOE|CASP3|CASP9|HMOX1|TNF|TP53
plasma lipoprotein particle clearance	Group2	APOE|HMOX1|LDLR
regulation of platelet activation	Group1	APOE|F2|SELP
positive regulation of anion transport	Group0	APOE|CFTR|TNFSF11

TABLE 5: Biological functions of *Gardeniae fructus* on BPSD

Function	Groups	Group Genes
regulation of amyloid-beta formation	Group2	APOE|CASP3|TNF
regulation of membrane protein ectodomain proteolysis	Group1	APOE|IL10|TNF
regulation of nitric oxide biosynthetic process	Group0	ICAM1|IL10|TNF

3.3. *"Compounds-targets-BPSD" network of memantine.* Patients with moderate to severe AD exhibit relatively severe cognitive and psychological symptoms. N-Methyl-D-aspartic acid (NMDA) is one of the main therapeutic options. Memantine is the most common therapeutic choice for NMDA[14]. In our study, memantine was used as the reference drug. We established the "compounds-targets-BPSD" network of memantine to determine its potential mechanism in BPSD, especially for the neurotransmitter receptor. It was found that DRD2 and HTR2A were the neurotransmitter receptors associated with BPSD.

3.4. Results of molecular docking. Serotonin and its receptors, particularly the HTR2A, play roles in cognitive behaviors and psychiatric conditions such as depression, schizophrenia, and AD[4]. A multitarget-directed ligand, acting on HTR2A and DRD2, has been shown to exert an anti-aggressive and antipsychotic activity and is, therefore, a promising therapeutic option for BPSD [15]. In our study, herbs active ingredients were docked with DRD2 and HTR2A. The 3D structures of the active ingredients were downloaded from the TCMSP database in .mol2 format. They were later converted to .pdb format using the Open Babel GUI software[16]. The local search parameters and rigid filenames of the macromolecule models were used when docking. The software's default values were set as our docking parameters. Grid box sizes were: DRD2: x-dimension: 62, y-dimension: 84, z-dimension: 126, spacing: 0.375, X center: 17.469, y center: 7.307, z center: 2.7. HTR2A: x-dimension: 126, y-dimension: 62, z-dimension: 84, spacing: 0.497, X center: 30.895, y center: 0.518, z center: 63.697. These parameters were minimally adjusted during docking.

We found that beta-sitosterol exhibited the lowest docking energy with DRD2 (-9.58 Kcal/mol) and HTR2A (-8.2 Kcal/mol) when compared to the other compounds, including memantine and Risperdal. Stigmasterol, chelerythrine, and campesterol also exhibited a lower docking energy than memantine and Risperdal. *Phellodendri chinrnsis cortex* was shown to contain most of these compounds. (Table 6 and Figure 6).

TABLE 6: The docking results of the active ingredients with DRD2 and HTR2A

Name	DRD2 (PDB ID:6CM4) binding energy (Kcal/mol)	HTR2A (PDB ID:6A93) binding energy (Kcal/mol)
memantine	-6.72	-6.02
Risperdal	-7.71	-7.9
beta-sitosterol	-9.58	-8.2
stigmasterol	-9.17	-8.9
chelerythrine	-8.1	-8.12
campesterol	-7.37	-8.67
berberine	-7.87	-7.61
Oroxylin a	-7.38	-6.42
acacetin	-7.31	-5.8
Sudan III	-6.87	-6.95
baicalein	-6.7	-6.0
kaempferol	-6.33	-5.49
wogonin	-5.68	-5.95
quercetin	-5.82	-5.04
panicllin	-5.53	-5.74
coptisine	-	-
rutaecarpine	-	-
fumarine	-	-

Note: "-" indicates that the result has not been calculated.

4. Discussion

TCM plays an important role in medical diagnosis and treatments. Based on the TCM theory, Chinese formulas contain a mixture of herbs, combined based on the following combination principle; "monarch (Jun), minister (Chen), assistant (Zuo), and guide (Shi)" meaning that herbs play primary, secondary, auxiliary, or harmonic roles, respectively[17]. Primary herbs are substances that provide the main therapeutic thrust. Secondary herbs enhance or assist the therapeutic actions of the primary. The rest have the following functions: treat accompanying symptoms, moderate the harshness or toxicity of the primary ones, guide the medicine to the proper organs, or exert a harmonizing effect [18]. In HLJDD, *Coptidis rhizoma* plays the monarch role, *Scutellariae radix* plays the minister role while *Phellodendri chinrnsis cortex* and *Gardeniae fructus* play the assistant and guide roles, respectively. However, this combination is not suitable for treating BPSD. We studied drugs from the molecular perspective, and proposed a novel method for testing the combination of formulas. We found that *Scutellariae radix* and *Phellodendri chinrnsis cortex* are the principal herbs, while *Coptidis rhizoma* and *Gardeniae fructus* are the assistant herbs.

In AD, the abnormal accumulation of amyloid-β released from amyloid precursor protein and neuroinflammation are its partially pathologic hallmarks. Amyloid-β accumulation also causes indirect injuries to neurons by inducing neuroinflammation[19]. Microglia, the resident innate immune cells in the brain, are important in AD immune responses. They act as sentinel and protective cells, but may become inappropriately reactive in AD to drive neuropathology[20]. With advances in age, microglia exhibit enhanced sensitivity to inflammatory stimuli, similar to that observed in brains with ongoing neurodegeneration[21]. The lipopolysaccharide (LPS), a gram-negative bacterial endotoxin released from the cell wall, mediates inflammation in the body, involving in regulating the expression of potential inflammatory factors[22]. Studies have linked schizophrenia with neuroinflammatory conditions and microglia, which have been correlated to the pathogenesis of schizophrenia. Neuroinflammatory changes observed in schizophrenia involve abnormal astrocyte functions[23]. In our study, *Scutellaria baicalensis* was shown to play anti-inflammatory roles, including regulating neuroinflammatory responses, mediating lipopolysaccharide-mediated signaling pathways, cellular response to interleukin-6, and regulation of interferon - α production during BPSD treatment. *Gardeniae fructus* was shown to regulate amyloid-beta formation in BPSD therapy.

Smooth muscle cell proliferation, especially vascular smooth muscle cells, are essential during cell growth or injury[24]. Blood vessels with vascular smooth muscle cells play an important role in normal brain functions. Apart from supplying adequate blood, they help maintain its structural integrity and function. Shortages in cerebral blood flow and blood-brain barrier dysfunction are early findings in neurodegenerative disorders. Cerebral blood flow shortage, impaired cerebrovascular reactivity, and impaired hemodynamic responses are increasingly prevalent in the early stages of AD[25]. Platelets, critical blood flow factors, are anucleate blood cells whose principal function is to stop bleeding by forming aggregates for hemostatic reactions. Platelet aggregates are also involved in pathological thrombosis and play an essential role in inflammation[26]. Nitric oxide (NO) is a small free radical molecule with an endothelium-derived relaxing factor. Adequate levels of NO in the vascular endothelium are critical for regulating blood flow and vasodilation. Moreover, NO plays a vital neuronal signaling role[27]. Cadmium, a metal that resembles zinc and calcium, is also crucial for neuronal

signaling. Cadmium exposure is associated with neurodegenerative diseases such as AD. It can alter neurotransmitters' release, cause oxidative stress, damage the mitochondria, and induce apoptosis[28]. In our study, *Scutellariae radix* was shown to positively regulate smooth muscle cell proliferation, endothelial cell proliferation, and blood vessel endothelial cell migration; *Coptidis rhizoma* was shown to mediate cellular responses to cadmium ion; *Phellodendri chinrnsis cortex* was shown to regulate platelet activation while *Gardeniae fructus* regulated the nitric oxide biosynthetic process.

Macroautophagy is an evolutionarily conserved dynamic pathway that functions primarily in a degradative manner. Various diseases are associated with macroautophagic dysregulation. Macroautophagy plays a critical role in cellular homeostasis. Insufficient or excessive macroautophagy can seriously compromise cell physiology[29]. *Coptidis rhizoma* was shown to negatively regulate macroautophagy while *Phellodendri chinrnsis cortex* regulated glial cell apoptosis.

Studies have documented that Nicotine might be involved in the pathophysiology of psychosis. Smoking is correlated with depression. In animal models, Nicotine exhibited anxiolytic properties. Depressed people are more likely to smoke and more likely to develop severe depressive episodes upon smoking cessation. Nicotine has also been observed to exhibit similar cognitive improvements in AD patients[30]. However, the relationship between smoking and AD has not been clearly elucidated[31]. In the study, *Coptidis rhizoma* and *Phellodendri chinrnsis cortex* can respond to nicotine.

It has been documented that serotoninergic, dopaminergic, and cholinergic systems are involved in BPSD pathogenesis, and the roles of HTR2A and DRD2 as therapeutic targets are evident[15]. We used molecular docking to determine the potential active ingredients that exhibited good binding activities to DRD2 and HTR2A. The best-docked compound was beta-sitosterol. The free binding energy of beta-sitosterol with DRD2 and HTR2A was -9.58 kcal/mol, -8.2 kcal/mol, respectively. Stigmasterol, chelerythrine, and campesterol also exhibited good binding activities. The active ingredients of *Phellodendri chinrnsis cortex* were found to be beta-sitosterol, Stigmasterol, chelerythrine, and campesterol. It is the only herb containing these five ingredients.

The TCM theory verifies our results. Triple energizers in TCM theory mean upper, middle, and lower energizers. They are the birth and channel to run for Qi, blood, thin, thick fluids, and essence. Moreover, they also contact five Zang-organs and six Fu-organs. *Scutellariae radix* affects the upper energizer, which consists of the brain and heart; *Phellodendri chinrnsis cortex* affects the lower energizer, which is the kidney and liver while *Coptidis rhizoma* influences the middle energizer that consists of the spleen and stomach. Kidney essence deficiency is a primary syndrome differentiation of AD in TCM theory. Due to the imbalance between yin and yang of liver functions, "Liver fire" is the largest contributor to BPSD[32, 33]. *Phellodendri chinrnsis cortex* acts on the lower energizer to purge the liver fire. "Su Wen" puts forward that "the mind is the monarch's official, and the gods come out of it." In the compendium of Materia Medica, Shizhen Li of the Ming Dynasty proposed that "the brain is the house of primordial God." *Scutellariae radix* works on the heart and brain, belonging to the upper energizer. In conclusion, based on the TCM theory, *Scutellariae radix* and *Phellodendri chinrnsis cortex* are HLJDD's primary herbs.

5. Conclusions

The therapeutic value of natural products in the management of BPSD has increased due to their clinical efficacy and insignificant side effects. Different types of compounds have been reviewed for their biological activities. Our results showed that *Scutellariae radix* has more molecular targets and biological processes involved in BPSD while *Phellodendri chinrnsis cortex* exhibited more well-docked compounds: poriferast-5-en-3beta-ol (beta-sitosterol), Stigmasterol, chelerythrine, and campesterol, which have a lower affinity for DRD2 and HTR2A. *Scutellariae radix* and *Phellodendri chinrnsis cortex* were found to have the primary compounds for treating BPSD. *Scutellariae radix* plays an anti-inflammatory role while *Phellodendri chinrnsis cortex* regulates apoptotic processes and response to nicotine. They both regulate blood vessels. *Coptidis rhizoma* and *Gardeniae fructus* play the assistant role in BPSD, and are involved in neuronal signaling.

Abbreviations

AD: Alzheimer's disease; BPSD: Behavioral and psychological symptoms in dementia; HLJDD: HuanglianJiedu decoction; DRD2: Dopamine D2 receptor; HTR2A: 5-hydroxytryptamine receptor 2A; TCM: traditional Chinese medicine; EPS: extrapyramidal severe side effects; TCMSP: TCM systems pharmacology database and analysis platform; OB: oral bioavailability; DL: drug-likeness; HL: half-life; STITCH: search tool for interactions of chemicals database; GO: Gene Ontology; D-CS: BPSD correlation score; NMDA: N-Methyl-D-aspartic acid; LPS: Lipopolysaccharide; NO: nitric oxide.

Declarations

Data Availability

The data used to support the findings of this study are available from the first author upon request.

Competing interests

The authors declare no competing financial interest.

Authors' contributions

Suya Ma wrote the manuscript. XW constructed the pharmacological networks. Jing Shi revised the manuscript. All authors were responsible for reviewing data. All authors read and approved the final manuscript.

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References

1 N. Zhang, L. Li, P. Wang et al., "Pharmacokinetics of the main compounds absorbed into blood after oral administration of Liu Wei Di Huang Wan, a typical combinatorial intervention of Chinese medical formula," *J Nat Med*, vol. 67, no. 1,pp.36-41,2013.

2 S. Chakraborty, J. C. Lennon, S. A. Malkaram, Y. Zeng, D. W. Fisher, H. Dong, "Serotonergic system, cognition, and BPSD in Alzheimer's disease," *NEUROSCIENCE LETTERS*, vol. 704, no.,pp.36-44,2019.

3 N. Eissa, A. Sadeq, A. Sasse, B. Sadek, "Role of Neuroinflammation in Autism Spectrum Disorder and the Emergence of Brain Histaminergic System. Lessons Also for BPSD?" *Frontiers in Pharmacology*, vol. 11, no.,pp.886,2020.

4 L. Tang, Y. Wang, Y. Chen et al., "The Association between 5HT2A T102C and Behavioral and Psychological Symptoms of Dementia in Alzheimer's Disease: A Meta-Analysis," *Biomed Research International*, vol. 2017, no.,pp.5320135,2017.

5 S. Lopez-Leon, A. C. Janssens, L. A. Gonzalez-Zuloeta et al., "Meta-analyses of genetic studies on major depressive disorder," *Mol Psychiatry*, vol. 13, no. 8,pp.772-85,2008.

6 B. M, P. E, "The role of Serotonin Receptors in Alzheimer's disease," *Opera Medica & Physiologica*, vol., no. ,pp.89-98.

7 C. H. Lin, H. Y. Lane, "The Role of N-Methyl-D-Aspartate Receptor Neurotransmission and Precision Medicine in Behavioral and Psychological Symptoms of Dementia," *Frontiers in Pharmacology*, vol. 10, no.,pp.540,2019.

8 Y. Chen, Y. Xian, Z. Lai, S. Loo, W. Y. Chan, Z. X. Lin, "Anti-inflammatory and anti-allergic effects and underlying mechanisms of Huang-Lian-Jie-Du extract: Implication for atopic dermatitis treatment," *JOURNAL OF ETHNOPHARMACOLOGY*, vol. 185, no.,pp.41-52,2016.

9 J Shi, J Ni, J Tian. et al., "A systematic review on heat-clearing and toxicity-removing therapy for Alzheimer's disease," *Bei Jing Zhong Yi Yao Da Xue Xue Bao*, vol. 42, no. 08,pp.667-72,2019.

10 G. Stelzer, N. Rosen, I. Plaschkes et al., "The GeneCards Suite: From Gene Data Mining to Disease Genome Sequence Analyses," *Curr Protoc Bioinformatics*, vol. 54, no.,pp.1-30,2016.

11 P. Shannon, A. Markiel, O. Ozier et al., "Cytoscape: a software environment for integrated models of biomolecular interaction networks," *GENOME RESEARCH*, vol. 13, no. 11,pp.2498-504,2003.

12 G. Bindea, B. Mlecnik, H. Hackl et al., "ClueGO: a Cytoscape plug-in to decipher functionally grouped gene ontology and pathway annotation networks," *BIOINFORMATICS*, vol. 25, no. 8,pp.1091-3,2009.

13 M. F. Sanner, "Python: a programming language for software integration and development," *JOURNAL OF MOLECULAR GRAPHICS & MODELLING*, vol. 17, no. 1,pp.57-61,1999.

14 R. Chen, P. T. Chan, H. Chu et al., "Treatment effects between monotherapy of donepezil versus combination with memantine for Alzheimer disease: A meta-analysis," *PLoS One*, vol. 12, no. 8,pp.e183586,2017.

15 A. Bucki, M. Marcinkowska, J. Sniecikowska et al., "Multifunctional 6-fluoro-3-[3-(pyrrolidin-1-yl)propyl]-1,2-benzoxazoles targeting behavioral and psychological symptoms of dementia (BPSD)," *EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY*, vol. 191, no.,pp.112149,2020.

16 N. M. O'Boyle, M. Banck, C. A. James, C. Morley, T. Vandermeersch, G. R. Hutchison, "Open Babel: An open chemical toolbox," *J Cheminform*, vol. 3, no.,pp.33,2011.

17 L. Ma, X. Zhang, X. Xu et al., "Compatibility principle in the Tanyu Tongzhi Formula revealed by a cell-based analysis," *JOURNAL OF ETHNOPHARMACOLOGY*, vol. 231, no.,pp.507-15,2019.

18 R. Yuan, Y. Lin, "Traditional Chinese medicine: an approach to scientific proof and clinical validation," *Pharmacol Ther*, vol. 86, no. 2,pp.191-8,2000.

19 R. Fujikawa, S. Higuchi, M. Nakatsuji et al., "Deficiency in EP4 Receptor-Associated Protein Ameliorates Abnormal Anxiety-Like Behavior and Brain Inflammation in a Mouse Model of Alzheimer Disease," *AMERICAN JOURNAL OF PATHOLOGY*, vol. 187, no. 8,pp.1848-54,2017.

20 C. Villegas-Llerena, A. Phillips, P. Garcia-Reitboeck, J. Hardy, J. M. Pocock, "Microglial genes regulating neuroinflammation in the progression of Alzheimer's disease," *CURRENT OPINION IN NEUROBIOLOGY*, vol. 36, no.,pp.74-81,2016.

21 F. Yin, H. Sancheti, I. Patil, E. Cadenas, "Energy metabolism and inflammation in brain aging and Alzheimer's disease," *Free Radic Biol Med*, vol. 100, no.,pp.108-22,2016.

22 L. Guo, H. Zhang, W. Li, D. Zhan, M. Wang, "N-acetyl cysteine inhibits lipopolysaccharide-mediated induction of interleukin-6 synthesis in MC3T3-E1 cells through the NF-kB signaling pathway," *ARCHIVES OF ORAL BIOLOGY*, vol. 93, no.,pp.149-54,2018.

23 F. V. Gomes, R. Llorente, B. E. Del, M. P. Viveros, M. Lopez-Gallardo, F. S. Guimaraes, "Decreased glial reactivity could be involved in the antipsychotic-like effect of cannabidiol," *SCHIZOPHRENIA RESEARCH*, vol. 164, no. 1-3,pp.155-63,2015.

24 Y. Baumer, S. McCurdy, M. Alcala et al., "CD98 regulates vascular smooth muscle cell proliferation in atherosclerosis," *ATHEROSCLEROSIS*, vol. 256, no.,pp.105-14,2017.

25 M. D. Sweeney, K. Kisler, A. Montagne, A. W. Toga, B. V. Zlokovic, "The role of brain vasculature in neurodegenerative disorders," *NATURE NEUROSCIENCE*, vol. 21, no. 10,pp.1318-31,2018.

26 Y. Zhou, A. Yasumoto, C. Lei et al., "Intelligent classification of platelet aggregates by agonist type," *eLife*, vol. 9, no.,2020.

27 J. Tejero, S. Shiva, M. T. Gladwin, "Sources of Vascular Nitric Oxide and Reactive Oxygen Species and Their Regulation," *PHYSIOLOGICAL REVIEWS*, vol. 99, no. 1,pp.311-79,2019.

28 M. Forcella, P. Lau, M. Oldani et al., "Neuronal specific and non-specific responses to cadmium possibly involved in neurodegeneration: A toxicogenomics study in a human neuronal cell model," *NEUROTOXICOLOGY*, vol. 76, no.,pp.162-73,2020.

29 X. Wen, D. J. Klionsky, "An overview of macroautophagy in yeast," *JOURNAL OF MOLECULAR BIOLOGY*, vol. 428, no. 9 Pt A,pp.1681-99,2016.

30 D. Paterson, A. Nordberg, "Neuronal nicotinic receptors in the human brain," *PROGRESS IN NEUROBIOLOGY*, vol. 61, no. 1,pp.75-111,2000.

31 T. Teaktong, A. J. Graham, M. Johnson, J. A. Court, E. K. Perry, "Selective changes in nicotinic acetylcholine receptor subtypes related to tobacco smoking: an immunohistochemical study," *Neuropathol Appl Neurobiol*, vol. 30, no. 3,pp.243-54,2004.

32 J. Tian, J. Shi, M. Wei et al., "Chinese herbal medicine Qinggongshoutao for the treatment of amnestic mild cognitive impairment: A 52-week randomized controlled trial," *Alzheimers Dement (N Y)*, vol. 5, no.,pp.441-9,2019.

33 J. Shi, M. Wei, J. Ni et al., "Tianzhi granule improves cognition and BPSD of vascular dementia: a randomized controlled trial," *Journal of Translational Medicine*, vol. 18, no. 1,pp.76,2020.

Figures

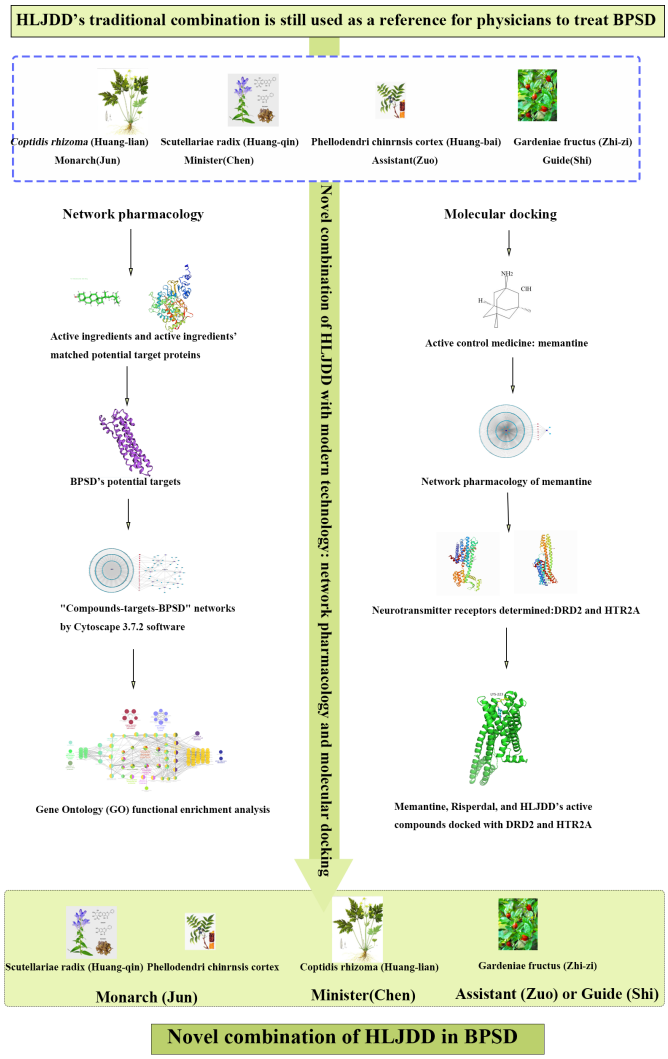

Figure 1

Schematic presentation of the method to discover the novel combination of HLJDD in treating BPSD.

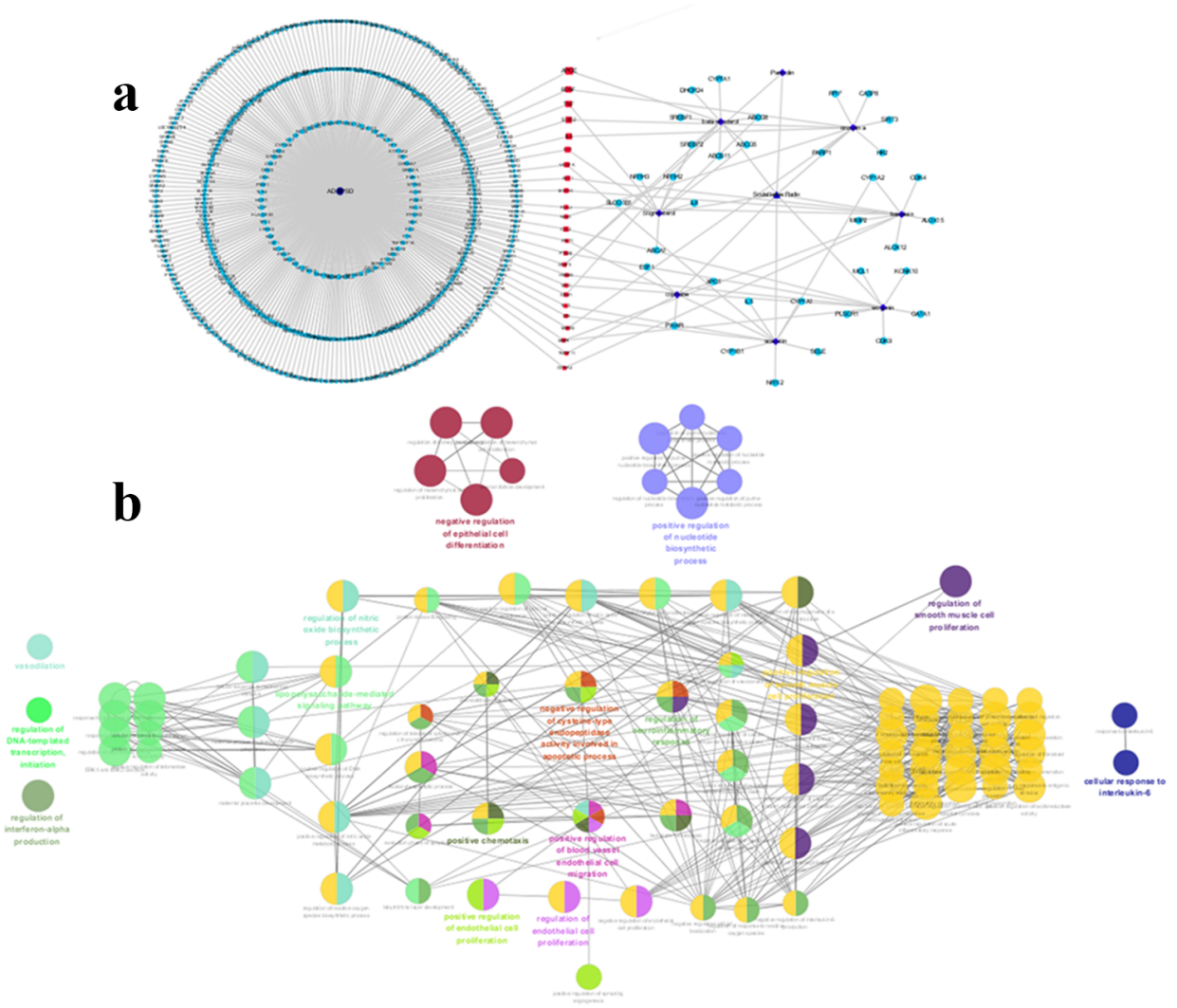

Figure 2

Schematic presentation of Scutellariae radix's potential mechanism in treating BPSD. a The "compounds-targets-BPSD" network of Scutellariae radix. This systematic approach successfully revealed 24 key protein targets related to BPSD: APOE ((BPSD correlation score, D-CS): 156.68), TNF (D-CS: 114.77), SOD2 (D-CS: 114.23), IL 6 (D-CS: 105.12), BDNF (D-CS: 104.54), IL 10 (D-CS: 86.21), VEGFA (D-CS: 72.66), AKT1 (D-CS: 66.45), CCL2 (D-CS: 66.37), MAPK1 (D-CS: 52.1), PLAU (D-CS: 41.02), CASP3 (D-CS: 37.17), ICAM1 (D-CS: 37.08), PTGS2 (D-CS: 35.32), NOS1 (D-CS: 32.33), MMP9 (D-CS: 30.39), MYC (D-CS: 30.15), IL 13 (D-CS: 29.05), MAPK 3 (D-CS: 26.17), HMGB 1 (D-CS: 23.14), JUN (D-CS: 22.83), STAT1 (D-CS: 20.14), TNFSF 11 (D-CS: 18.98), CYP3A4 (D-CS: 17.31). b GO biological processes analysis of Scutellariae radix. Scutellariae radix's key protein targets involved 156 biological processes, such as positive regulation of smooth muscle cell proliferation, lipopolysaccharide-mediated signaling pathway, and neuroinflammatory responses.

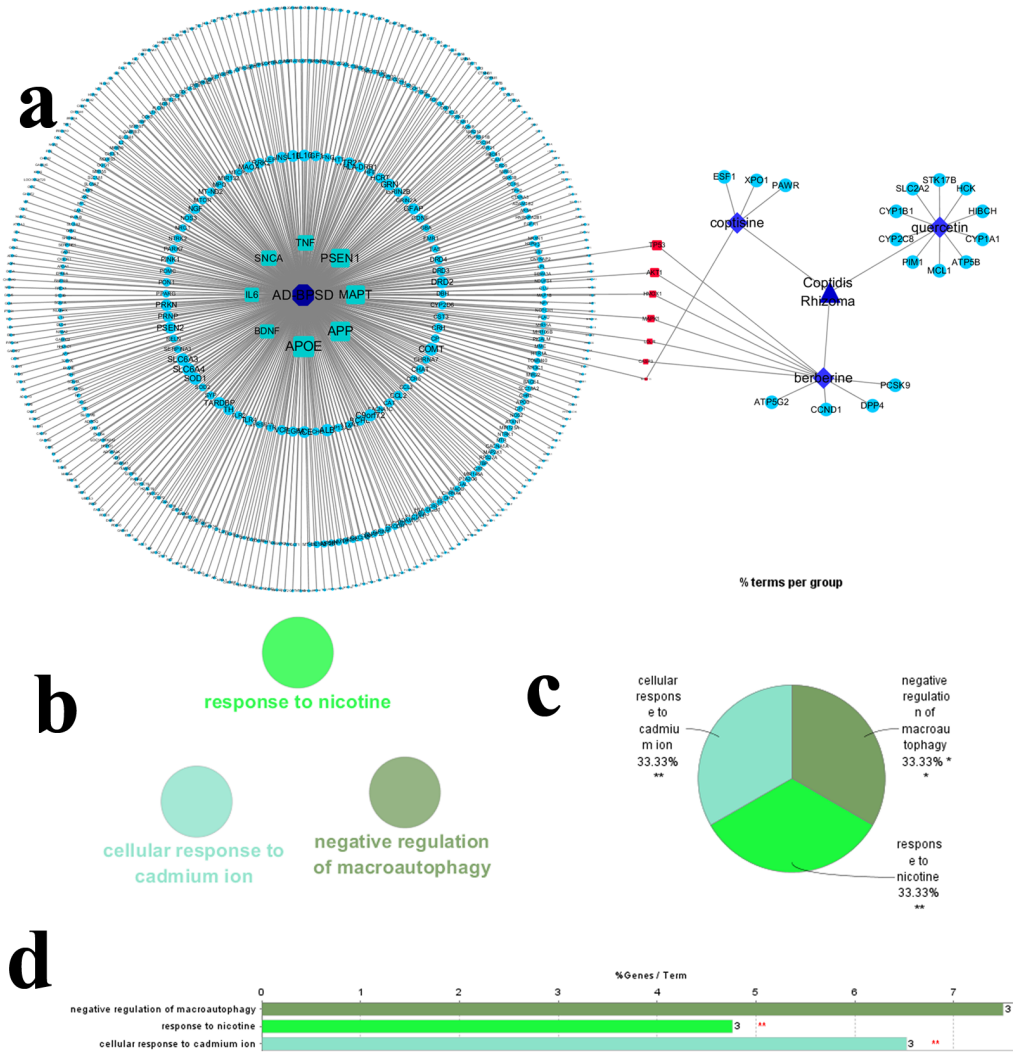

Figure 3

Schematic presentation of Coptidis rhizoma's potential mechanisms in treating BPSD. a The "compounds-targets-BPSD" network of Coptidis rhizoma. Seven key protein targets were associated with BPSD: TP53 (D-CS: 72.85), AKT 1 (D-CS: 66.45), HMOX 1 (D-CS: 50.08), LDLR (D-CS: 40.09), MAPK 1 (D-CS: 52.1), CASP 3 (D-CS: 37.17). TNFSF 11 (D-CS: 18.98). b, c, d GO biological processes analysis of Coptidis rhizoma. The key protein targets of Coptidis rhizoma are involved in three biological processes; negative regulation of macroautophagy, responses to nicotine, and cellular responses to cadmium ions.

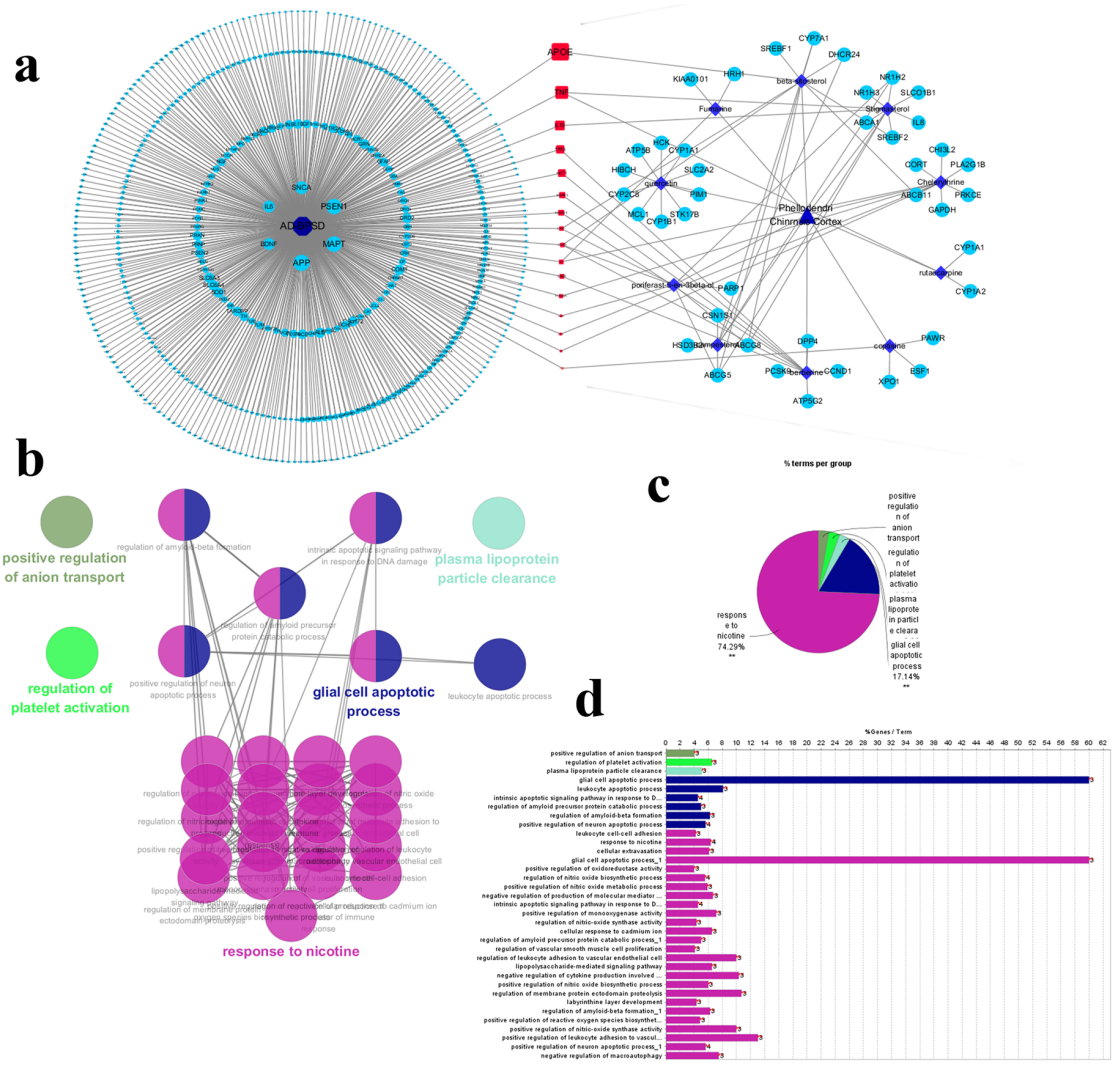

Figure 4

Schematic presentation of Phellodendri chinrnsis cortex's potential mechanisms in BPSD. a The "compounds-targets-BPSD" network of Phellodendri chinrnsis cortex. Fifteen key protein targets were involved in BPSD: APOE (D-CS: 156.68), TNF (D-CS: 114.77), IL 10 (D-CS: 86.21), TP 53 (D-CS: 72.85), AKT 1 (D-CS: 66.45), MAPK 1 (D-CS: 52.1), HMOX 1 (D-CS: 50.08), LDLR (D-CS: 40.09), F 2 (D-CS: 38.83), CASP 3 (D-CS: 37.17), ICAM 1 (D-CS: 37.08), PLA2G2A (D-CS: 33.98), CFTR (D-CS: 21.04), CASP 9 (D-CS: 20.76), TNFSF 11 (D-CS: 18.98), SELP (D-CS: 17.65). b, c, d GO biological process analysis of Phellodendri chinrnsis cortex. Phellodendri chinrnsis cortex's key protein targets involved 35 biological processes including responses to nicotine, glial cell apoptotic processes, and positive regulation of anion transport.

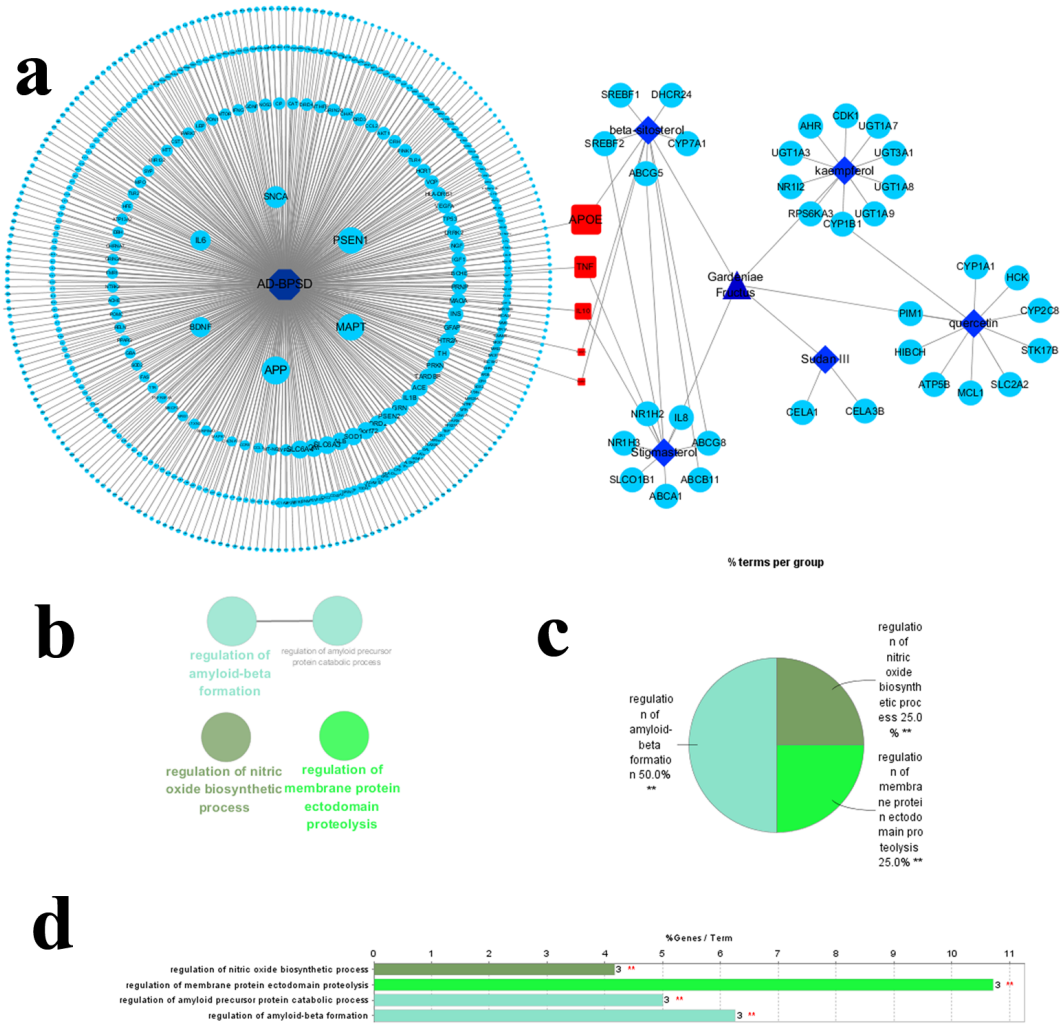

Figure 5

Schematic presentation of Gardeniae fructus's potential mechanisms in BPSD. a The "compounds-targets-BPSD" Gardeniae fructus network. Five key protein targets were identified. They are APOE (D-CS: 156.68), TNF (D-CS: 114.77), IL 10 (D-CS: 86.21), CASP 3 (D-CS: 37.17), ICAM 1 (D-CS: 37.08). b, c, d GO biological processes analysis of Gardeniae fructus. Gardeniae fructus's key protein targets are involved in 4 biological processes; regulation of amyloid precursor protein catabolic processes, regulation of membrane protein ectodomain proteolysis, regulation of nitric oxide biosynthetic processes, and regulation of amyloid-beta formation.

Figure 6

Schematic 3D presentation of the molecular docking model with DRD2 and HTR2A