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Network pharmacology-based study on the mechanism of action for herbal medicines in Alzheimer treatment

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Ginsenoside Rg5 (PubChem CID: 44416768)
Ginsenoside Rg3 (PubChem CID: 9918693)
Ginsenoside Rg2 (PubChem CID: 75412551)
Tenuifolin (PubChem CID: 21588226)
Tanshinone IIA (PubChem CID: 164676)
Salvianolic acid A (PubChem CID: 5281793)
Salvianolic acid B (PubChem CID: 11629084)
Cryptotanshinone (PubChem CID: 160254)
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ABSTRACT

Ethnopharmacological relevance: Alzheimer's disease (AD), as the most common type of dementia, has brought a heavy economic burden to healthcare system around the world. However, currently there is still lack of effective treatment for AD patients. Herbal medicines, featured as multiple herbs, ingredients and targets, have accumulated a great deal of valuable experience in treating AD although the exact molecular mechanisms are still unclear.

Materials and methods: In this investigation, we proposed a network pharmacology-based method, which combined large-scale text-mining, drug-likeness filtering, target prediction and network analysis to decipher the mechanisms of action for the most widely studied medicinal herbs in AD treatment.

Results: The text mining of PubMed resulted in 10 herbs exhibiting significant correlations with AD. Subsequently, after drug-likeness filtering, 1016 compounds were remaining for 10 herbs, followed by structure clustering to sum up chemical scaffolds of herb ingredients. Based on target prediction results performed by our in-house protocol named AlzhCPI, compound-target (C-T) and target-pathway (T-P) networks were constructed to decipher the mechanism of action for anti-AD herbs.

Conclusions: Overall, this approach provided a novel strategy to explore the mechanisms of herbal medicine from a holistic perspective.

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disease which currently affects over 46.8 million people worldwide, characterized by the presence of extracellular deposits of insoluble amyloid-β plaques, neurofibrillary tangles (NFT) and neuronal loss (Alzheimer's, 2015; Wood and Cummings, 2004). Clinically, AD manifests as progressive memory loss followed by a gradual cognitive decline.

Abbreviations: AD, Alzheimer's disease; NFT, neurofibrillary tangles; ChE, cholinesterase; NMDA, N-methyl-D-aspartate; TCM, Traditional Chinese Medicine; MOA, mechanisms of action; TCMSP, traditional Chinese medicine systems pharmacology; TCMID, traditional Chinese medicine integrative database; MW, molecular weight; HBAs, the number of H acceptors; HBDs, the number of H donors; logPo/w, Log octanol/water partition coefficient; b_rotN, the number of rotable bonds; TPSA, topological polar surface area; logS, Log solubility in water; rings, the number of rings; PCA, principal component analysis; RMS, root-mean-square; mt-QSAR, multi-target quantitative structure-activity relationship; C-T network, compound-target network; T-P network, target-pathway network; *G. biloba*, *Ginkgo biloba*; *H. serrata*, *Huperzia serrate*; *S. officinalis*, *Salvia officinalis*; *C. longa*, *Curcuma longa*; *C. chinensis*, *Coptis chinensis*; *P. ginseng*, *Panax ginseng*; *P. tenuifolia*, *Polygala tenuifolia*; *S. miltiorrhiza*, *Salvia miltiorrhiza*; *C. sativus*, *Crocus sativus*; ADMET, absorption, distribution, metabolism, excretion and toxicity; PTGS2, cyclooxygenase-2; ACHE, acetylcholinesterase

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While partially effective in reducing modest symptom, there are no disease-modifying drugs available to halt progression of AD (Rafii and Aisen, 2015). Considering that AD is a multifactorial disease, monotherapy following a single-target principle is not suitable (Carmo Carreiras et al., 2013). In order to develop a successful disease-modifying treatment, scientists have been searching for various combination therapeutic strategies that apply drugs with different mechanisms, such as cholinesterase (ChE) inhibitors in conjunction with cerebrolysin (Alvarez et al., 2011), memantine (Gauthier and Molinuevo, 2013; Howard et al., 2012), and N-methyl-D-aspartate (NMDA) agonists in combination with Vitamin D (Annweiler and Beauchet, 2012; Annweiler et al., 2014). Long-term efficacy evaluation shows that combination therapy slows cognitive and functional decline in AD compared to ChE inhibitor monotherapy and no treatment (Atri et al., 2008; Zhu et al., 2013).

Traditional Chinese Medicine (TCM) has been extensively used for centuries in the treatment of various diseases for the people of Asia. With holistic theory and rich experience in multicomponent therapeutics, TCM offers bright prospects for the prevention and treatment of complex diseases such as AD in a systematic way (Liu et al., 2014; Wu et al., 2011). As being supported by cellular and animal model studies, herbal medicines such as *Ginkgo biloba* (*G. biloba*) (Luo et al., 2002), *Huperzia serrate (H. serrata)* (Desilets et al., 2009) and *Salvia officinalis (S. officinalis)* (Akhondzadeh et al., 2003) were being used in AD as a new pathway for improvement of memory and cognitive function (Anekonda and Reddy, 2005; Sun et al., 2013; Yan et al., 2007).

Being an emerging area of pharmacology, network pharmacology emphasizes the concept of "network target, multicomponent therapeutics," highlighting a holistic thought also shared by TCM (Zhang et al., 2013; Zhao et al., 2010). It affords new possibilities for identifying multi-scale mechanisms of action (MOA) of herbal medicines to treat AD. To explore the mechanism of anti-AD herb, Cao's group performed text mining of PubMed for anti-AD herbs, their ingredients as well as their corresponding targets based on applicable bioinformatics resources and methodologies (Sun et al., 2012). Though the results provided valuable support to explain the MOA of herb medicines intervening AD to some extent, there were still not enough for understanding the complex interactions at different levels. On the one hand, limited herbs as well as ingredients were mined from literatures (from 1995 to 2011), resulting in deficient ingredients collection; On the other hand, targets for herbal ingredients was identified from HIT (Ye et al., 2011), a target database that only covers known targets of 586 herbal compounds, thus the targets for many compounds cannot be collected or predicted.

In this study, a more wide-scale text mining of PubMed for anti-AD references was performed to explore potential anti-AD herbs, and then typical herb ingredients as alternative treatment for AD were summarized. Subsequently, all the herb ingredients were retrieved from online databases such as traditional Chinese medicine systems pharmacology (TCMSP) database (Ru et al., 2014), and drug-likeness analysis was carried out to filter the compounds for further study. Furthermore, network pharmacology approach was used to explore the chemical-protein interactions and potential active ingredients identification for treating AD. Finally, based on network analyses, we interpreted the multi-scale mechanisms of action of herbs in AD management (Fig. 1).

2. Materials and methods

2.1. Constructing herbs and ingredients database for AD

To obtain anti-AD herbs, we conducted a large-scale text mining of PubMed and the manual extraction in the English literatures using the 'herbal medicine' and 'Alzheimer' as search terms. Considering that different herbs were studied to different extent, a variable R was introduced to balance this bias and further evaluated the association between them and AD. R represents the ratio of (AD-herb-related papers)/(herb-related papers). *P*-value was employed to further appraise the chance probability of co-occurrences of each herb and AD in at least *k* articles.

$$P=1-\sum_{i=0}^{k-1} f(i)=1-\sum_{i=0}^{k-1}\frac{\binom{K}{i}\binom{N-K}{n-i}}{\binom{N}{n}}$$

where N represents the total number of articles in PubMed (25.0 million articles, access time: October 7, 2015), *K* stands for the number of references related to AD (81,758 articles), n is the number of articles about one single herb, *k* shows the volume of papers about the effects of corresponding herbs on AD. *P*-value suggests the consequence of relevance between each herb and AD (significant correlation when $P < 0.01$) (Tavazoie et al., 1999; Zhang et al., 2014).

Subsequently, all ingredients of anti-AD herbs were collected from TCMSP database (Ru et al., 2014), TimTec plant extracts database (http://www.timtec.net/), and traditional Chinese medicine integrative (TCMID) database (Xue et al., 2012). In view of the fact that only lycodine alkaloids were identified as active ingredients toward AD for *H. serrata* (Yuan et al., 2012), here other chemical constituents were neglected for this herb.

2.2. Chemical space and drug-likeness calculation

To explore chemical space and drug-likeness of ingredients for AD, 8 physicochemical properties were calculated using Molecular Operating Environment (MOE). The properties included molecular weight (MW), the number of H acceptors (HBAs), the number of H donors (HBDs), Log octanol/water partition coefficient (logP(o/w)), the number of rotable bonds (b_rotN), topological polar surface area (TPSA), Log solubility in water (logS), and the number of rings (rings). Subsequently, 1,630 FDA-approved drugs were downloaded from DrugBank (Wishart et al., 2006), and the same properties were calculated. Principal component analysis (PCA) (Fang et al., 2014; Ma and Dai, 2011) was conducted with the calculated properties. The three largest principal components (PC1, PC2, and PC3) were chosen to compare the structural diversity between anti-AD ingredients and drugs for the scatter plot.

2.3. Drug-likeness filtering and clustering analysis

Drug-likeness filtering was explored based on Lipinski's rule of five (Lipinski, 2004). The rule considers that one compound possesses good absorption or permeation more likely if the molecule has (1) less than five hydrogen-bond donors, (2) less than ten hydrogen-bond acceptors, (3) a molecular weight lower than 500 Da, and (4) a calculated log P (ClogP) lower than five. In this study, anti-AD ingredients that satisfied more than three criteria above were kept for further study.

After drug-likeness filtering, structure clustering analysis was performed based on MDL public keys using the Cluster ligands module in Discovery Studio 4.0. Clustering is based on the root-mean-square (RMS) difference of the Tanimoto distance for fingerprinting. It assigns a set of molecules into subsets or clusters so that each compound possesses similar properties in the same cluster.

2.4. Target profile prediction and target selection

The compound target profiles were predicted using our *in-house* protocol named AlzhCPI (Fang et al., 2015). AlzhCPI predicts CPI spectrum based on multi-target quantitative structure-activity relationship (mt-QSAR) method, which integrated the chemical and pharmacological information derived from the BindingDB database (Liu et al., 2007). AlzhCPI can predict the activity of 51 key targets related to AD.

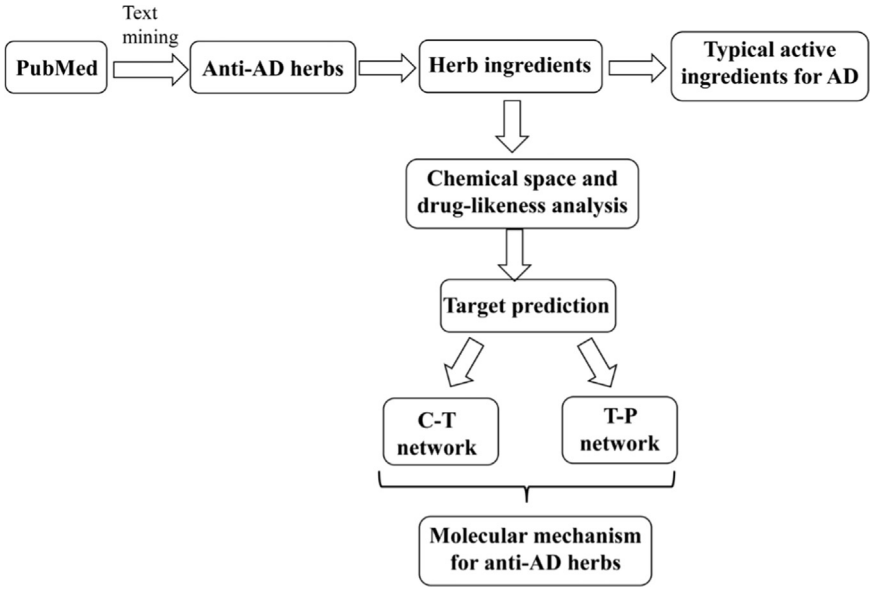

Fig. 1. Workflow for network pharmacology analysis of anti-AD herbal mechanism.

Among 51 NB_ECFP6 models in AlzhCPI, the average of the area under the receiver operating characteristic curve (AUC) for the 5-fold cross-validation is 0.995, whereas that of AUC for the test sets is 0.978.

In this work, 51 NB_ECFP6 models were applied to predict the targets of anti-AD ingredients. Chemical-protein interaction was defined as a potential interaction if the compound could satisfy the following conditions: (1) it was predicted active by corresponding model; (2) the Estategood value of compound was higher than 0.5.

2.5. Network construction

The herbs which include multiple active ingredients might target multiple proteins in the biological network and then the biological system would attain new equilibrium, resulting in reducing the harmful effects (Janga and Tzakos, 2009). To further explore the multi-scale mechanisms of action of herbal ingredients in treating AD, two types of networks were constructed by Cytoscape 3.2.0 (Shannon et al., 2003): (1) compound-target network (C-T network). (2) target-pathway network (T-P network). In the graphical network, each component such as compound, protein and pathway was described by node, and the interactions were encoded by edges. For each node in interaction network, two features were calculated to measure its topological property: (1) 'Degree' was defined as the number of links to node i; (2) 'Node betweenness' was defined as the number of shortest paths between pairs of nodes that run through node i.

Firstly, C-T network was generated based on active ingredients and their corresponding targets. After network analysis, the key targets were further mapped onto KEGG database (http://www.genome.jp/kegg/) (Kanehisa and Goto, 2000) for extracting the canonical pathways that were highly associated with these proteins. Then T-P network was constructed to reflect a global view of the interactions between targets and anti-AD therapy-associated pathways.

3. Results and discussion

3.1. Anti-AD herbs and their ingredients

Text mining results indicated the top 10 herbs ($P \ll 0.01$) possessed significant correlations with Alzheimer (Table 1). Among them, *G. biloba* obtained the optimal *P*-value (1.68E−253) and the second highest R (7.19%), while *H. serrata* got the second best *P*-value (8.73E-35) and the highest R (21.3%). The results were in accordance with Cao's previous study (Sun et al., 2012), which also identified that *G. biloba* and *H. serrata* were the top two anti-AD herbs. However, among 10 herbs, there were 6 herbs were not identified in Cao's mining results. They were *Curcuma longa (C. longa), Panax ginseng (P. ginseng), Coptis chinensis (C. chinensis), Polygala tenuifolia (P. tenuifolia), Crocus sativus (C. sativus)*, and *Salvia miltiorrhiza (S. miltiorrhiza)*. This was because a more extensive scope of herbs and references were considered in this work.

Table 1
Statistics and association analysis between top 10 herbs and AD.

Herb Name	Abbreviation	*k*	*n*	R (%)	*P*-value
Ginkgo biloba	*G. biloba*	3699	266	7.19	1.68E−253
Huperzia serrata	*H. serrata*	108	23	21.30	8.73E−35
Curcuma longa	*C. longa*	2442	29	1.19	4.84E−09
Panax ginseng	*P. ginseng*	5350	42	0.79	2.50E−07
Coptis chinensis	*C. chinensis*	434	11	2.53	2.52E−07
Melissa officinalis	*M. officinalis*	1689	20	1.18	1.03E−06
Ginkgo folium	*G. folium*	558	11	1.97	2.74E−06
Polygala tenuifolia	*P. tenuifolia*	132	6	4.55	5.20E−06
Crocus sativus	*C. sativus*	613	11	1.79	6.50E−06
Salvia miltiorrhiza	*S. miltiorrhiza*	1944	18	0.93	7.27E−05

Further research suggested that these 10 herbal medicines or their corresponding herbal constituents were widely used for treating AD all around the world. Here we summarized 13 typical compounds (Fig. S1-2) from the 6 herbs which were not included in Cao's results as alternative treatment for AD (Table 2) by retrieving references from PubMed. For instance, curcumin, as a hydrophobic polyphenol isolated from *C. longa*, was reported to be involved in antioxidant, anti-inflammatory, metal chelators, anti-amyloid, anti-tau, and neuroprotective activities (Kim et al., 2014). Berberine, an isoquinoline alkaloid isolated from *C. chinensis*, could reduce Aβ levels by modulating APP processing (Asai et al., 2007) and suppress amyloid-beta-induced inflammatory response in microglia by inhibiting NF-kB and MAPK signaling pathways *in vitro* (Jia et al., 2012), and ameliorates β-amyloid pathology, gliosis, and cognitive impairment *in vivo* (Durairajan et al., 2012). Ginsenoside Rd, one of the main active ingredients in *P. ginseng*, decreased tau phosphorylation in AD rats model (Liu et al., 2012). Other active ingredients such as Ginsenoside Rg1, Rg2, and Rg3 were also reported to be neuroprotective against cytotoxicity *in vitro*. For example, Ginsenoside Rg1 inhibited beta-secretase activity *in vitro* and protected against Aβ-induced cytotoxicity

Table 2
Summary of findings on typical herb ingredients from 6 mined anti-AD herbs as alternative treatment for AD.

Compound	Herb Name	Reference	Model	Mechanism
Curcumin	*C. longa*	PMID: 23901044	SK-N-SH cell (neuroblastoma)	Protect neuronal-like cells against acrolein by restoring Akt and redox signaling pathways.
Curcumin	*C. longa*	PMID: 24188406	SH-SY5Y cell	Attenuate amyloid-beta-induced tau hyperphosphorylation involving PTEN/Akt/GSK-3beta signaling pathway
Curcumin	*C. longa*	PMID: 22328947	rat primary hippocampal neurons	Decrease ROS level and protected from intracellular Aβ toxicity
Curcumin	*C. longa*	PMID: 22180352	pAPPswe-transfected SH -SY5Y cell	Mediate presenilin-1 activity to reduce beta-amyloid production
Curcumin	*C. longa*	PMID: 23954730	Wistar rat	Suppress beta-amyloid-induced cognitive impairments by mediating BDNF and Akt/GSK-3 beta signaling pathway
Curcumin	*C. longa*	PMID: 26651837	rat primary hippocampal neurons	Protect against ET-1-mediated cell death through blocking an increase in c-Jun levels.
berberine	*C. chinensis*	PMID: 17125739	human neuroglioma H4 cells	Reduce Abeta levels by modulating APP processing
berberine	*C. chinensis*	PMID: 22943182	Murine primary cells; microglia cells; BV2 cells	Suppress amyloid-beta-induced inflammatory response in microglia by inhibiting NF-kB and MAPK signaling pathways.
berberine	*C. chinensis*	PMID: 22152059	HEK293 cells	Decrease the production of beta-amyloid40/42 by inhibiting the expression of BACE via activation of the ERK1/2 pathway.
berberine	*C. chinensis*	PMID: 22459600	TgCRND8 mice	Ameliorates β-amyloid pathology, gliosis, and cognitive impairment
berberine	*C. chinensis*	PMID: 22459600	N2a-SwedAPP cells	Regulat APP processing and the hyperphosphorylation of tau via the Akt/GSK3 signaling pathway
berberine	*C. chinensis*	PMID: 26446867	ICV-STZ-induced sporadic dementia rats	Significantly attenuate behavioral, biochemical, cellular, and histological alterations
ginsenoside Rg1	*P. ginseng*	PMID: 20183297	PC12 cells	Inhibit beta-secretase activity and protect against Abeta-induced cytotoxicity.
ginsenoside Rg1	*P. ginseng*	PMID: 22982182	SK-N-SH neuroblastoma cells	Attenuate tau phosphorylation and the involvement of p38 pathway activation.
ginsenoside Rg1	*P. ginseng*	PMID: 22617143	SK-N-SH neuroblastoma cells	Protect against Aβ(1–40)-induced neuronal injury and apoptosis, likely through its anti-inflammatory mechanism.
ginsenoside Rg1	*P. ginseng*	PMID: 22381145	Cortical neurons from C57BL/6 mouse fetuses	Attenuate Aβ-induced neuronal death through the suppression of intracellular mitochondrial oxidative stress
ginsenoside Rg1	*P. ginseng*	PMID: 21438847	Primary cultured hippocampal neurons	Protect against cell apoptosis induced by β-amyloid protein probably by up-regulating the ratio of Bcl-2/Bax
ginsenoside Rg1	*P. ginseng*	PMID: 22015470	Tg mAPP mice	Ameliorate amyloid pathology, modulate APP process, improve cognition, and activate PKA/CREB signaling.
ginsenoside Rg1	*P. ginseng*	PMID: 23588117	Sprague Dawley rats	Possiblely decrease OKA-induced memory impairment through GSK3β/tau signaling pathway and/or attenuating Aβ formation.
ginsenoside Rg1	*P. ginseng*	PMID: 25340298	NG108-15 murine neuroglial cell	Suppress the signaling transduction pathway of TLR3 and TLR4, and decrease the inflammation factors induced by Aβ25–35
Ginsenoside Rd	*P. ginseng*	PMID: 24792734	Sprague-Dawley rats	Attenuate tau protein phosphorylation via the PI3K/AKT/GSK-3β pathway after transient forebrain ischemia.
Ginsenoside Rd	*P. ginseng*	PMID: 21945003	Sprague Dawley rats; cultured cortical neurons	Decrease OA-induced the hyperphosphorylation of tau by the increase in activities of PP-2A
Ginsenoside Rg5	*P. ginseng*	PMID: 24503167	STZ-induced memory impaired rats	Improve cognitive dysfunction and beta-amyloid deposition via attenuating neuroinflammatory responses.
Ginsenoside Rg3	*P. ginseng*	PMID: 19222911	SweAPP-transfected-SK-N-SH cells	Promote beta-amyloid peptide degradation by enhancing gene expression of neprilysin
ginsenoside Rg2	*P. ginseng*	PMID: 17257792	PC12 cells	Protect against glutamate-induced neurotoxicity through mechanisms related to anti-oxidation and anti-apoptosis.
Tenuifolin	*P. tenuifolia*	PMID:19208093	COS-7 cells	Decrease Abeta secretion without altering the ratio of Abeta1-40 and Abeta-42.
Tenuifolin	*P. tenuifolia*	PMID: 25444865	PC12 cells; C57BL/6J mice injected Aβ25-35	Possess neuroprotective effects against Aβ25-35-induced apoptosis in PC12 cells and significantly improved the cognitive deficits in vivo.
Tanshinone IIA	*S. miltiorrhiza*	PMID: 20800073	rat cortical neurons	Protect against Aβ25-35-induced neurotoxicity through its antioxidative potential.
Tanshinone IIA	*S. miltiorrhiza*	PMID: 24859152	injection of the amyloid beta protein (Aβ) in rats	Inhibit iNOS, MMP-2 and NF-κBp65 transcription and translation in AD rats
Salvianolic acid A	*S. miltiorrhiza*	PMID: 23703159	SH-SY5Y cells; transgenic Caenorhabditis elegans	Significantly inhibit Aβ self-aggregation and blocks the formation of ROS in vitro; alleviates Aβ-induced paralysis in vivo
Salvianolic acid B	*S. miltiorrhiza*	PMID: 23461850	Mice injected with Aβ25–35 peptide	Exert neuroprotective activity via anti-inflammatory and anti-oxidative effects
Cryptotanshinone	*S. miltiorrhiza*	PMID: 19774505	scopolamine-induced AD rats	Improve task learning in rats with scopolamine-induced cognitive impairment as a dual cholinesterase inhibitor
Cryptotanshinone	*S. miltiorrhiza*	PMID: 19154776	APP/PS1 mice; rat cortical neuronal cells	Improve the cognitive ability in vivo and promote APP metabolism toward the non-amyloidogenic products pathway in vitro
Crocin	*C. sativus*	PMID: 26484504	male albino Wistar rats	Significantly improve the memory impairment caused by beta amyloid injection; restore apoptotic biomarkers induced by Aβ
Crocin	*C. sativus*	PMID: 20683499	STZ-icv in male rats	Significantly prevent the cognitive impairments following icv injection of STZ

Table 3
Statistics of molecular descriptors of herb ingredients and FDA-approved drugs.

	Herb ingredients				Drugs			
Descriptors	MEAN	MAX	MIN	Median	MEAN	MAX	MIN	Median
b_rotN	5.28 ± 6.11	50	0	3	6.21 ± 6.04	58	0	5
lip_acc	4.23 ± 5.51	46	0	2	6.36 ± 5.41	52	0	5
lip_don	1.99 ± 3.22	30	0	1	2.75 ± 3.69	28	0	2
logP(o/w)	3.56 ± 3.30	21.95	−8.198	3.05	2.22 ± 2.62	13.68	−13.42	2.42
logS	(−4.56) ± 3.01	1.95	−24.44	−3.96	(−3.72) ± 2.56	2.53	−18.57	−3.61
rings	2.29 ± 2.12	18	0	2	2.79 ± 1.78	15	0	3
TPSA	70.12 ± 88.75	777.98	0	40.13	92.82 ± 86.42	785.86	0	74.6
Weight	311.75 ± 213.93	1727.60	45.02	264.32	376.02 ± 224.92	1817.72	4	332.46

in PC12 cells (Wang and Du, 2009), while Ginsenoside Rg2 showed protective effects against glutamate-induced neurotoxicity in PC12 cells (Li et al., 2007). Ginsenoside Rg3 promoted beta-amyloid peptide degradation by enhancing gene expression of neprilysin (Yang et al., 2009).

After the top 10 anti-AD herbs were identified, a total of 1,149 compounds were finally collected from multiple database sources. Among them, *G. folium* had the largest numbers of chemicals (250 compounds), following by *C. longa* with 222 chemicals and *S. miltiorrhiza* possessing 206 molecules.

3.2. Drug-likeness properties and chemical space of compounds among top 10 herbs

The drug-likeness properties of a compound play a crucial role in its absorption, distribution, metabolism, excretion and toxicity (ADMET) properties. Comparing important molecular descriptors of anti-herb ingredients with FDA-approved drugs can provide insight into the ADMET properties of the ingredients. Here, 8 physicochemical characteristics of the ingredients and FDA-approved drugs are listed in Table 3. Except for logS and rings, the distributions of other descriptors for two groups of compounds are given in Fig. 2A–F. As shown in Table 3, except for logP(o/w), the statistical means of descriptors of herb ingredients are smaller than those of FDA-approved drugs. Fig. 2 indicates the overall distribution of descriptors is similar between herb ingredients and approved drugs. The majority of two groups of compounds are located within the scope of "rule of five". However, compared with approved drugs, the higher frequency of logP(o/w) are distributed beyond the scope of −5 to 5 (Fig. 2D), which might indicate that herb ingredients possess the poorer absorption than approved drugs.

Principal component analysis was further conducted to give visual illustration in chemical space. As seen in Fig. 3, compared with the distribution of herb ingredients, the distribution of approved drugs is scattered more widely in chemical space. At the same time, there are obvious overlaps between the two groups of compounds in chemical space, which implies that many herb ingredients have drug potential.

3.3. Drug-likeness filtering and clustering analysis

After the drug-likeness filtering, 1016 compounds (as given in Table S1) were kept for further study, which obeyed three criteria of Lipinski's rule at least. The detailed distribution information of 1016 compounds on the 10 herbs are given in Table 4. As seen in Table 4, both of *G. biloba* (the fruits of *Ginkgo*) and *G. folium* (the leaves of *Ginkgo*) belong to ginkgoaceae, which contains 294 (63+231) out of 1016 compounds in total.

Clustering analysis was performed to give an overview of chemical

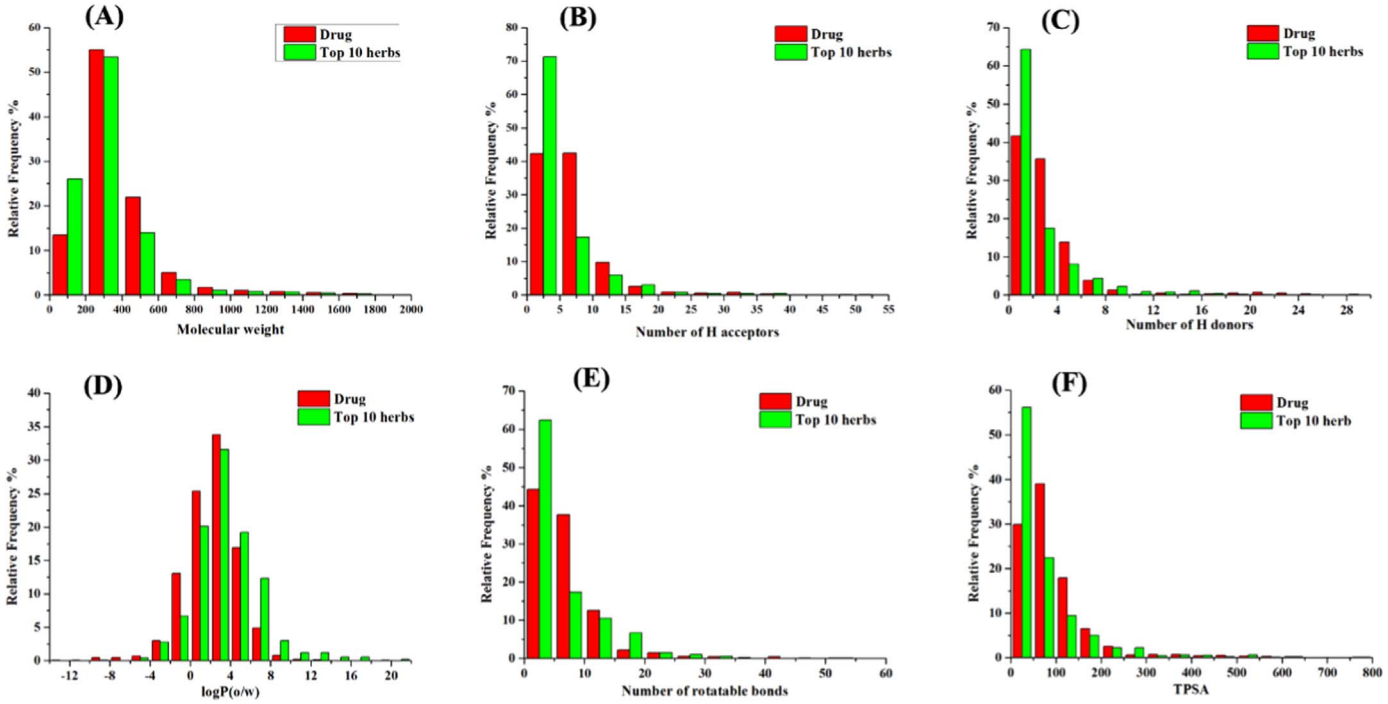

Fig. 2. Distribution of six molecular descriptors of herb ingredients and approved drugs.

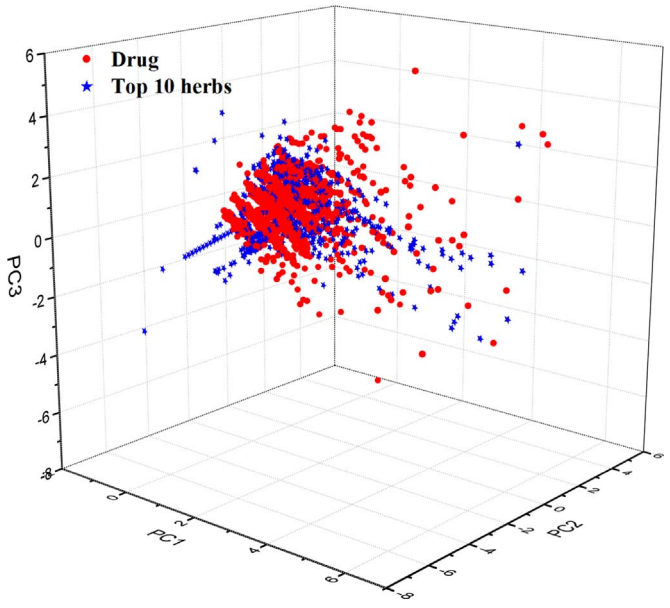

Fig. 3. The distribution in chemical space according to principal component analysis of herb ingredients and FDA-approved drugs. The red dots and blue stars represent FDA-approved drugs and herb ingredients, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 4
The detailed distribution information of 1016 compounds together with chemical IDs.

Herb Name	Number of chemicals	Chemical IDs
Ginkgo biloba	63	GB01-GB63
Huperzia serrata	9	HS01-H09
Curcuma longa	221	CR001-CR221
Panax ginseng	145	PG001-PG145
Coptis chinensis	30	HL01-HL30
Melissa officinalis	41	MO01-MO41
Ginkgo folium	231	GF001-GF231
Polygala tenuifolia	27	PT01-PT27
Crocus sativus	62	CS01-CS62
Salvia miltiorrhiza	187	SM001-SM187

scaffolds of herb ingredients. As shown in Fig. 4A, 1016 compounds are clustered into 10 groups, and each group has similar chemical features. Among them, cluster 8 (Cluster center: PG126) has the largest numbers of chemicals (294 compounds) mainly featured as steroids, following by cluster 2 (Cluster center: GB32) with 214 molecules characterized by unsaturated fatty acids or alkyls. The structures of cluster center are also shown in Fig. 4B. Other compounds in the same cluster possess similar properties with the structure of cluster center.

3.4. Network pharmacology

As an empirical system of multicomponent therapeutic, herb medicines exert extensive biological and pharmacological effects through multiple compounds and targets. To uncover the mechanism of action between herb ingredients and targets toward AD, we constructed the compound-target (C-T) network based on 1016 herb ingredients and potential targets. As shown in Fig. 5, the C-T network embodies 936 nodes (895 ingredients and 41 potential targets) and 2397 C-T interactions (Table S2). Network analysis suggests that the average number of targets for each ingredient is 2.68, while the average degree for each target is 58.46. Nearly half of compounds (452 out of 895 compounds) are linked with greater than or equal to two target, indicating the multi-target properties of herbal ingredients.

We also investigated the distributions of 2397 C-T interactions on the 10 herbs. As suggested in Fig. 6, *G. folium* has the largest number of C-T interactions (660, 28%), following by *C. longa* with 404 C-T interactions (17%) and *S. miltiorrhiza* possessing 336 interactions (14%). The results might imply that the three herbs contain more active ingredients in treating AD compared with other herbs.

After deleting compound nodes of which degree is lower than or equal to 4, 173 compound nodes that can target at more than 4 proteins simultaneously and 35 potential targets were kept. As seen in Fig. 7, the compound nodes are arranged by circles according to their degrees. Circle 1st represents compound nodes of which degree are greater than or equal to 10, following by circle 2nd (degree=7 to 9) and circle 3rd (degree=5 or 6). Among them, SM040, also named GF140 (luteolin, PubChem CID:5280445), and GF106 (chrysoeriol, PubChem CID:5280666), the compounds extracted from *S. miltiorrhiza* and *G. folium*, of which scaffold belong to flavones, have the largest number of potential targets (degree=12) (Fig. 8). In fact, luteolin was reported to inhibit glycogen synthase kinase 3(GSK3, IC_{50}=0.8 μM, PMID 15689157) (Lu et al., 2005), cyclin-dependent kinase 5 (CDK5, IC_{50}=3.8 μM, PMID 15689157), $A\beta_{1-42}$ aggregation (IC_{50}=6.4 μM, PMID 24878198) (Churches et al., 2014), lipoxygenase (ALOX, IC_{50}=3.2 μM PMID 16143531) (Rao et al., 2005), and TNF-alpha (TNF, IC_{50}=5.8 μM, PMID 12951092) production in RBL-2H3 cells (Matsuda et al., 2003), while chrysoeriol was reported to inhibit TNF-

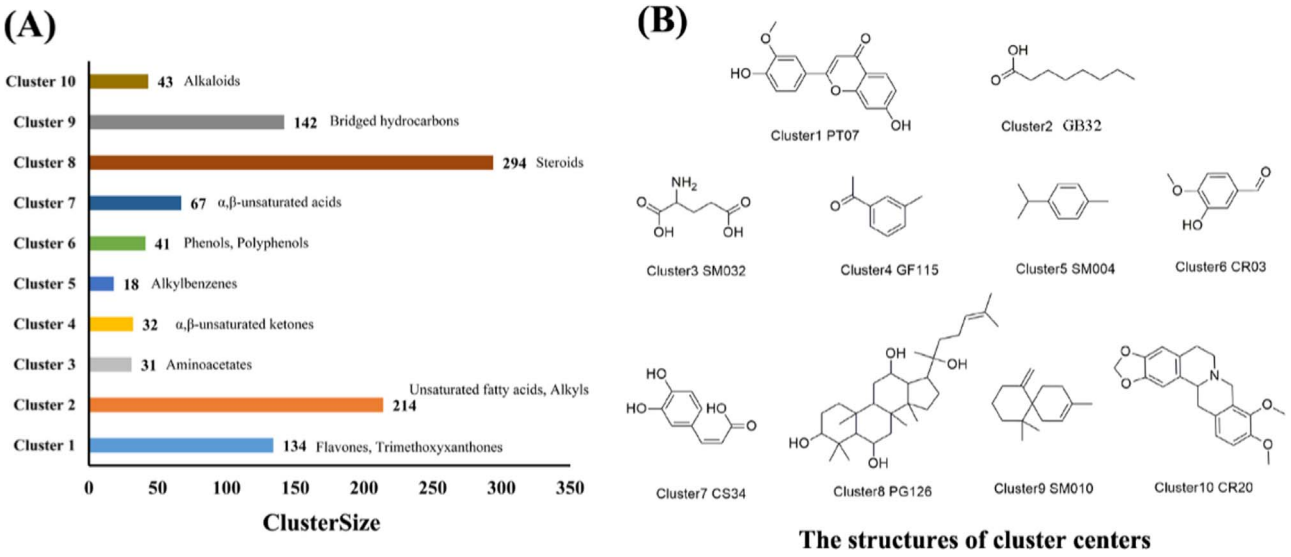

Fig. 4. (A) Structure clustering of 1016 herb ingredients based on MDL public keys; (B) the structures of cluster center for each cluster.

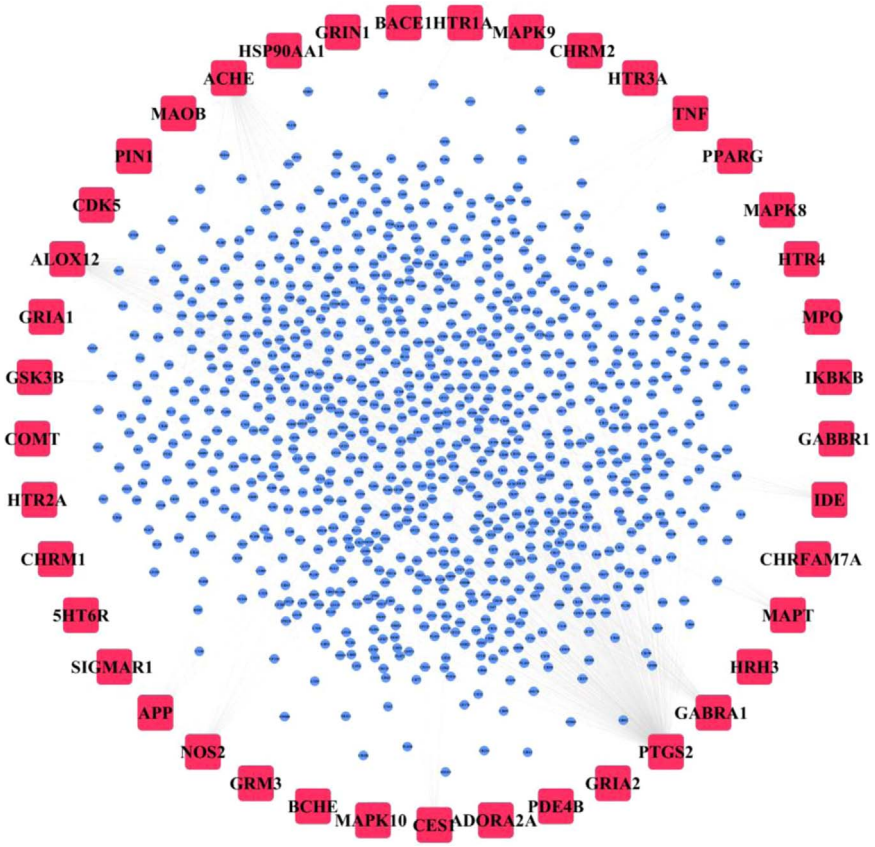

Fig. 5. The whole compound-target network of herb ingredients (filled in light blue, and targets (filled in red). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

alpha (TNF, IC_{50}=22 μM, PMID 10096854) expression in LPS-stimulated human monocytes (Manthey et al., 1999). All of these activities above are consistent with our predicted results, which indicates the reliability of target prediction.

Considering that most of 1016 ingredients have low concentrations in herbs, here we also focused on 20 typical herb ingredients (including 13 in Tables 2 and 7 in Cao's article (Sun et al., 2012)) among the top 10 herbs, and explored their potential targets. As depicted in Fig. 9, the degree analysis suggested that the target could interact with multiple molecules (4.06 compounds per target on average), and one compound could also target several proteins related to AD (3.25 targets per compound on average). From a holistic perspective, 20 compounds can target 16 proteins related to AD. Among 20 compounds, both of quercetin (PubChem CID: 5280343) and kaempferol (PubChem CID: 5280863) were predicted active against 10 targets, respectively, followed by curcumin (PubChem CID: 969516) against 9 targets. Among 16 targets, cyclooxygenase-2 (PTGS2) could interact with 17 compounds, followed by acetylcholinesterase (ACHE) with 13 compounds.

To further decipher the action mechanism of potential targets, the

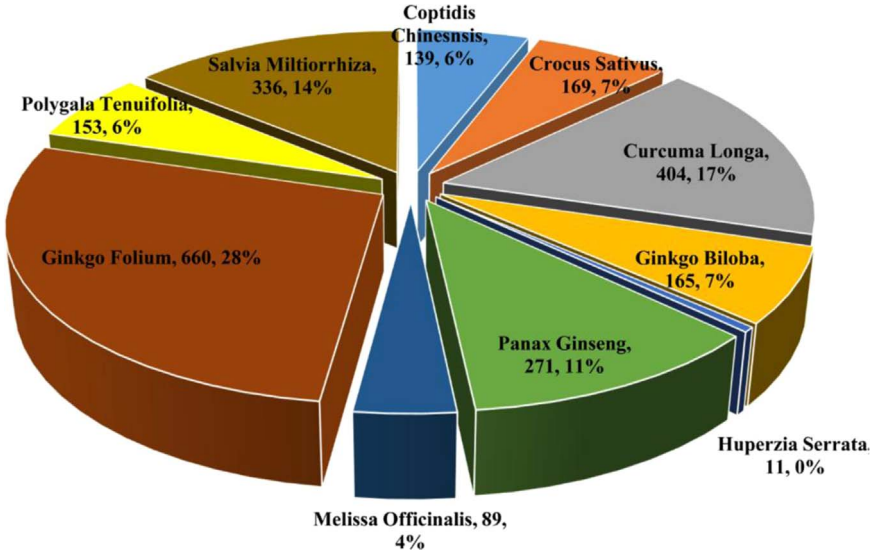

Fig. 6. The distributions of 2,397 C-T interactions on the 10 herbs.

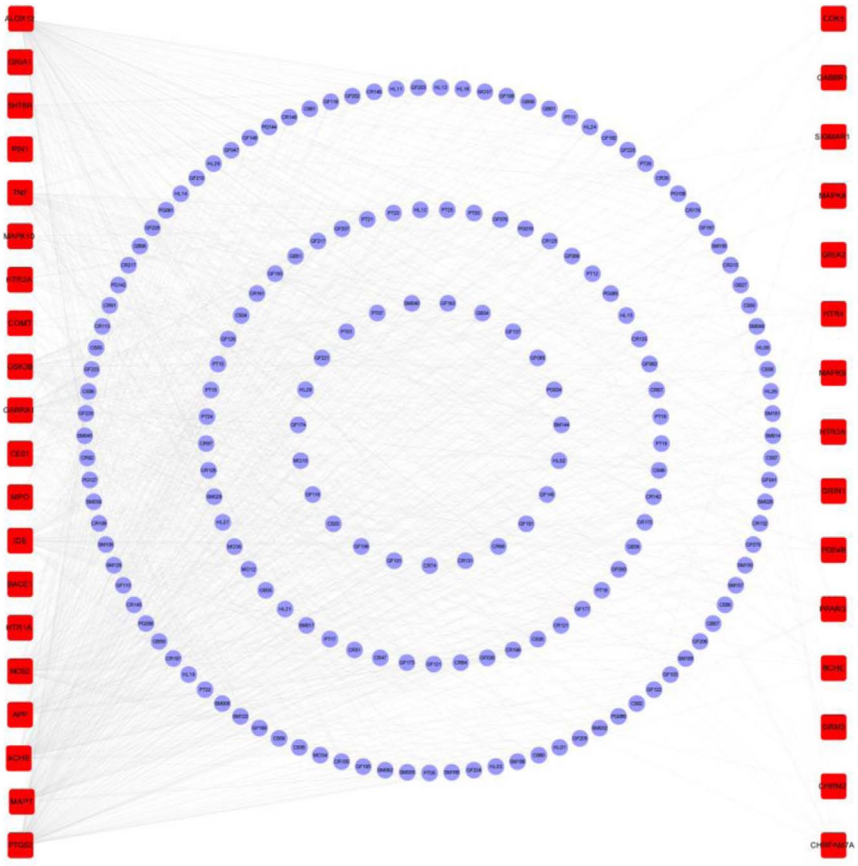

Fig. 7. The compound-target network of herb ingredients (filled in light blue) of which degree is greater than or equal to 5 and targets (filled in red). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

targets of which degree lower than 5 in Fig. 7 are deleted, resulting in 24 potential targets (displayed in Table S3). Subsequently, ClueGO, a Cytoscape plugin, was used to identify biological interpretation and molecular functions for the 24 targets. As shown in Fig. 10A, the biological processes of the targets are mainly consisted of three groups: learning or memory, negative regulation of synaptic and serotonin receptor signaling pathway. In Fig. 10B, the main molecular functions are classified into three categories: beta-amyloid binding, serotonin receptor activity and extracellular ligand-gated ion channel activity.

To better understand the major pathways among the 10 herbs for Alzheimer therapy, we retrieved the 24 potential targets on KEGG database for extracting the canonical pathways, then built T-P network. As seen in Fig. 11, the network contains 143 nodes (23 proteins and 120 pathways) and 222 edges, which has an average degree of 9.65 per target and 1.85 per pathway, respectively. The results suggest that most of pathways are involved in a small number of targets, while nearly half of the targets (11 out of 23) locate in multiple pathways (n≥5).

After deleting the pathways that involved in less than or equal to two targets, the new network is composed of 46 nodes (24 pathways and 22 proteins) and 94 edges. The name of 24 pathways and 22

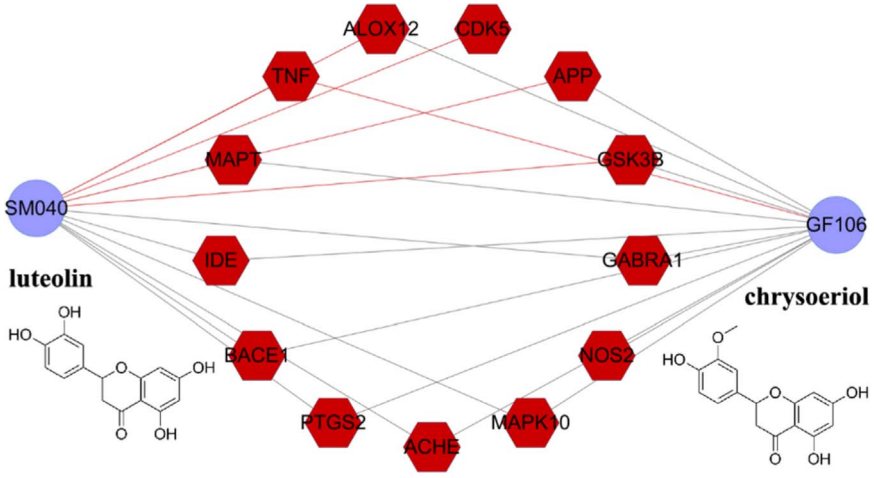

Fig. 8. The compounds-target network of herb ingredients (SM040 and GF106, filled in light blue) and targets (filled in red). The red edge stands for the bioassay activity validated by experiments, and the gray edge represents for the remaining predicted interactions which merit further validation by experiments. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

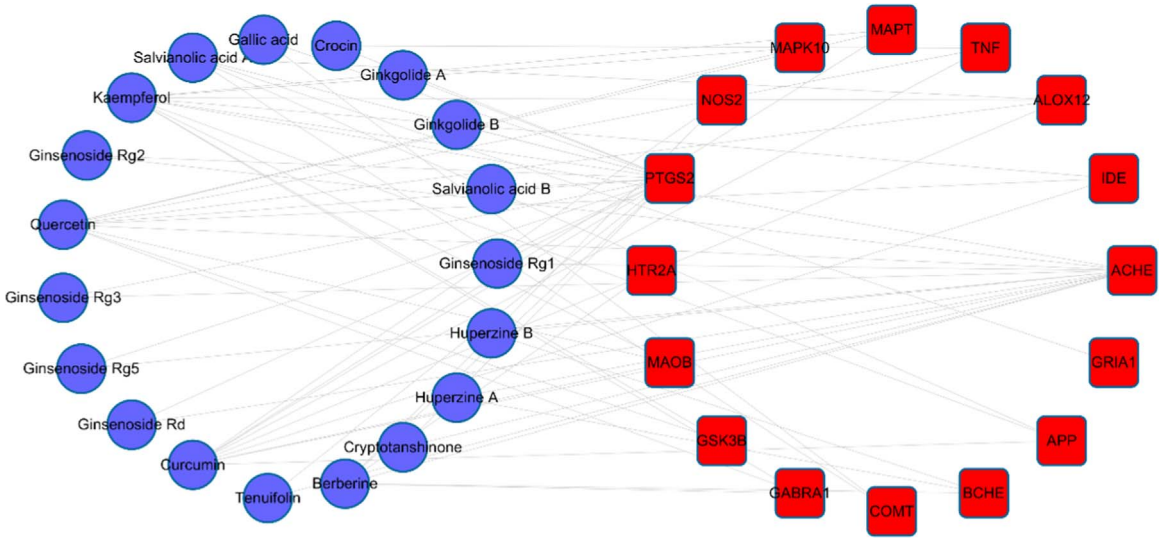

Fig. 9. The compounds-target network of 20 typical herb ingredients (ellipse) and targets (round rectangle).

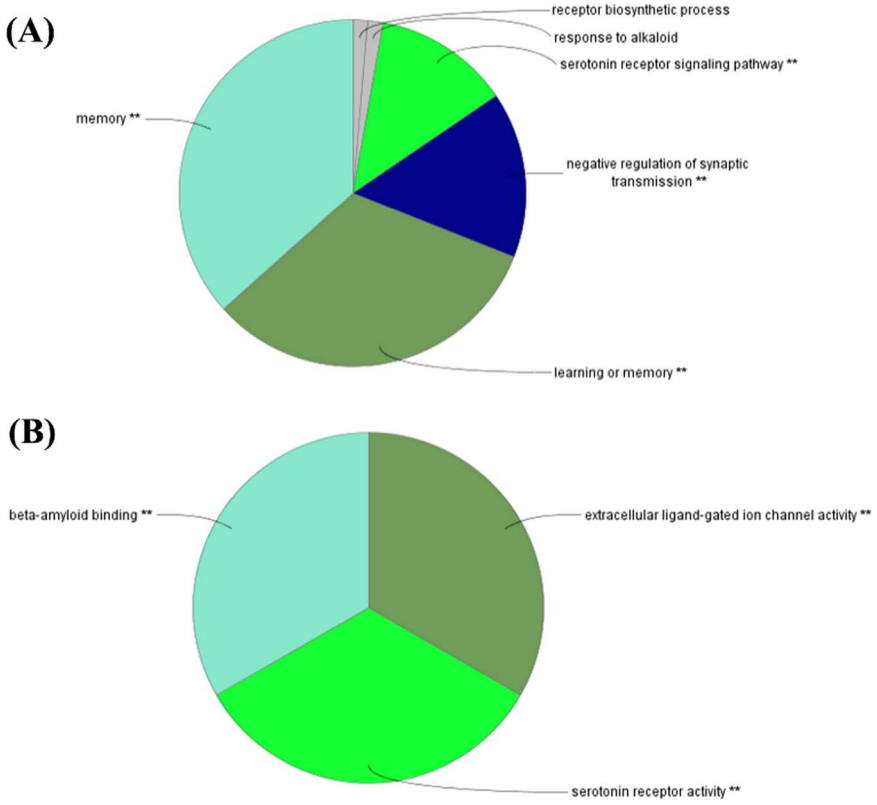

Fig. 10. ClueGO analysis of the 24 potential targets. (A) Representative biological processes among targets. (B) Representative molecular functions among targets.

proteins involved in are given in Table 5. The results shows that these pathways intensively connected to targets, such as Alzheimer's disease, neuroactive ligand-receptor interaction, serotonergic synapse, metabolic pathways, calcium signaling pathway, cAMP signaling pathway, pathways in cancer, inflammation, and so on.

Actually, these pathways have already been testified and tightly linked to the pathogenesis of AD (Anand et al., 2014). For example, the pathway neuroactive ligand-receptor interaction (hsa04080) could modulate the neurotransmitters such as glutamate, serotonin, cholinergic receptors, and histamine receptor, which plays an important role in cognition and memory. The calcium hypothesis states that disruption of Ca^{2+} signaling pathway (hsa04020) /homeostasis underlies the pathogenesis of AD. It was proposed that Aβ peptides form Ca^{2+} permeable pores and bind to and modulate NMDAR, AMPAR, mGluR5 and VGCC, leading to the overfilling of neurons with calcium ions (Bezprozvanny, 2009).

4. Conclusion

Network pharmacology has become a useful tool in identifying alternative targets for herbal medicines and developing multi-target drugs. It focuses on the complex interactions in biological systems from a holistic perspective, rather than altering the single molecular component. To date, the lack of satisfactory treatment remains a challenge for AD in the modern medicine. Traditional Chinese Medicines have accumulated rich theories and valuable experience in

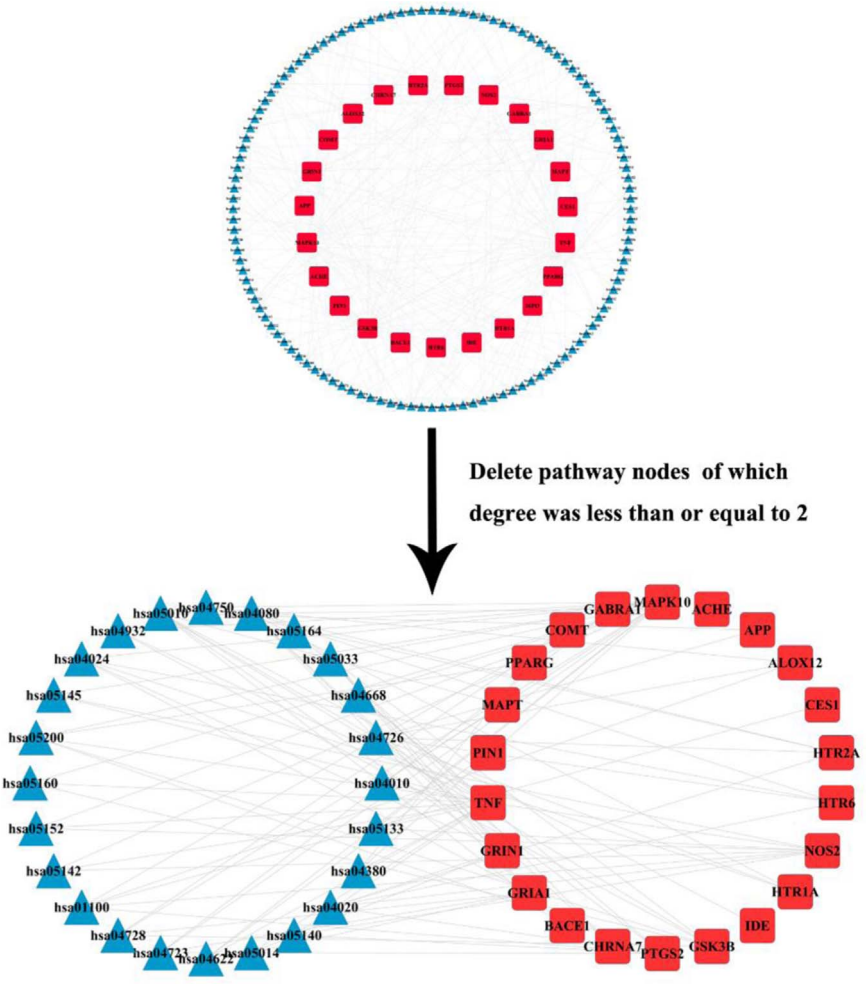

Fig. 11. Target-pathway network of anti-AD herbs where red nodes represent the targets and turquoise nodes signify pathways.

Table 5
The 24 pathways and 22 proteins involved in.

Pathway_ID	Related proteins	Number of genes	Pathway name
hsa04080	GABRA1, GRIA1, HTR6, HTR1A, HTR2A, CHRNA7, GRIN1	7	Neuroactive ligand-receptor interaction
hsa05010	IDE, MAPT, APP, TNF, BACE1, GSK3B, GRIN1	7	Alzheimer's disease
hsa04726	ALOX12, PTGS2, APP, HTR6, HTR1A, HTR2A	6	Serotonergic synapse
hsa01100	ALOX12, PTGS2, COMT, NOS2, CES1	5	Metabolic pathways
hsa04020	NOS2, HTR6, HTR2A, CHRNA7, GRIN1	5	Calcium signaling pathway
hsa04024	GRIA1, HTR6, MAPK10, HTR1A, GRIN1	5	cAMP signaling pathway
hsa05200	PTGS2, NOS2, MAPK10, GSK3B, PPARG	5	Pathways in cancer
hsa04723	GABRA1, PTGS2, GRIA1, MAPK10	4	Retrograde endocannabinoid signaling
hsa04728	COMT, GRIA1, MAPK10, GSK3B	4	Dopaminergic synapse
hsa05033	GABRA1, GRIA1, CHRNA7, GRIN1	4	Nicotine addiction
hsa04010	MAPT, TNF, MAPK10	3	MAPK signaling pathway
hsa04380	TNF, MAPK10, PPARG	3	Osteoclast differentiation
hsa04622	TNF, MAPK10, PIN1	3	RIG-I-like receptor signaling pathway
hsa04668	PTGS2, TNF, MAPK10	3	TNF signaling pathway
hsa04750	ALOX12, MAPK10, HTR2A	3	Inflammatory mediator regulation of TRP channels
hsa04932	TNF, MAPK10, GSK3B	3	Non-alcoholic fatty liver disease (NAFLD)
hsa05014	TNF, GRIA1, GRIN1	3	Amyotrophic lateral sclerosis (ALS)
hsa05133	TNF, NOS2, MAPK10	3	Pertussis
hsa05140	PTGS2, TNF, NOS2	3	Leishmaniasis
hsa05142	TNF, NOS2, MAPK10	3	Chagas disease (American trypanosomiasis)
hsa05145	TNF, NOS2, MAPK10	3	Toxoplasmosis
hsa05152	TNF, NOS2, MAPK10	3	Tuberculosis
hsa05160	TNF, MAPK10, GSK3B	3	Hepatitis C
hsa05164	TNF, MAPK10, GSK3B	3	Influenza A

the prevention and treatment of AD. Thus, based on network pharmacology, efforts should be paid to understand what the active herbal ingredients is, and how they interact to treat AD.

In this study, we applied a network pharmacology method, which integrated wide-scale text-mining, drug-likeness filtering, target prediction and network analysis to dissect the underlying molecular mechanism of the most widely studied medicinal herbs for AD treatment. The main findings are summarized as follows:

(1) A large-scale text mining results shows that 10 herbs such as *G. biloba, H. serrata, C. longa*, etc. possess significant correlations with AD.
(2) Chemical space and drug-likeness properties indicate that many herb ingredients have drug potential.
(3) After the drug-likeness filtering, 1016 compounds were kept for 10 herbs, followed by structure clustering to visualize chemical scaffolds of herb ingredients.
(4) Based on the results of target fishing, C-T network exhibits that these 10 anti-AD herbs probably acts on 41 targets, and then T-P network suggests that these herb ingredients have therapeutic benefits in AD through multi-pathway such as modulating neuroactive ligand-receptor interaction, calcium signaling pathway, and inflammation-related proteins. Our results provided new insights into understanding the mechanism of action for anti-AD herbs.

Conflict of interest

All the authors do not have any conflicts of interest.

Author contributions

Conceived and designed the experiments: Jiansong Fang (fangjs@gzucm.edu.cn), Wen Tan (went@scut.edu.cn) and Qi Wang (wqitcm@qq.com); Manuscript writing: Jiansong Fang; Data acquisition/interpretation: Ling Wang (lingwang@scut.edu.cn), Tian Wu (674450883@qq.com), Cong Yang (754995975@qq.com), Li Gao (gaoli5945lily@yeah.net), Haobin Cai (vinsonodin@163.com); Manuscript revision/review: Junhui Liu (451947085@qq.com), Shuhuan Fang (fangshuhuan@gzucm.edu.cn), Yunbo Chen (ybchengz@gzucm.edu.cn).

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.jep.2016.11.034.

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