Computational and Structural Biotechnology Journal 23 (2024) 506–519

Contents lists available at ScienceDirect

Computational and Structural Biotechnology Journal

journal homepage: www.elsevier.com/locate/csbj

Network-based identification and mechanism exploration of active ingredients against Alzheimer's disease via targeting endoplasmic reticulum stress from traditional chinese medicine

Zhao Dai [a,1], Tian Hu [a,1], Junwen Wei [a], Xue Wang [a], Chuipu Cai [a], Yong Gu [b], Yunhui Hu [c], Wenjia Wang [c], Qihui Wu [b,*], Jiansong Fang [a,*]

[a] Science and Technology Innovation Center, Guangzhou University of Chinese Medicine, Guangzhou 510405, China
[b] Clinical Research Center, Hainan Provincial Hospital of Traditional Chinese Medicine, Hainan Medical University, Haikou 570100, China
[c] Tasly Pharmaceutical Group Co., Ltd., Tianjin 300402, China

ARTICLE INFO

Keywords:
Alzheimer's disease
Anti-AD ingredients
Kidney-tonifying herbs
Endoplasmic reticulum stress
Endophenotype network

ABSTRACT

Alzheimer's disease is a neurodegenerative disease that leads to dementia and poses a serious threat to the health of the elderly. Traditional Chinese medicine (TCM) presents as a promising novel therapeutic therapy for preventing and treating dementia. Studies have shown that natural products derived from kidney-tonifying herbs can effectively inhibit AD. Furthermore, endoplasmic reticulum (ER) stress is a critical factor in the pathology of AD. Regulation of ER stress is a crucial approach to prevent and treat AD. Thus, in this study, we first collected kidney-tonifying herbs, integrated chemical ingredients from multiple TCM databases, and constructed a comprehensive drug-target network. Subsequently, we employed the endophenotype network (network proximity) method to identify potential active ingredients in kidney-tonifying herbs that prevented AD via regulating ER stress. By combining the predicted outcomes, we discovered that 32 natural products could ameliorate AD pathology via regulating ER stress. After a comprehensive evaluation of the multi-network model and systematic pharmacological analyses, we further selected several promising compounds for in vitro testing in the APP-SH-SY5Y cell model. Experimental results showed that echinacoside and danthron were able to effectively reduce ER stress-mediated neuronal apoptosis by inhibiting the expression levels of BIP, p-PERK, ATF6, and CHOP in APP-SH-SY5Y cells. Overall, this study utilized the endophenotype network to preliminarily decipher the effective material basis and potential molecular mechanism of kidney-tonifying Chinese medicine for prevention and treatment of AD.

1. Introduction

Alzheimer's disease (AD) is the most prevalent dementia. It is primarily characterized by cognitive impairment, which affects the physical and mental well-being of patients[1]. A statistical study in 2020 of China estimated that 65.2% (9.83 million / 15.07 million) of dementia patients aged 60 and older were AD patients[2]. To date, there are still no approved drugs that can actually reverse the progression of AD. Despite the initial approvals of aducanumab and GV-971 for the treatment of AD in the US and China[3], controversies persist surrounding these two drugs. Thus, the identification of potential and effective therapeutic drugs for the prevention and treatment of AD is highly significant.

According to the theory of Traditional Chinese Medicine (TCM), insufficient kidney essence can lead to dementia[4]. The utilization of kidney-tonifying herbs represents one of the fundamental therapeutic approaches within TCM for treating dementia. Indeed, contemporary pharmacological investigations have demonstrated the potential therapeutic efficacy of various bioactive components derived from kidney-tonifying herbs against AD. For example, Lycium barbarum polysaccharides, extracted from the kidney-tonifying herb Lycium chinense (GouQiZi), ameliorate spatial memory deficits by negatively regulating the apoptotic signaling cascade to play a neuroprotective role [5]. Osthole, a natural coumarin derivative derived from the

* Corresponding authors.
E-mail addresses: wuqh@hainmc.edu.cn (Q. Wu), fangjs@gzucm.edu.cn (J. Fang).
[1] Zhao Dai and Tian Hu contributed equally to this work.

https://doi.org/10.1016/j.csbj.2023.12.017
Received 11 August 2023; Received in revised form 14 December 2023; Accepted 16 December 2023
Available online 20 December 2023

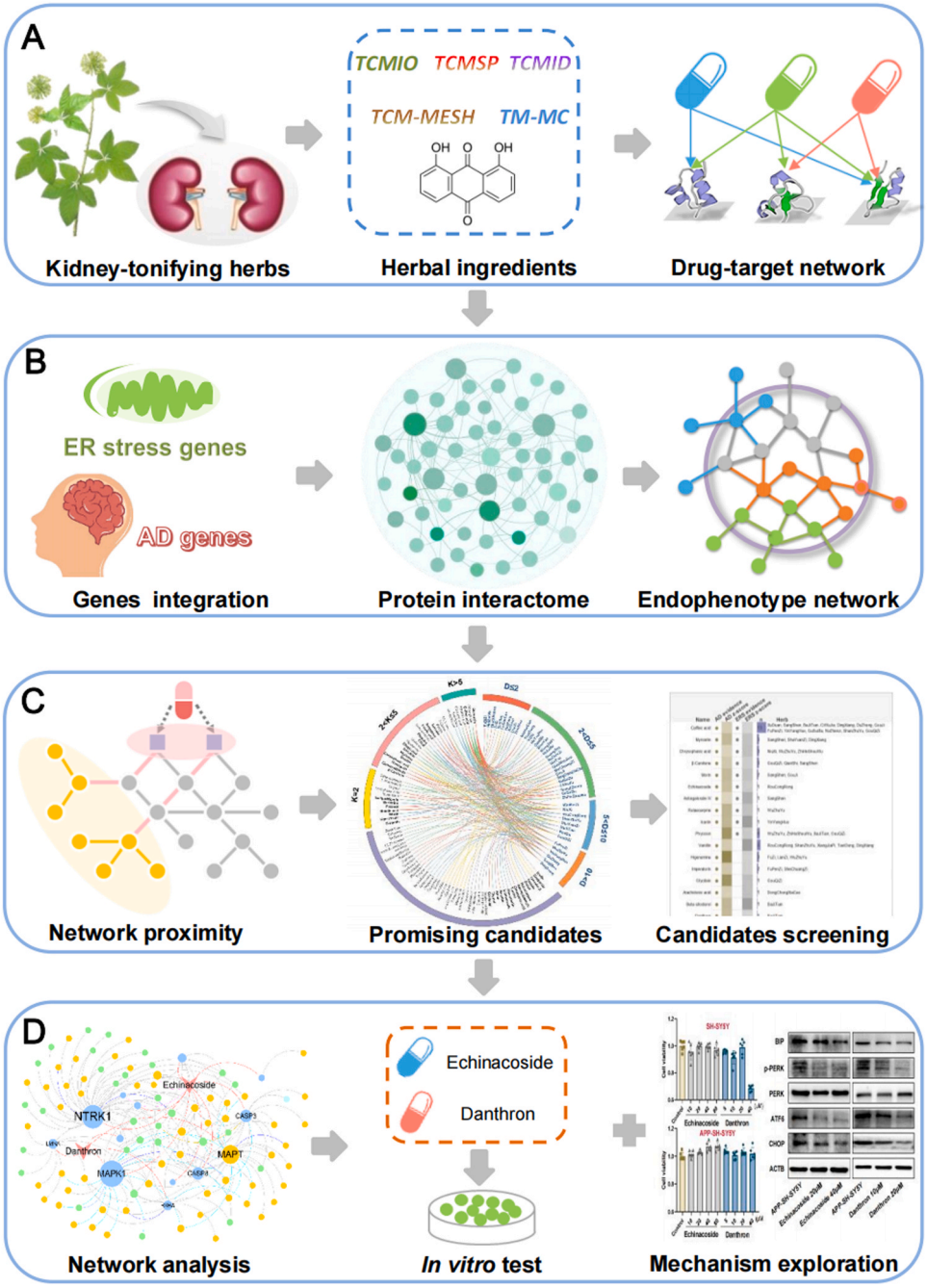


Fig. 1. The overall workflow of this study. (a) Construction of a drug-target network; (b) Endophenotype disease network framework by assembling the drug-target network, AD disease endophenotype network, and human protein-protein interactome; (c) Identification of the potential candidates in kidney-tonifying herbs through the approach of network proximity; (d) Experimental validation and mechanism exploration of potential drug candidates.

kidney-tonifying herb Cnidium monnieri (L.) Cusson (SheChuangZi), has shown promising outcomes in the treatment of AD. Study demonstrated its ability to promote neuronal differentiation by upregulating microRNA-9 in an AD cell model and to alleviate impairment in hippocampal neurons in AD mouse model[6].

As one of the most conserved stress responses, literature has demonstrated the contributory role of ER stress in the progression of AD [7]. For example, a recent experimental study has shown the neuroprotective effects of the Huangpu Tongqiao capsule against AD, elucidating its impact on the apoptosis pathway mediated by ER stress[8]. Moreover, several studies have implied that luteolin exhibits the potential to ameliorate cognitive deficits in mouse models of AD through the inhibition of ER stress-dependent neuroinflammation[9]. Therefore, the regulation of ER stress might have promising potential for the treatment of AD.

The endophenotype network is a molecular network capable of characterizing the intermediate genetic characteristics of an independent biological system's function, which is an effective strategy to explore the drug-disease or disease-disease correlation through the utilization of the network proximity method [10,11]. The hypothesis surrounding the endophenotype network within AD posits that AD involves various pathological mechanisms, each of which can be considered as an endophenotype, and these phenotypes are simultaneously involved in the pathogenesis of multiple diseases[12]. Recently, Fang et al. developed a methodology based on endophenotype disease modules, which combined with large-scale data mining of clinical electronic medical

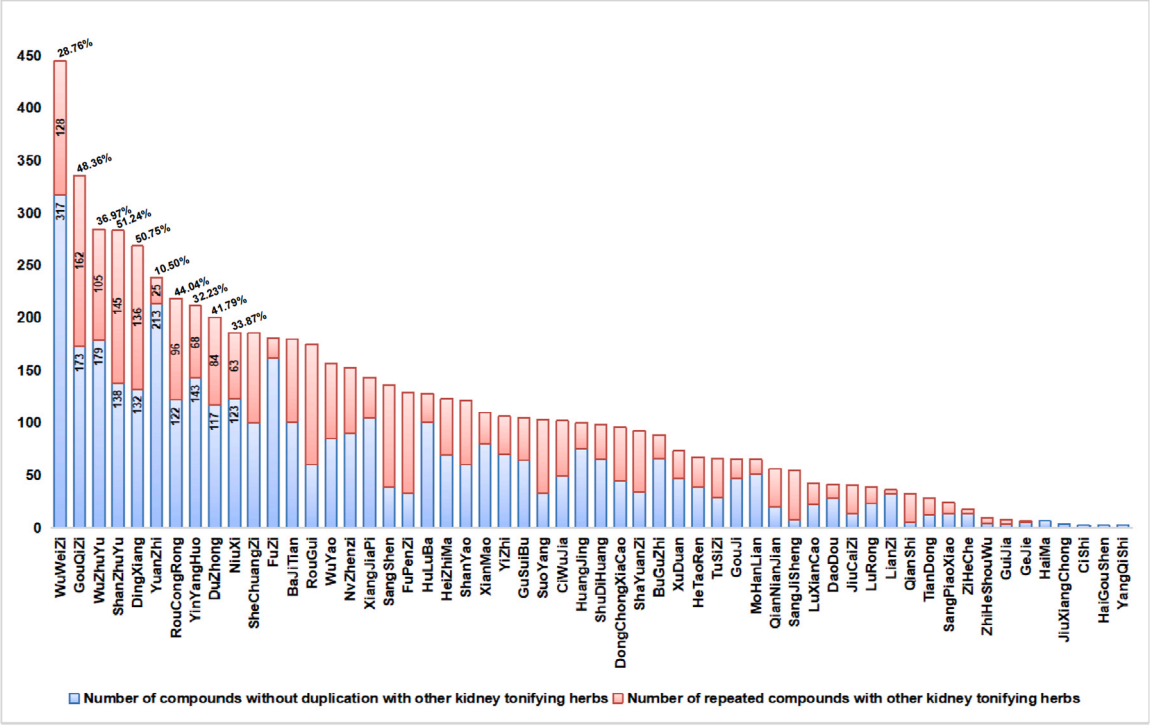


Fig. 2. Chemical components analysis of the 56 kidney-tonifying herbs from the Chinese Pharmacopoeia.

records for drug repurposing, and identified sildenafil as a potential drug for the treatment of AD[13].

In this study, we initially compiled the components of kidney-tonifying herbs and constructed a drug-target network (Fig. 1a) from multiple data sources. Next, we integrated the AD disease genes and ER stress endophenotype genes and developed an endophenotype disease network framework by assembling the drug-target (D-T) network, the AD disease network and the human protein-protein interactome (Fig. 1b). We further utilized the network proximity approach to discover the underlying anti-AD components targeting ER stress within kidney-tonifying herbs (Fig. 1c). Finally, we prioritized the most promising components, performed systematic pharmacological analysis, and validated their anti-AD effects and associated mechanisms of action (MOA) (Fig. 1d).

2. Materials and methods

2.1. Construction of D-T network of ingredients in kidney-tonifying herbs

Initially, kidney-tonifying herbs were identified from the *Chinese Pharmacopoeia*. Kidney-tonifying herbs were defined as those classified under the renal meridian in the *Chinese Pharmacopoeia*, with explicit documentation in the sections on functionality and indications confirming their kidney-tonifying properties. To construct the D-T network of kidney-tonifying herbs, we integrated the ingredients of kidney-tonifying herbs from the five authoritative databases, namely, the TCMIO database (http://tcmio.xielab.net/)[14], TCMID database (http://www.megabionet.org/tcmid/)[15], TCMSP database (https://old.tcmsp-e.com/tcmsp.php)[16], TCM-MESH database (http://mesh.tcm.microbioinformatics.org/)[17] and TM-MC database (https://tm-mc.kr/)[18]. We removed the duplicate ingredients according to their InChIKeys among different databases using the Open Babel software [19].

Furthermore, the targets of herbal ingredients were integrated based on three kinds of data sources: 1) Data on D-T interactions (DTIs) involving human proteins were sourced from the ChEMBL database (https://www.ebi.ac.uk/chembl/)[20], BindingDB (https://www.bindingdb.org/bind/index.jsp)[21] and STITCH (http://stitch.embl.de.)[22] database; 2) Information on ingredient-target interactions was extracted from the HIT database (http://lifecenter.sgst.cn/hit/)[23] and TCMID database[15]; 3) manual curation of the relevant pharmacological literature data of TCM compounds for kidney-tonifying herbs published in pharmacological or comprehensive journals during 2008 to 2017[24].

2.2. Integration of AD disease genes and ER stress genes

We first downloaded a list of AD disease genes data from the AlzGPS database (https://alzgps.lerner.ccf.org/)[25]. The list of AD disease genes was integrated from multiple data sources, including the large-scale genome-wide association studies (GWAS) analyses, the Human Gene Mutation Database (HGMD) database[26], DisGeNET database (https://www.disgenet.org/)[27] (score $\geq$ 0.2), MalaCards database (https://previous.malacards.org/pages/info)[28] and the Open Targets database (https://www.opentargets.org/)[29] (score $\geq$ 0.7, owning the literature evidence). Finally, 144 AD disease genes were obtained (Table S1).

The endophenotype ER stress genes were collected from the QuickGO database (https://www.ebi.ac.uk/QuickGO/). Three sets of ER stress-related genes were acquired, including GO:0051082, GO:0034976 and GO:0030968. We then removed the duplicated genes and obtained 57 ER stress genes (Table S1).

2.3. Physicochemical properties calculation

The physicochemical property results of the chemical ingredients in kidney-tonifying herbs were obtained via the SwissADME database (http://www.swissadme.ch/)[30]. For large molecules with more than 200 characters per SMILES, ADMETlab 2.0 (https://admetmesh.scbdd.com/)[31] was used to evaluate their physicochemical properties. Six physicochemical properties were calculated, including the molecular lipophilicity (logP), molecular solubility (logS), molecular weight (MW),

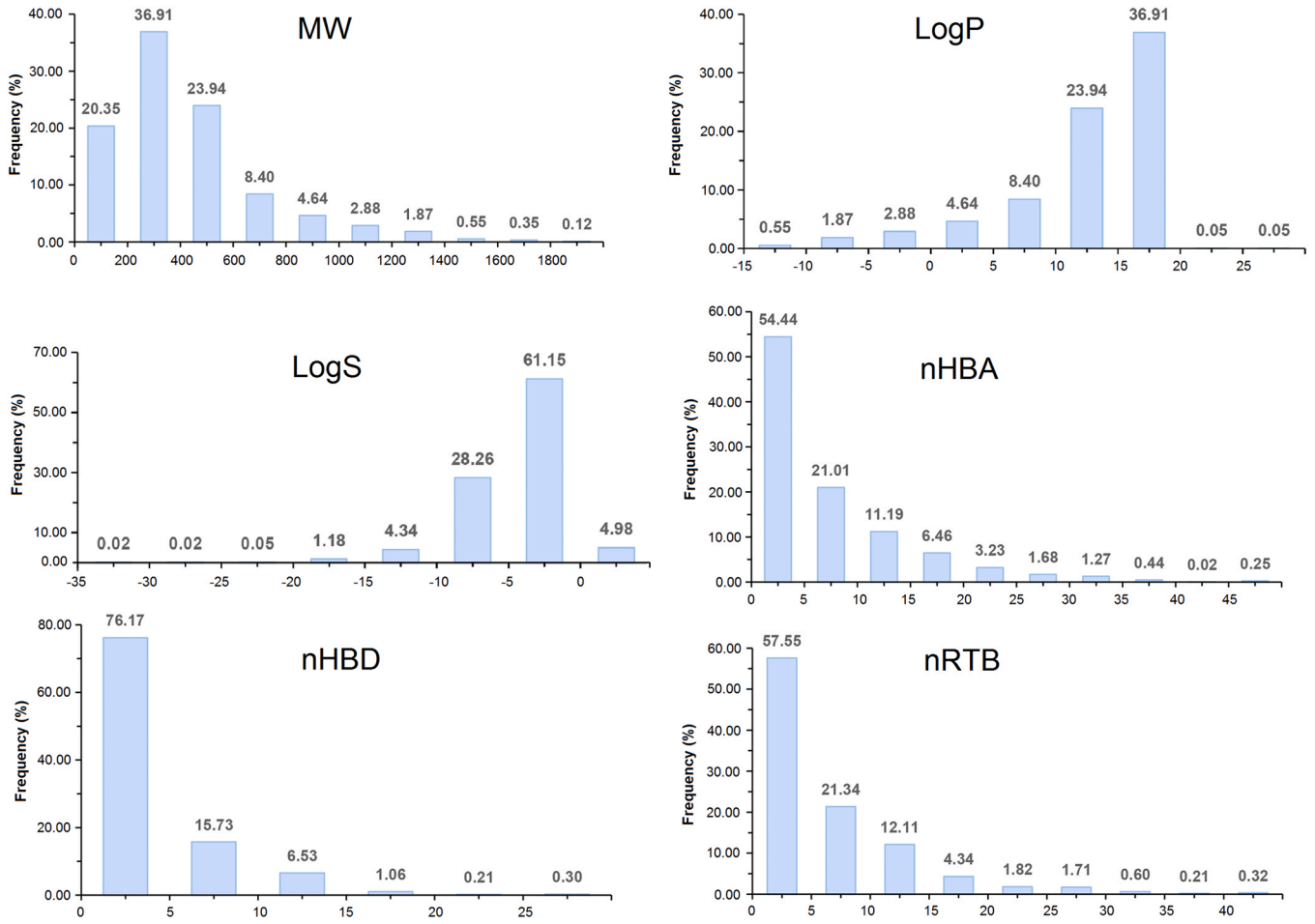


Fig. 3. Distributions of six physicochemical properties of chemical components in 56 kidney-tonifying herbs. These physicochemical properties include the molecular weight (MW), molecular lipophilicity (logP), molecular solubility (logS), the number of hydrogen bond acceptors (nHBA), the number of hydrogen bond donors (nHBD) and the number of rotatable bonds (nRTB).

number of hydrogen bond acceptors (nHBA), number of hydrogen bond donors (nHBD) and number of rotatable bonds (nRTB).

2.4. Network proximity approach

The background of human protein interactions was obtained by integrating different types of protein-protein interactions (PPIs) and experimental evidence from different bioinformatics databases[32]. The network proximity method was then used to measure the network distance between the AD/ER stress protein module (T) and the drug target module (C) in the context of protein interaction. The nearest distance d_{TC} represents the distance of the protein module to the drug module. The formula is as follows:

$$\langle d_{TC}\rangle = \frac{1}{||T||+||C||}\left(\sum_{t\in T}\min_{c\in C}d(t,c)+\sum_{c\in C}\min_{t\in T}d(t,c)\right)$$

where $d(t,c)$ represents the shortest distance between the AD/ER stress gene t and drug target c.

The network distance (Z-score, z_d)between T and C was calculated by randomly selecting two groups of proteins from the PPI network that both matched the original protein sets in size and distribution. The formula is as follows:

$$z_d = \frac{d-\overline{d}}{\sigma_d}$$

We considered Z < -1.5 and $P < 0.05$ to be statistically significant. Detailed descriptions of this approach to screen potential drug candidates can be found in previous literature[33].

2.5. Experimental validation

2.5.1. Chemicals and reagents

Echinacoside (# T1716) and Danthron (# T0800) were purchased from Targetmol (Shanghai, China). The following antibodies were used for Western blots and immunofluorescence: Heat Shock Protein Family A Member 5 (BIP) antibody (# AF0729, Affinity Biosciences), Phosphorylated Protein Kinase R-like Endoplasmic Reticulum Kinase (p-PERK) antibody (# DF7576, Affinity Biosciences), Protein Kinase R-like Endoplasmic Reticulum Kinase (PERK) antibody (# AF5304, Affinity Biosciences), Activating Transcription Factor 6 (ATF6) antibody (# DF6009, Affinity Biosciences), DNA Damage Inducible Transcript 3 (CHOP) antibody (# AF6277, Affinity Biosciences), and beta Actin (ACTB) antibody (# AF7018, Affinity Biosciences).

2.5.2. Cell viability assay

The two cell lines used for the in vitro experiment were SH-SY5Y and APP-SH-SY5Y. The former is a commonly used neuronal cell model, while the latter has the ability to mimic the pathogenesis of AD attributed to Aβ accumulation. Both of these two cells were cultured in a 96-well plate for 24 h and treated with 2 natural products (echinacoside and danthron) for 24 h. Perform the steps outlined in the MTT operation protocol. Finally, the absorbance was read at 490 nm.

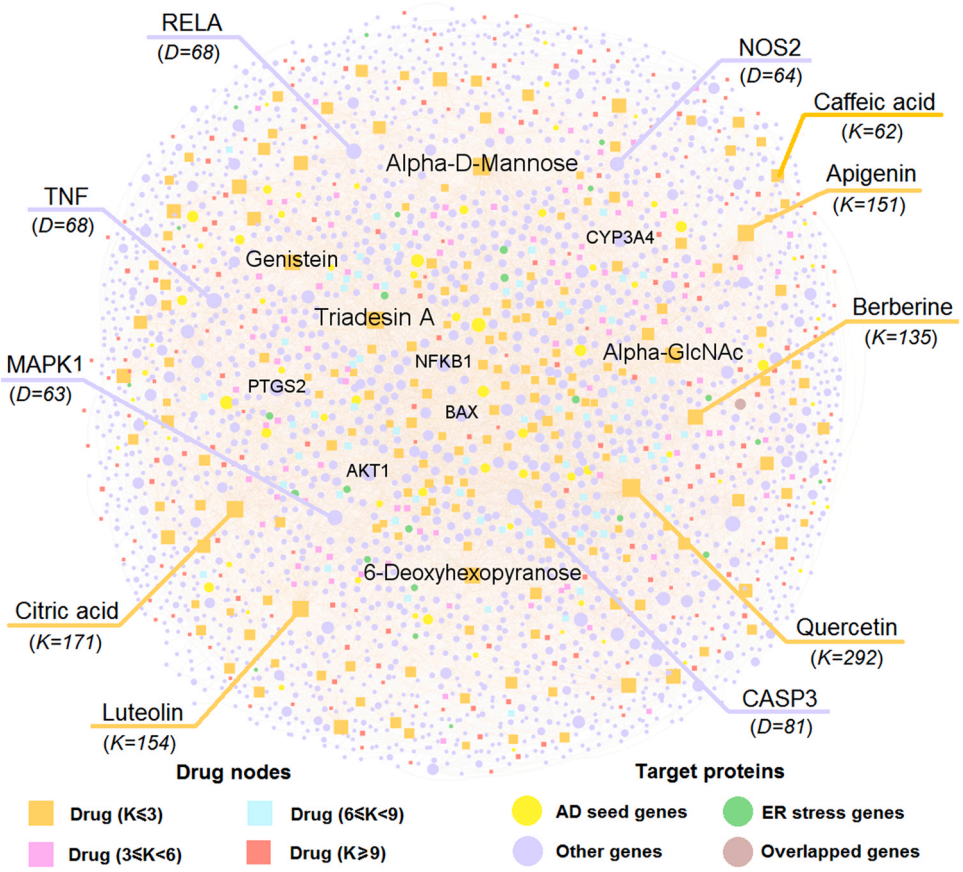


Fig. 4. Drug-target network for ingredients of 56 kidney-tonifying herbs. The network connects 548 natural products to 2255 target proteins. The font size of the labels and the size of the nodes are proportional to the degree. The natural products are ranked by the degree number and the labels of the top ten natural products and genes with the highest degrees are shown. The network was generated by the Gephi[37] (version 0.9.2).

2.5.3. *Western blot assay*

Equal volumes and masses of proteins were loaded on 10–15% SDS-PAGE gels and blotted onto methanol-activated polyvinylidene fluoride (PVDF) membranes (Millipore, USA). The membranes were exposed to the blocking solution (5% BSA) and incubated for one hour at room temperature. After this step, primary and secondary antibodies were applied to the membranes and then visualised.

using ChemiDocXRS + (Bio-RAD, USA). Image J was used for density analysis.

2.5.4. *Immunofluorescence assay*

The cells in the 24-well plate were washed, fixed, permeabilized, incubated according to the immunofluorescence kit protocol, and finally stained with DAPI (DNA stain), sealed, and observed using a fluorescence microscope.

3. Results

3.1. *Kidney-tonifying herbs and chemical components analysis*

We first collected 56 kidney-tonifying herbs from the Chinese Pharmacopoeia (version: 2015) and subsequently integrated their chemical ingredients from the five authoritative databases (see Material and Method 2.1), resulting in 4338 kidney-tonifying TCM chemical ingredients after removing duplicates. To explore the composition similarity of the 56 kidney-tonifying herbs, we further performed the chemical components analysis on these herbs. As shown in Fig. 2, there are multiple common chemical components among the 56 kidney-tonifying herbs. The top 10 herbs with the highest number of chemical components and their corresponding component overlap rates were as follows: Schisandra chinensis (Turcz.) Baill. (WuWeiZi, 445, 28.76%), Lycium barbarum L. (GouQiZi, 335, 48.36%), Tetradium ruticarpum (A. Juss.) T.G.Hartley (WuZhuYu, 284, 36.97%), Cornus officinalis Siebold & Zucc. (ShanZhuYu, 283, 51.24%), Syzygium aromaticum (L.) Merr. & L.M.Perry (DingXiang, 268, 50.75%), Polygala tenuifolia Willd. (YuanZhi, 238, 10.50%), Cistanche deserticola Ma (RouCongRong, 218, 44.04%), Epimedium sagittatum (Siebold & Zucc.) Maxim. (YinYangHuo, 211, 32.23%), Eucommia ulmoides Oliv. (DuZhong, 201, 41.79%) and Achyranthes bidentata Blume (NiuXi, 186, 33.87%). This result preliminarily explains the reason why kidney-tonifying Chinese herbs are generally effective for AD, which may attribute to the similar active component groups, thus exerting similar anti-AD effects.

3.2. *Physicochemical properties analysis of chemical components in kidney-tonifying herbs*

The physicochemical properties of various chemical components can provide significant and valuable information on druggability. MW was considered to be a simple estimate of molecular size, and LogP and LogS represented the lipophilicity and solubility of a compound. Moreover, nHBA and nHBD usually represented the hydrogen bonding ability while nRTB represented the number of rotatable bonds. As shown in Fig. 3, the MWs of most chemical components were distributed between 0 and 600 with a total frequency of 81.2%, indicating that most of the chemical components in kidney-tonifying herbs were small molecule compounds. Among these compounds, the highest frequencies of LogP and LogS were 36.91% and 61.15%, respectively, distributed between 15 and 20 and between − 5 and 0. As for the nHBA, nHBD and nRTB, it was obvious that all of them were widely distributed. Intriguingly, we found that the highest frequency of nHBA (54.44%), nHBD (76.17%) and nRTB

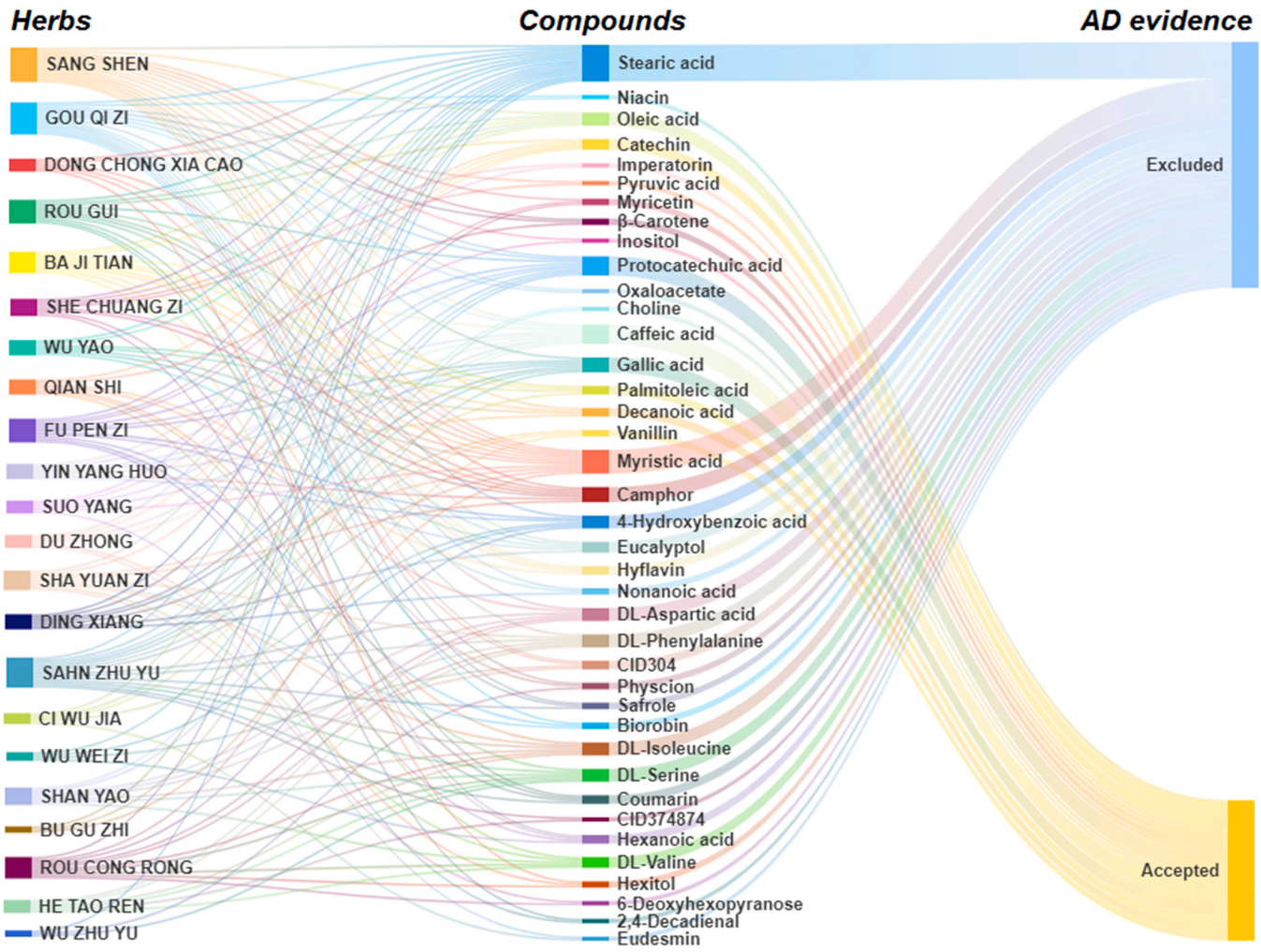


Fig. 5. Sankey diagram illustrating 39 potential anti-AD compounds from 22 kidney-tonifying herbs identified by the network proximity approach. The 22 kidney-tonifying herbs are derived from 52 kidney-tonifying herbs containing more than 5 potential anti-AD compounds. The 39 anti-AD compounds identified by more than or equal to 22 kidney-tonifying herbs are listed. The network was performed by Omicshare webserver (https://www.omicshare.com/tools).

(57.55%) were all distributed between 0 and 5, suggesting the high consistency of the distribution of the chemical components in kidney-tonifying herbs. Overall, most of the natural products had good physicochemical properties.

3.3. D-T network analysis of herbal ingredients in kidney-tonifying herbs

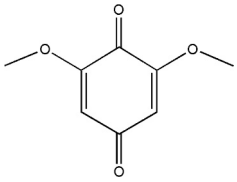
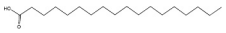
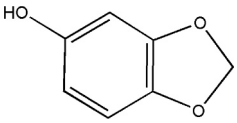
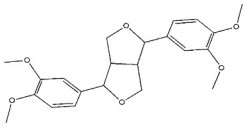
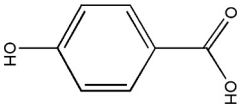
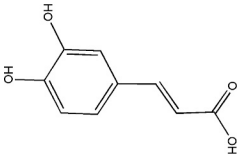
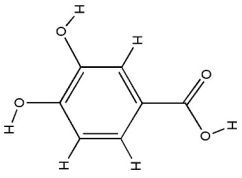
Next, we conducted a comprehensive D-T network for kidney-tonifying herbs (Fig. 4) by assembling the DTIs data from several public databases and literature. This network included 7582 DTIs, interacting 548 natural products with 2255 target proteins (Table S2). Fig. 4 shows that most of the kidney-tonifying compounds interact with different proteins, with an average target degree of 4.1 (K) for each compound. The top 10 in the 548 natural products with the highest degree number are: quercetin (CID5280343, K=292), triadesin A (CID238, K=216), α-D-Mannose (CID185698, K=214), citric acid (CID311, K=171), luteolin (CID5280445, K=154), apigenin (CID5280443, K=151), 6-deoxypyranose (CID840, K=142), berberine (CID2353, K= 135), alpha-GlcNAc (CID82313, K=127) and caffeic acid (CID689043, K=62). These natural products likely have potential therapeutic effects on AD. For example, network analysis shows that luteolin has 154 target connections, including 12 AD-related genes, 2 ER stress genes, and 1 overlap. A recent study demonstrated that luteolin can alleviate learning and memory impairments in transgenic AD mouse models through inhibiting ER stress as well as improving neuroinflammation[9].

Meanwhile, among the 2255 target proteins, CASP3 (D=81) had the largest number of compound interactions, followed by RELA (D=68) and TNF (D=68). Literature evidence suggests the role of these targets in AD. For instance, it has been shown that the systemic upregulation of TNF-α accelerates a pro-inflammatory environment in the brain, contributing to the development of AD-like pathology and cognitive dysfunction[34]. Several members of the caspase family of proteases (e.g., caspase-3) are involved in apoptosis during AD progression. For example, alterations in caspase-3 levels were found in AD patient samples, suggesting the importance of CASP3[35]. Overall, the D-T network analysis indicates that the natural products from the kidney-tonifying herbs have multiple pharmacological properties[36], which can act on various AD-related and ER stress genes. Therefore, we next attempted to identify the natural products with potential anti-AD effects through targeting ER stress in kidney-tonifying Chinese herbs by integrating the D-T network and disease endophenotype network.

3.4. Identification of potential anti-AD ingredients in kidney-tonifying herbs

In this part, we used the network proximity method to identify the anti-AD ingredients in kidney-tonifying herbs. Our network prediction model prioritized 104 TCM compounds that were significantly correlated with the AD endophenotype network ($P < 0.05$) using the network

Table 1
7 potential anti-AD compounds in Eleutherococcus senticosus (Rupr. & Maxim.) Maxim. (CiWuJia).

Name	Structure	PubChem CID	MW	MF	PMID
2,6-Dimethoxy-1,4-benzoquinone		68262	168.15 g/mol	C8H8O4	N/A
Stearic acid		5281	284.5 g/mol	C18H36O2	N/A
Sesamol		68289	138.12 g/mol	C7H6O3	25449035
Eudesmin		234823	386.4 g/mol	C22H26O6	N/A
4-Hydroxybenzoic acid		135	138.12 g/mol	C7H6O3	N/A
Caffeic acid		689043	180.16 g/mol	C9H8O4	33432954; 27430591
Protocatechuic acid		72	154.12 g/mol	C7H6O4	32679284

Note: MW represents the molecular weight while MF denotes the molecular formula.

proximity score threshold of $Z < -1.5$ (Table S3). Interestingly, we found that 41 out of the predicted compounds (39.4%) had in vitro or in vivo AD literature evidence (Table S3) after in-depth literature search, indicating that our network model had high predictive accuracy. For example, caffeic acid was reported to significantly alleviate Aβ-induced toxicity, increase lifespan, reduce body paralysis, and ameliorate reproductive defects in Caenorhabditis elegans models[38]. Moreover, an in vitro study revealed that gallic acid showed potent anti-amyloidogenic properties in a cell membrane-like environment, highlighting its potential in the prevention and treatment of AD[39].

We then integrated these 104 potential anti-AD compounds and found that they were attributed to 52 kidney-tonifying herbs listed in Chinese Pharmacopoeia. Among them, a total of 22 kidney-tonifying herbs contained more than 5 potential anti-AD compounds (e.g., CiWuJia, Fig. 5), and the top 7 kidney-tonifying herbs with the highest number of anti-AD compounds were Morus alba L. (SangShen, n = 24), Lycium barbarum L. (GouQiZi, n = 18), Cornus officinalis Siebold & Zucc. (ShanZhuYu, n = 15), Gynochthodes officinalis (F.C.How) Razafim. & B.Bremer (BaJiTian, n = 15), Neolitsea cassia (L.) Kosterm. (RouGui, n = 13), Rubus idaeus Linn. (FuPenZi, n = 12), Cistanche deserticola Ma (RouCongRong, n = 12). In addition, we found that there was a total of 39 anti-AD compounds identified by more than or equal to 2 kidney-tonifying herbs (Fig. 5), indicating their significance against AD.

Taking Eleutherococcus senticosus (Rupr. & Maxim.) Maxim. (CiWuJia, n = 7) as an example, network proximity predicted that CiWuJia contained 7 potential anti-AD compounds, among which 3 compounds were confirmed to have anti-AD effects (Table 1). Indeed, Experimental literature evidence has shown that CiWuJia and its active components can significantly improve the cognitive impairment and learning and memory ability. For instance, the ethanol extract of Acanthopanax koreanum (EEAK) has been shown to ameliorate the cognitive dysfunction in mice, and the potential mechanism is related to the activation of Akt, CaMKII and CREB in the hippocampus[40]. Moreover, the active ingredients of CiWuJia (eleutheroside B or E) may improve cognitive function related to learning and memory in aged rats

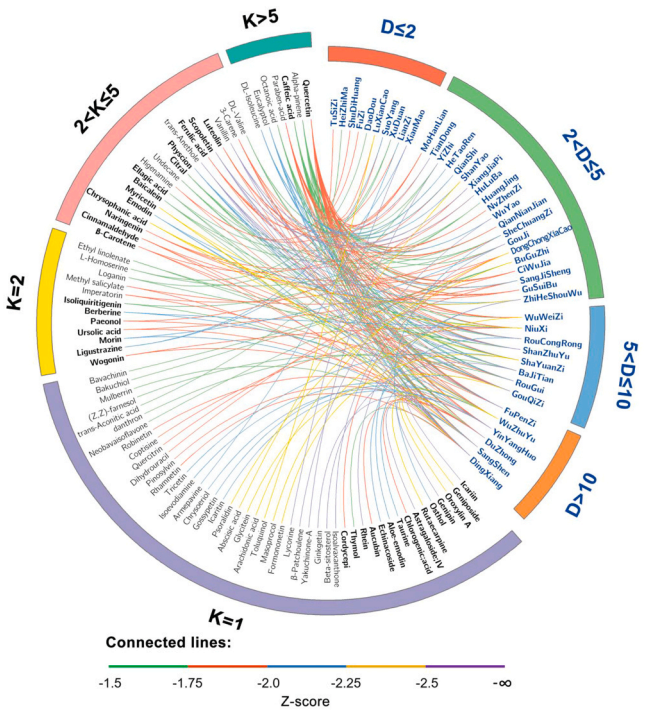


Fig. 6. The potential compounds targeting endoplasmic reticulum stress from kidney-tonifying herbs identified by network proximity approach. The compounds with experimental evidence are marked in bold. Each endoplasmic reticulum stress-regulated compound belongs to a specific number of kidney-tonifying herbs denoted by K, while D denotes the number of endoplasmic reticulum stress-regulated compounds present in each kidney-tonifying herb. The diagram was developed by using Perl.

by activating cholinesterase or enhancing the reuse of choline to promote the synthesis of acetylcholine in hippocampal neurons[41].

3.5. Identification of potential ingredients modulating ER stress in kidney-tonifying herbs

The network proximity approach was also to predict the active ingredients that modulated ER stress in TCM kidney-tonifying herbs. Fig. 6 reveals that 85 TCM compounds derived from 44 kidney-tonifying herbs listed in the *Chinese Pharmacopoeia* have the potential to regulate ER stress. After a comprehensive literature review, we discovered that 37 (43.5%) had been confirmed to regulate ER stress (Table S4). For instance, ellagic acid was shown to induce apoptosis in bladder cancer cells by regulating the ER stress[42]. Among the 44 kidney-tonifying herbs, 34 out of them had three or more potentially active compounds related to ER stress, and 38 potentially active compounds existed in two or more species of kidney-tonifying herbs (Fig. 6).

As shown in Fig. 6 quercetin had the highest frequency and was present in 21 herbs, suggesting its potential effect on ER stress. Indeed, a recent study has demonstrated that quercetin could act on the AMPK/SIRT1 signaling pathway to alleviate ER stress, and relieve apoptosis and inflammation[43]. Quercetin was also reported to act on GADD34 in the brain to reduce eIF2α phosphorylation and ATF4 expression, thereby improving memory and delaying early memory decline in AD model mice[44]. Our findings indicate the presence of over 10 promising compounds that have the potential to regulate ER stress in six different herbs, specifically Syzygium aromaticum (L.) Merr. & L.M.Perry (DingXiang, n = 13), Eucommia ulmoides Oliv. (DuZhong, n = 12), Morus alba L. (SangShen, n = 12), Tetradium ruticarpum (A.Juss.) T.G. Hartley (WuZhuYu, n = 11), Rubus chingii Hu (FuPenZi, n = 11), and Epimedium sagittatum (Siebold & Zucc.) Maxim. (YinYangHuo, n = 11).

3.6. Identification of potential anti-AD compounds targeting ER stress for experimental validation

We further identified the potential anti-AD compounds targeting ER stress by integrating the results from Fig. 5 (Table S3) and Fig. 6 (Table S4), and found that 32 compounds had the potential for both anti-AD effect and regulating ER stress (Fig. 7). Following a systematic search of the literature, we confirm that 18 out of 32 compounds have demonstrated anti-AD effects or impact on ER stress (Table S5). Taking myricetin as an example, research found a significant increase in the hippocampal CA3 pyramidal neurons and an improvement in cognitive function related to learning and memory in AD rats, indicating that myricetin could be beneficial for the treatment of AD[45]. In addition, myricetin has been shown to protect β-cells against apoptosis by regulating ER stress[46]. To narrow the range of probable compounds for experimental verification, only those with both AD or ER stress regulation literature evidence and a Z-score value under − 2.0 ($Z < -2.0$) were retained, resulting in a total of 12 compounds. Drug uptake and distribution require plasma protein binding (PPB), while drugs that act on the central nervous system need to cross the blood-brain barrier (BBB) to reach the targets. We calculated the PPB and BBB of drugs via the ADMET lab2.0 (https://admetmesh.scbdd.com/). After evaluating of PPB, BBB, and literature evidence of drugs (Table S6), we selected 2 predictive compounds (echinacoside and danthron) to verify their anti-AD efficacy and ER-related molecular mechanism in vitro.

3.7. Systems pharmacology analysis of promising candidates

Next, we developed the D-T subnetwork and PPI network of the two promising candidates (echinacoside and danthron). Fig. 8a shows the network that comprises 15 DTIs and 156 PPIs, interacting with 56 AD genes, 30 ER stress genes, 2 overlapped genes and 12 other genes. Echinacoside, the major active component of TCM Cistanches Herba is demonstrated to possess numerous properties, such as anti-inflammatory, antioxidant and anti-apoptotic effects[47]. Fig. 8a indicates that echinacoside binds to 10 genes (e.g., CASP3) and 74 PPI partners. It was suggested that the ER stress inhibitor regulates CASP3, revealing its role in ER stress[48]. Additionally, CASP3 is involved in the AD pathological process. For instance, variations in CASP3 levels have been found in samples of AD patients[35]. As an anthraquinone derivative naturally extracted from rhubarb, danthron displays potential antitumor and antioxidant properties[49,50]. Network analysis shows that danthron interacts with 5 genes (e.g., LMNA) and 51 PPI partners. Multiple pieces of evidence have confirmed the role of LMNA in ER stress[51] and AD[52]. Intriguingly, we found that these two compounds both act on several genes, such as MAPK1, indicating that they might exert a synergistic effect on AD.

To investigate the molecular mechanism of echinacoside and danthron against AD, we conducted an enrichment analysis of all the affected genes. Fig. 8b shows that these genes are enriched in different biological processes (BPs), including the response to ER stress. Recent evidence in the literature shows that ER stress has been observed in both in vitro cellular models of AD and certain animal models of the disease [53]. Since both compounds act on ER stress and AD according to our predictive results, it is probable that echinacoside and danthron exert their therapeutic effects on AD through ER stress regulation. Additionally, these genes are also involved in multiple KEGG pathways, including apoptosis, implying a potential mechanism against AD (Fig. 8c). Apoptosis is known to be responsible for manifestations associated with AD under pathological conditions[54]. Interestingly, ER stress has been found to mediate the apoptotic pathway, presenting a new and promising therapeutic target for AD treatment[55].

3.8. Echinacoside and danthron downregulated ER stress-related proteins

Following the results mentioned above, we selected echinacoside

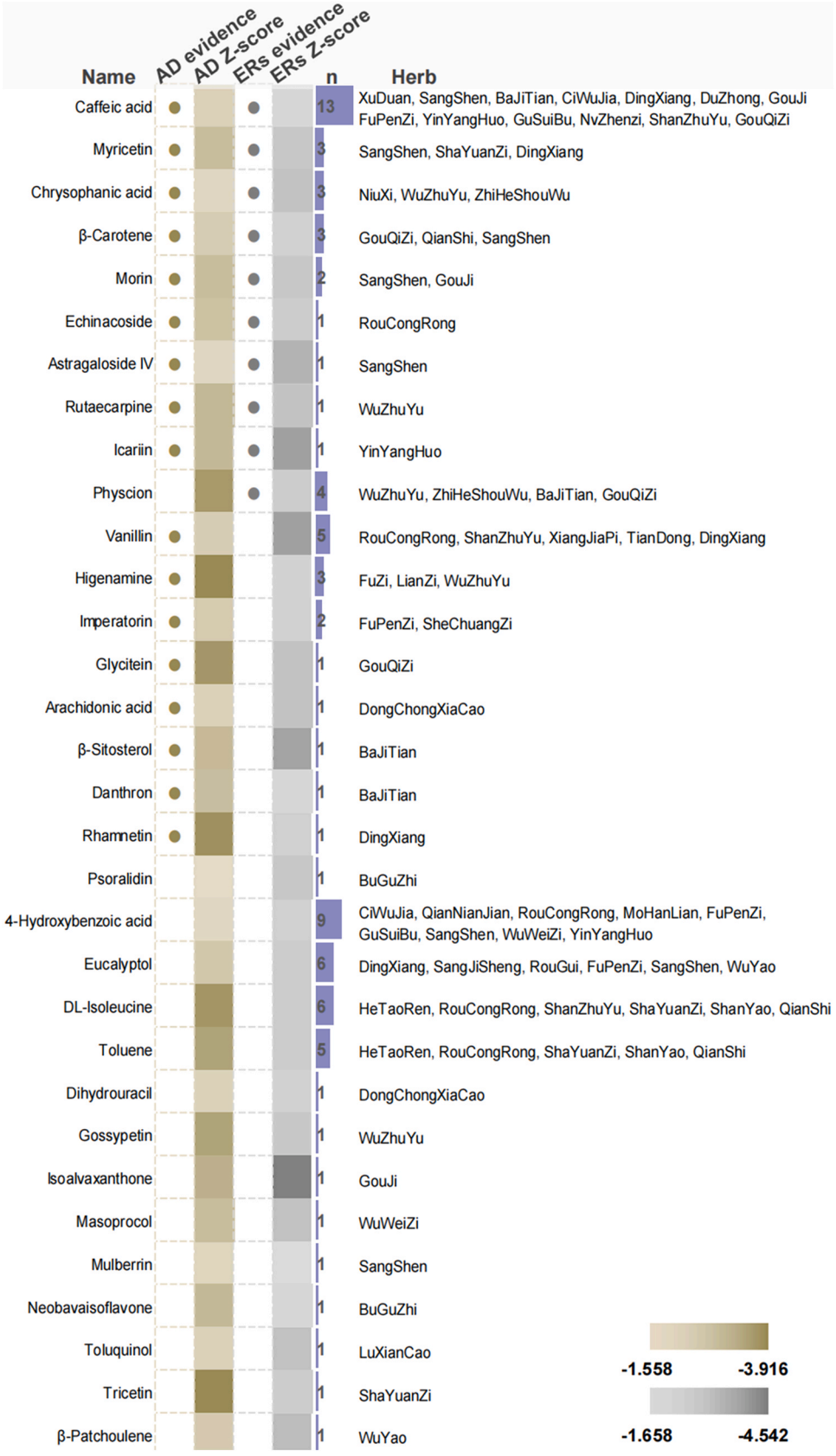


Fig. 7. Identification of potential anti-AD compounds targeting endoplasmic reticulum stress from kidney-tonifying herbs.

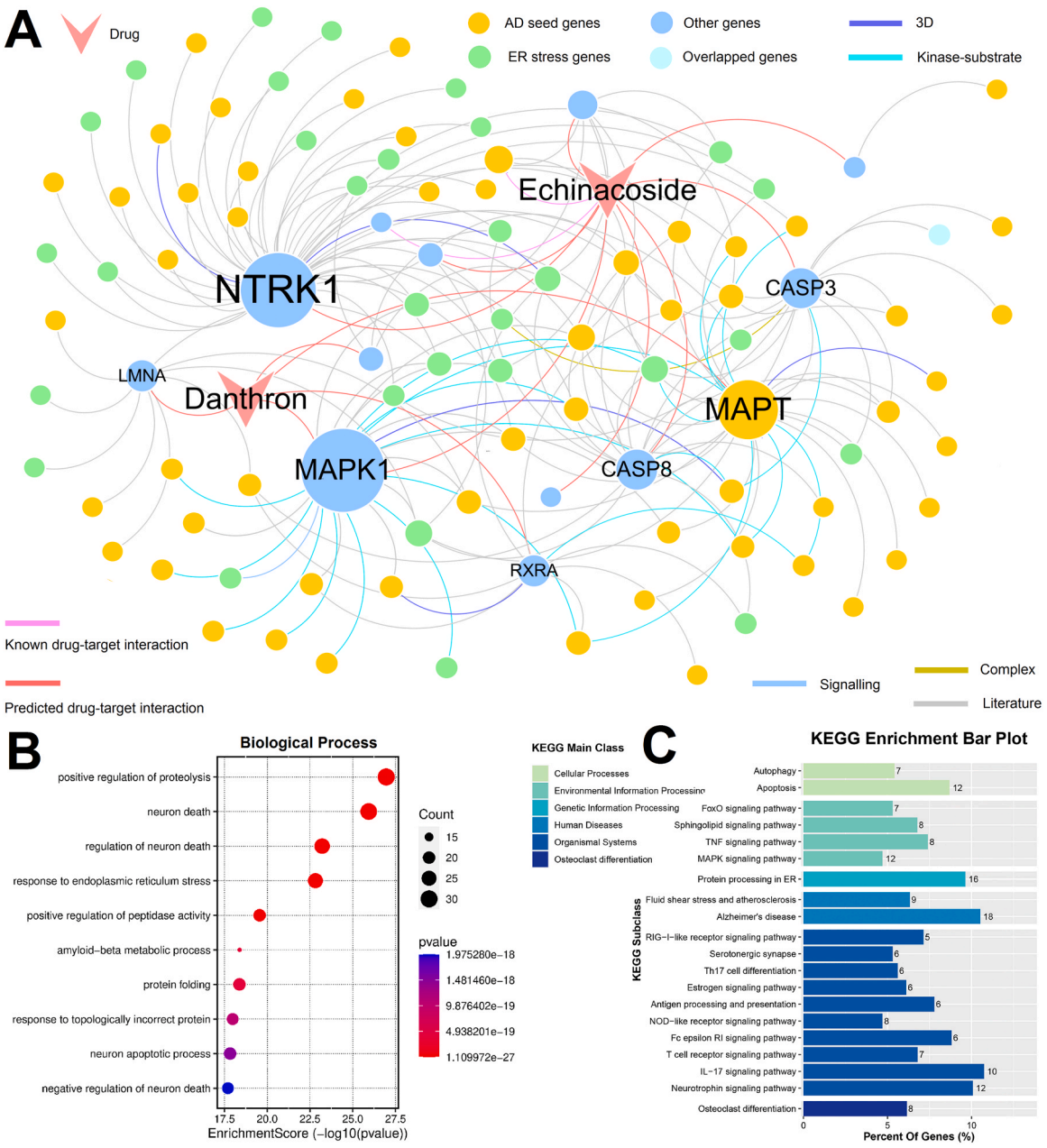


Fig. 8. Systems pharmacology analysis of echinacoside and danthron. The drug-target network consisted of 15 drug-target interactions and 156 protein-protein interactions (a). Biological process enrichment analysis and (b) KEGG pathways annotations results (c) were performed via the ClueGo plug-in in the Cytoscape software.

and danthron for cell viability detection and mechanism verification of ER stress, and drug concentrations were referred to in the previously published literature. It was evident from the data presented in Fig. 9a and b that neither echinacoside nor danthron caused toxicity to SH-SY5Y and APP-SH-SY5Y cell lines. The effects of echinacoside and danthron on ER stress proteins by western blot (Fig. 9c, d, and e). p-PERK ($P < 0.05$) and ATF6 ($P < 0.05$) were downregulated significantly by 40 μM of echinacoside. Furthermore, ATF6 was markedly downregulated by 20 μM of echinacoside ($P < 0.05$). Besides, we found that 10 μM of danthron significantly downregulated p-PERK ($P < 0.05$) and ATF6 ($P < 0.01$). Additionally, a dose of 20 μM of danthron notably downregulated BIP ($P < 0.05$), p-PERK ($P < 0.05$), ATF6 ($P < 0.001$) and CHOP ($P < 0.05$), respectively. Two proteins (p-PERK and ATF6) were then evaluated with immunofluorescence assay, which confirmed that the administration of echinacoside and danthron reduced the expression of p-PERK and ATF6 (Fig. 9f).

4. Discussion

Kidney tonification for the treatment of cognitive impairment is a well-established and effective method in TCM. There is a considerable amount of high-quality clinical evidence to support this effect[56]. A two-year, placebo-controlled, randomized trial demonstrated the long-term therapeutic effects of Bushen capsules can improve mild cognitive impairment[57]. In addition, a randomized, double-blind, placebo-controlled clinical trial demonstrated the efficacy of the kidney tonic formula "ba wei di huang wan" in the treatment of dementia [58]. The 2020 edition of the Guidelines for the diagnosis and treatment of AD in China recommends that the TCM treatment for AD adopts sequential kidney tonification therapy in the initial stage and

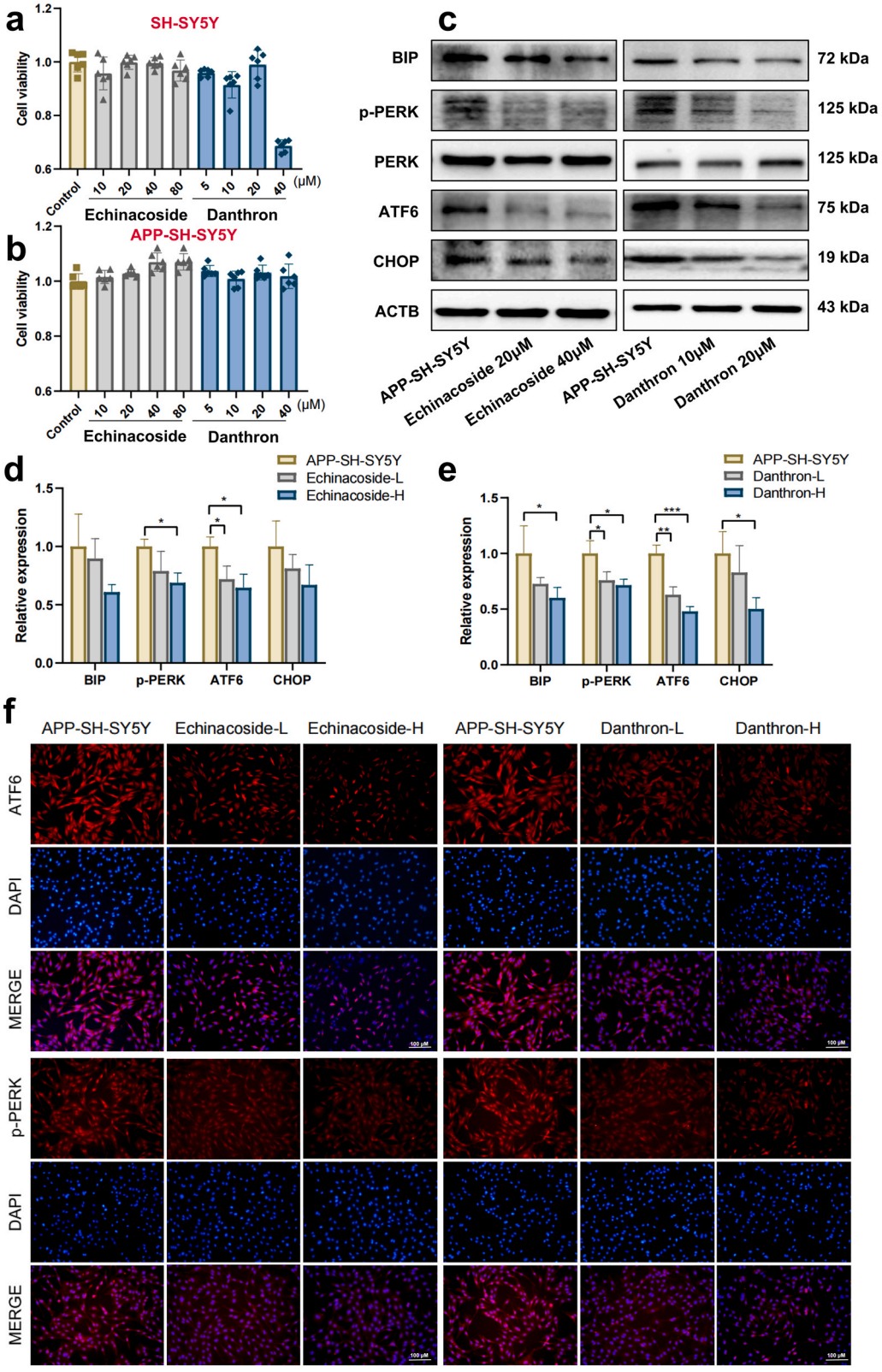


Fig. 9. Echinacoside and danthron downregulated endoplasmic reticulum stress-related proteins. Cell viability of echinacoside and danthron on SH-SY5Y and APP-SH-SY5Y cell lines was assessed using the MTT assay (a, b). Western blot results of ER stress-related proteins (BIP, p-PERK, PERK, ATF6, CHOP) were analyed through Western blot on APP-SH-SY5Y cell line (c, d, e). Immunofluorescence expression of ATF6 and p-PERK on APP-SH-SY5Y cell line (f). Data are presented as mean ± standard deviation. * $P < 0.05$, * * $P < 0.01$, * ** $P < 0.001$.

throughout the whole process, thereby highlighting the crucial role of kidney-tonifying herbs in modern AD treatment. Currently, there is still no comprehensive method that can entirely uncover the pharmacodynamic material basis of kidney-tonifying herbs for preventing and treating AD. Nonetheless, the endophenotype network method guided by the network medicine theory, provides the potential to decipher it.

Our study employed a network medicine framework to investigate the pharmacodynamic material basis and MOA of kidney-tonifying herbs in treating AD by regulating ER stress. Specifically, we constructed both the AD disease network and the D-T network of kidney-tonifying TCMs. We then identified potentially active ingredients in kidney-tonifying TCMs that could effectively treat AD and regulate ER stress by using the network proximity method. Furthermore, we integrated the anti-AD compounds that targeted ER stress and verified the anti-AD mechanisms of action through in vitro experimental validation.

Our study demonstrated that echinacoside and danthron regulated ER stress in AD cell models. Echinacoside is a natural phenylethanoid glycoside that has protective effects against neurodegenerative diseases. Recent study has shown that echinacea exerts neuroprotective effects by inhibiting the α-synuclein /TLR2/NF-κB/NLRP3 axis of microglia in models of Parkinson's disease (PD)[59]. In AD, echinacoside blocks amyloid deposition by inhibiting amyloid oligomerization, thereby improving cognitive dysfunction caused by $A\beta_{1-42}$[60]. In addition, echinacoside inhibits the increase of intracellular reactive oxygen species (ROS) induced by Aβ[61]. In terms of regulating ER stress. echinacoside has been proven to regulate ER stress in animal models of PD [62], osteoarthritis[63], heart failure[64] and so on. Danthron is a natural product derived from *salvia miltiorrhiza*. Study has shown that danthron may reduce $A\beta_{25-35}$ related neurotoxicity by inhibiting membrane lipid peroxidation and glutathione deprivation[65]. We validated the role of echinacoside and danthron in regulating ER stress in AD cell models. This study confirmed the role of danthron in regulating ER stress for the first time, and provided a useful reference for clarifying the efficacy of echinacoside and danthron.

Overall, our study presents three advantages that may be emphasised as follows. Firstly, the potential anti-AD ingredients with promising regulatory effects on ER stress in kidney-tonifying TCMs were systematically screened, which contributed to clarifying the pharmacodynamic material basis of kidney-tonifying TCMs against AD. Moreover, we have effectively verified that two promising compounds (echinacoside and danthron) can regulate ER stress to exhibit anti-AD effects. This provides important evidence for identifying and developing AD drug candidates derived from kidney-tonifying TCMs. Finally, the study utilized the endophenotype network method to investigate the effectiveness of kidney-tonifying TCMs against AD, which conformed to the Network pharmacology evaluation method guidance[11]. This approach is a beneficial attempt for developing new approaches to TCM network medicine, and it holds a significant reference value for the modernization of TCM theory.

However, the study has several limitations. Firstly, due to the limited information on disease genes and compound targets available through public databases and literature evidence, the incompleteness of the D-T network and disease network ramains unavoidable. To address this, the target prediction method based on network inference[66] could be introduced to expand the DTIs and help to identify more potential active compounds. Secondly, there is a need for further improvement of predictive network models, as there is a lack of information regarding the contents of compounds found in kidney-tonifying herbs, as well as the evaluation data of absorption, distribution, metabolism, excretion and toxicity (ADMET) properties of drugs within the body. In the future, we will select two or three kidney-tonifying TCMs and conduct qualitative and quantitative analyses on blood components using modern TCM analysis methods. Any redundant compounds will then be filtered out through ADMET property assessments. Lastly, we did not consider the synergistic effect of compounds or herbs[67,68] and just performed in vitro experiment. Thus, we will further investigate the synergistic effect of herbs and in vivo validation in next study.

In summary, this study combined the clinical advantages of TCMs with the cutting-edge network medicine approach to decipher the pharmacodynamic material basis and the potential MOAs of kidney-tonifying TCMs in preventing and treating AD, providing a new paradigm for the transformation of TCM research from empirical medicine to evidence-based medicine.

5. Conclusion

In this study, we used the network proximity approach to identify the potential anti-AD compounds regulating ER stress in kidney-tonifying herbs. We next validated the role of echinacoside and danthron in regulating ER stress in an in vitro AD model by combining literature studies and systematic pharmacological analyses. In general, this study has preliminarily interpreted the effective material basis and potential molecular mechanism of kidney-tonifying Chinese medicine for prevention and treatment of AD by using endophenotypic network approach.

Ethics approval and consent to participate

Not applicable.

Funding

This work was supported by the National Key Research and Development Program of China (2023YFF1204900; 2023YFF1204901), National Natural Science Foundation of China (Grant No. 82074278 82160956), Hainan Provincial Natural Science Foundation of China (Grant No. 822MS185), Hainan Province Science and Technology Special Fund (Grant No. ZDKJ2021034).

Declaration of Competing Interest

The authors do not have any potential conflicts of interest.

Acknowledgements

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

Competing interests

The authors declare that they have no competing interests.

Author contributions

Jiansong Fang and Qihui Wu designed the research; Zhao Dai and Tian Hu contributed equally to this work; Zhao Dai and Junwen Wei performed the research; Qihui Wu and Zhao Dai drafted the manuscript; Chuipu Cai, Yong Gu, Tian Hu, Xue Wang and Wentao Wei analyzed the data; Yunhui Hu, Wenjia Wang, Qihui Wu and Jiansong Fang revised the manuscript. All authors contributed to the production of this manuscript and have approved the final version.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.csbj.2023.12.017.

References

[1] Trejo-Lopez JA, Yachnis AT, Prokop S. Neuropathology of Alzheimer's Disease. Neurother: J Am Soc Exp Neurother 2022;19(1):173–85.
[2] Jia L, Du Y, Chu L, Zhang Z, Li F, et al. Prevalence, risk factors, and management of dementia and mild cognitive impairment in adults aged 60 years or older in China: a cross-sectional study. Lancet Public Health 2020;5(12):e661–71.
[3] Syed YY. Sodium oligomannate: first approval. Drugs 2020;80(4):441–4.
[4] Howes M-JR, Fang R, Houghton PJ. Effect of Chinese herbal medicine on Alzheimer's disease. Int Rev Neurobiol 2017;135:29–56.
[5] Lam C-S, Tipoe GL, So K-F, Fung M-L. Neuroprotective mechanism of Lycium barbarum polysaccharides against hippocampal-dependent spatial memory deficits in a rat model of obstructive sleep apnea. PloS One 2015;10(2):e0117990.
[6] Li S-H, Gao P, Wang L-T, Yan Y-H, Xia Y, et al. Osthole stimulated neural stem cells differentiation into neurons in an Alzheimer's disease cell model via upregulation of MicroRNA-9 and rescued the functional impairment of hippocampal neurons in APP/PS1 transgenic mice. Front Neurosci 2017;11:340.
[7] Ajoolabady A, Lindholm D, Ren J, Pratico D. ER stress and UPR in Alzheimer's disease: mechanisms, pathogenesis, treatments. Cell Death Dis 2022;13(8):706.
[8] Li X, Qin Y, Ye S, Song H, Zhou P, et al. Protective effect of Huangpu Tongqiao capsule against Alzheimer's disease through inhibiting the apoptosis pathway mediated by endoplasmic reticulum stress in vitro and in vivo. Saudi Pharm J: SPJ: Publ Saudi Pharm Soc 2022;30(11):1561–71.
[9] Kou J-J, Shi J-Z, He Y-Y, Hao J-J, Zhang H-Y, et al. Luteolin alleviates cognitive impairment in Alzheimer's disease mouse model via inhibiting endoplasmic reticulum stress-dependent neuroinflammation. Acta Pharmacol Sin 2022;43(4):840–9.
[10] Ghiassian SD, Menche J, Chasman DI, Giulianini F, Wang R, et al. Endophenotype network models: common core of complex diseases. Sci Rep 2016;6:27414.
[11] Hai-Yu Xu, Xin Wang, Hong-Jun Yang, Kuo Yang, Ming Yang, Hai-Yang Yu, Bo Zhang, Peng Zhang, Qian-Ru Zhang, Hui-Hui Zhao, Jing Zhao, S.L.Y.-T.C.Q.-Y.D.J.-Y.D.X.-C.D.Y.-J.H.X.-X.L.Q.-F.L.M.N.R.-W.X.Z.X, (2021), Network Pharmacology Evaluation Method Guidance-Draft. World Journal of Traditional Chinese Medicine.
[12] Fang J, Pieper AA, Nussinov R, Lee G, Bekris L, et al. Harnessing endophenotypes and network medicine for Alzheimer's drug repurposing. Med Res Rev 2020.
[13] Fang J, Zhang P, Zhou Y, Chiang C-W, Tan J, et al. Endophenotype-based in silico network medicine discovery combined with insurance record data mining identifies sildenafil as a candidate drug for Alzheimer's disease. Nat Aging 2021;1(12):1175–88.
[14] Liu Z, Cai C, Du J, Liu B, Cui L, et al. TCMIO: a comprehensive database of traditional chinese medicine on immuno-oncology. Front Pharmacol 2020;11:439.
[15] Xue R, Fang Z, Zhang M, Yi Z, Wen C, et al. TCMID: traditional chinese medicine integrative database for herb molecular mechanism analysis. Nucleic Acids Res 2013;41(Database issue):D1089–95.
[16] Ru J, Li P, Wang J, Zhou W, Li B, et al. TCMSP: a database of systems pharmacology for drug discovery from herbal medicines. J Chemin- 2014;6:13.
[17] Zhang RZ, Yu SJ, Bai H, Ning K. TCM-Mesh: the database and analytical system for network pharmacology analysis for TCM preparations. Sci Rep 2017;7(1):2821.
[18] Kim SK, Nam S, Jang H, Kim A, Lee JJ. TM-MC: a database of medicinal materials and chemical compounds in Northeast Asian traditional medicine. BMC Complement Alter Med 2015;15:218.
[19] O'Boyle NM, Banck M, James CA, Morley C, Vandermeersch T, et al. Open Babel: an open chemical toolbox. J Chemin- 2011;3:33.
[20] Mendez D, Gaulton A, Bento AP, Chambers J, De Veij M, et al. ChEMBL: towards direct deposition of bioassay data. Nucleic Acids Res 2019;47(D1):D930–40.
[21] Gilson MK, Liu T, Baitaluk M, Nicola G, Hwang L, et al. BindingDB in 2015: a public database for medicinal chemistry, computational chemistry and systems pharmacology. Nucleic Acids Res 2016;44(Database issue):D1045–53.
[22] Szklarczyk D, Santos A, Mering CV, Jensen LJ, Bork P, et al. STITCH 5: augmenting protein–chemical interaction networks with tissue and affinity data. Nucleic Acids Res 2016;44(Database issue):D380–4.
[23] Ye H, Ye L, Kang H, Zhang D, Tao L, et al. HIT: linking herbal active ingredients to targets. Nucleic Acids Res 2011;39(Database issue):D1055.
[24] Huang Y, Fang J, Lu W, Wang Z, Wang Q, et al. A systems pharmacology approach uncovers wogonoside as an angiogenesis inhibitor of triple-negative breast cancer by targeting hedgehog signaling. Cell Chem Biol 2019;26(8).
[25] Zhou Y, Fang J, Bekris LM, Kim YH, Pieper AA, et al. AlzGPS: a genome-wide positioning systems platform to catalyze multi-omics for Alzheimer's drug discovery. Alzheimer'S Res Ther 2021;13(1):24.
[26] Stenson PD, Mort M, Ball EV, Evans K, Hayden M, et al. The human gene mutation database: towards a comprehensive repository of inherited mutation data for medical research, genetic diagnosis and next-generation sequencing studies. Hum Genet 2017;136(6):665–77.
[27] Pinero J, Bravo à, Queralt-Rosinach N, Gutiérrez-Sacristán A, Deu-Pons J, et al. DisGeNET: a comprehensive platform integrating information on human disease-associated genes and variants. Nucleic Acids Res 2017;45(Database issue):D833–9.
[28] Rappaport N, Twik M, Plaschkes I, Nudel R, Iny Stein T, et al. MalaCards: an amalgamated human disease compendium with diverse clinical and genetic annotation and structured search. Nucleic Acids Res 2017;45(D1):D877–87.
[29] Koscielny G, An P, Carvalho-Silva D, Cham JA, Fumis L, et al. Open targets: a platform for therapeutic target identification and validation. Nucleic Acids Res 2017;45(D1):D985–94.
[30] Daina A, Michielin O, Zoete V. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci Rep 2017;7:42717.
[31] Xiong G, Wu Z, Yi J, Fu L, Yang Z, et al. ADMETlab 2.0: an integrated online platform for accurate and comprehensive predictions of ADMET properties. Nucleic Acids Res 2021;49(W1).
[32] Cheng F, Lu W, Liu C, Fang J, Hou Y, et al. A genome-wide positioning systems network algorithm for in silico drug repurposing. Nat Commun 2019;10(1):3476.
[33] Guney E, Menche J, Vidal M, Barabasi AL. Network-based in silico drug efficacy screening. Nat Commun 2016;7:10331.
[34] Wang RP-H, Huang J, Chan KWY, Leung WK, Goto T, et al. IL-1β and TNF-α play an important role in modulating the risk of periodontitis and Alzheimer's disease. J Neuroinflamm 2023;20(1):71.
[35] Khezri MR, Ghasemnejad-Berenji M, Moloodsouri D. The PI3K/AKT signaling pathway and caspase-3 in alzheimer's disease: which one is the beginner? J Alzheimer'S Dis: JAD 2023;92(2):391–3.
[36] Fang J, Liu C, Wang Q, Lin P, Cheng F. In silico polypharmacology of natural products. Brief Bioinforma 2018;19(6):1153–71.
[37] Bastian, M., Heymann, S. and Jacomy, M., *Gephi: An Open Source Software for Exploring and Manipulating Networks*. 2009.
[38] Li H, Yu X, Li C, Ma L, Zhao Z, et al. Caffeic acid protects against Aβ toxicity and prolongs lifespan in Caenorhabditis elegans models. Food Funct 2021;12(3):1219–31.
[39] Andrade S, Loureiro JA, Pereira MdC. Influence of in vitro neuronal membranes on the anti-amyloidogenic activity of gallic acid: Implication for the therapy of Alzheimer's disease. Arch Biochem Biophys 2021;711:109022.
[40] Lee S, Park HJ, Jeon SJ, Kim E, Lee HE, et al. Cognitive ameliorating effect of acanthopanax koreanum against scopolamine-induced memory impairment in mice. Phytother Res: PTR 2017;31(3):425–32.
[41] Huang D, Hu Z, Yu Z. Eleutheroside B or E enhances learning and memory in experimentally aged rats. Neural Regeneration. Research 2013;8(12):1103–12.
[42] Ho C-C, Huang A-C, Yu C-S, Lien J-C, Wu S-H, et al. Ellagic acid induces apoptosis in TSGH8301 human bladder cancer cells through the endoplasmic reticulum stress- and mitochondria-dependent signaling pathways. Environ Toxicol 2014;29(11):1262–74.
[43] Jin T, Zhang Y, Botchway BOA, Huang M, Lu Q, et al. Quercetin activates the Sestrin2/AMPK/SIRT1 axis to improve amyotrophic lateral sclerosis. Biomed Pharmacother = Biomedecine Pharmacother 2023;161:114515.
[44] Hayakawa M, Itoh M, Ohta K, Li S, Ueda M, et al. Quercetin reduces eIF2α phosphorylation by GADD34 induction. Neurobiol Aging 2015;36(9):2509–18.
[45] Ramezani M, Darbandi N, Khodagholi F, Hashemi A. Myricetin protects hippocampal CA3 pyramidal neurons and improves learning and memory impairments in rats with Alzheimer's disease. Neural Regen Res 2016;11(12):1976–80.
[46] Karunakaran U, Elumalai S, Moon JS, Jeon JH, Kim ND, et al. Myricetin protects against high glucose-induced β-cell apoptosis by attenuating endoplasmic reticulum stress via inactivation of cyclin-dependent kinase 5. Diabetes Metab J 2019;43(2):192–205.
[47] Ni Y, Zhang J, Zhu W, Duan Y, Bai H, et al. Echinacoside inhibited cardiomyocyte pyroptosis and improved heart function of HF rats induced by isoproterenol via suppressing NADPH/ROS/ER stress. J Cell Mol Med 2022;26(21):5414–25.
[48] Chiu K-W, Chen H-Y, Chen C-L, Hsieh C-P, Huang Y-F. Attenuation of endoplasmic reticulum stress enhances carvacrol-induced apoptosis in osteosarcoma cell lines. Life (Basel, Switz) 2023;13(3).
[49] Ma C, Wang Z, Xia R, Wei L, Zhang C, et al. Danthron ameliorates obesity and MAFLD through activating the interplay between PPARα/RXRα heterodimer and adiponectin receptor 2. Biomed Pharmacother = Biomedecine Pharmacother 2021;137:111344.
[50] Vidal I, Torres-Vargas JA, Sánchez JM, Trigal M, García-Caballero M, et al. Danthron, an anthraquinone isolated from a marine fungus, is a new inhibitor of angiogenesis exhibiting interesting antitumor and antioxidant properties. Antioxid (Basel, Switz) 2023;12(5).
[51] Elenbaas JS, Bragazzi Cunha J, Azuero-Dajud R, Nelson B, Oral EA, et al. Lamin A/C maintains exocrine pancreas homeostasis by regulating stability of RB and activity of E2F. Gastroenterology 2018;154(6).
[52] Méndez-López I, Blanco-Luquin I, Sánchez-Ruiz de Gordoa J, Urdánoz-Casado A, Roldán M, et al. Hippocampal LMNA gene expression is increased in late-stage Alzheimer's disease. Int J Mol Sci 2019;20(4).
[53] Lim D, Tapella L, Dematteis G, Genazzani AA, Corazzari M, et al. The endoplasmic reticulum stress and unfolded protein response in Alzheimer's disease: a calcium dyshomeostasis perspective. Ageing Res Rev 2023;87:101914.
[54] Kumari, S., R. Dhapola, and D.H. Reddy, (2023), Apoptosis in Alzheimer's disease: insight into the signaling pathways and therapeutic avenues. Apoptosis: an International Journal On Programmed Cell Death.
[55] Gao H, Lei X, Ye S, Ye T, Hua R, et al. Genistein attenuates memory impairment in Alzheimer's disease via ERS-mediated apoptotic pathway in vivo and in vitro. J Nutr Biochem 2022;109:109118.
[56] Li F., Z.Y., Zhang T., Zhang T., Liu L., Wang P., (2019), Efficacy and safety of traditional Chinese medicine kidney-nourishing formula for Alzheimer's disease in

comparison with donepezil: a systematic review and meta-analysis. Traditional Medicine Research.
[57] Zhang J, Liu Z, Zhang H, Yang C, Li H, et al. A two-year treatment of amnestic mild cognitive impairment using a compound chinese medicine: a placebo controlled randomized trial. Sci Rep 2016;6:28982.
[58] Iwasaki K, Kobayashi S, Chimura Y, Taguchi M, Inoue K, et al. A randomized, double-blind, placebo-controlled clinical trial of the Chinese herbal medicine "ba wei di huang wan" in the treatment of dementia. J Am Geriatr Soc 2004;52(9):1518–21.
[59] Wang W, Jiang S, Zhao Y, Zhu G. Echinacoside: a promising active natural products and pharmacological agents. Pharm Res 2023;197:106951.
[60] Shiao YJ, Su MH, Lin HC, Wu CR. Echinacoside ameliorates the memory impairment and cholinergic deficit induced by amyloid beta peptides via the inhibition of amyloid deposition and toxicology. Food Funct 2017;8(6):2283–94.
[61] Zhang D, Li H, Wang JB. Echinacoside inhibits amyloid fibrillization of HEWL and protects against Abeta-induced neurotoxicity. Int J Biol Macromol 2015;72:243–53.
[62] Zhang Y, Long H, Zhou F, Zhu W, Ruan J, et al. Echinacoside's nigrostriatal dopaminergic protection against 6-OHDA-Induced endoplasmic reticulum stress through reducing the accumulation of Seipin. J Cell Mol Med 2017;21(12):3761–75.
[63] Lin Z, Teng C, Ni L, Zhang Z, Lu X, et al. Echinacoside upregulates Sirt1 to suppress endoplasmic reticulum stress and inhibit extracellular matrix degradation in vitro and ameliorates osteoarthritis in vivo. Oxid Med Cell Longev 2021;2021:3137066.
[64] Ni Y, Zhang J, Zhu W, Duan Y, Bai H, et al. Echinacoside inhibited cardiomyocyte pyroptosis and improved heart function of HF rats induced by isoproterenol via suppressing NADPH/ROS/ER stress. J Cell Mol Med 2022;26(21):5414–25.
[65] Kwon YS, Koh JY, Song DK, Kim HC, Kwon MS, et al. Danthron inhibits the neurotoxicity induced by various compounds causing oxidative damages including beta-amyloid (25-35) in primary cortical cultures. Biol Pharm Bull 2004;27(5):723–6.
[66] Fang J, Wu Z, Cai C, Wang Q, Tang Y, et al. Quantitative and systems pharmacology. 1. In Silico prediction of drug-target interaction of natural products to enable of new targeted cancer therapy. J Chem Inf Model 2017;57:2657–71.
[67] Guo Y, Bao C, Ma D, Cao Y, Li Y, et al. Network-based combinatorial crispr-cas9 screens identify synergistic modules in human cells. ACS Synth Biol 2019;8(3):482–90.
[68] Li S, Zhang B, Zhang N. Network target for screening synergistic drug combinations with application to traditional Chinese medicine. BMC Syst Biol 2011;5(Suppl 1):S10. Suppl 1.