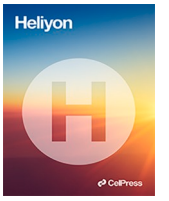
Heliyon 9 (2023) e17603

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Repositioning of clinically approved drug Bazi Bushen capsule for treatment of Aizheimer's disease using network pharmacology approach and *in vitro* experimental validation

Tongxing Wang [a,b,1], Meng Chen [a,b,1], Huixin Li [a,b], Guoyuan Ding [a,b], Yanfei Song [a,b], Bin Hou [a,b], Bing Yao [a,b], Zhixin Wang [a,b], Yunlong Hou [a,b], Junqing Liang [a,b,*], Cong Wei [a,b,c,**], Zhenhua Jia [a,b,c,***]

[a] *National Key Laboratory of Collateral Disease Research and Innovative Chinese Medicine, Shijiazhuang, 050035, PR China*
[b] *Hebei Yiling Pharmaceutical Research Institute, Key Laboratory of State Administration of TCM (Cardio-Cerebral Vessel Collateral Diseases), Shijiazhuang, 050035, PR China*
[c] *Key Disciplines of State Administration of TCM for Collateral Disease, Shijiazhuang, 050035, PR China*

A R T I C L E I N F O

Keywords:
Drug repositioning
Bazi Bushen capsule
Network pharmacology
Aizheimer's disease
Network targets
Mechanism of action

A B S T R A C T

Aims: To explore the new indications and key mechanism of Bazi Bushen capsule (BZBS) by network pharmacology and *in vitro* experiment.
Methods: The ingredients library of BZBS was constructed by retrieving multiple TCM databases. The potential target profiles of the components were predicted by target prediction algorithms based on different principles, and validated by using known activity data. The target spectrum of BZBS with high reliability was screened by considering the source of the targets and the node degree in compound-target (*C*-T) network. Subsequently, new indications for BZBS were predicted by disease ontology (DO) enrichment analysis and initially validated by GO and KEGG pathway enrichment analysis. Furthermore, the target sets of BZBS acting on AD signaling pathway were identified by intersection analysis. Based on STRING database, the PPI network of target was constructed and their node degree was calculated. Two Alzheimer's disease (AD) cell models, BV-2 and SH-SY5Y, were used to preliminarily verify the anti-AD efficacy and mechanism of BZBS *in vitro*.
Results: In total, 1499 non-repeated ingredients were obtained from 16 herbs in BZBS formula, and 1320 BZBS targets with high confidence were predicted. Disease enrichment results strongly suggested that BZBS formula has the potential to be used in the treatment of AD. GO and KEGG enrichment results provide a preliminary verification of this point. Among them, 113 functional targets of BZBS belong to AD pathway. A PPI network containing 113 functional targets and 1051 edges for the treatment of AD was constructed. *In vitro* experiments showed that BZBS could

* Corresponding author. National Key Laboratory of Collateral Disease Research and Innovative Chinese Medicine, Shijiazhuang, 050035, PR China.
** Corresponding author. National Key Laboratory of Collateral Disease Research and Innovative Chinese Medicine, Shijiazhuang, 050035, PR China.
*** Corresponding author. National Key Laboratory of Collateral Disease Research and Innovative Chinese Medicine, Shijiazhuang, 050035, PR China.
E-mail addresses: liangjunqing@yiling.cn (J. Liang), weitcm@163.com (C. Wei), jzhjiazhenhua@163.com (Z. Jia).
[1] Tongxing Wang and Meng Chen contributed equally to this work.

https://doi.org/10.1016/j.heliyon.2023.e17603
Received 5 February 2023; Received in revised form 17 June 2023; Accepted 22 June 2023
Available online 27 June 2023

significantly reduce the release of TNF-α and IL-6 and the expression of COX-2 and PSEN1 in $A\beta_{25-35}$-induced BV-2 cells, which may be related to the regulation of $ERK_{1/2}$/NF-κB signaling pathway. BZBS reduced the apoptosis rate of $A\beta_{25-35}$ induced SH-SY5Y cells, significantly increased mitochondrial membrane potential, reduced the expression of Caspase3 active fragment and PSEN1, and increased the expression of IDE. This may be related to the regulation of GSK-3β/β-catenin signaling pathway.

Conclusions: BZBS formula has a potential use in the treatment of AD, which is achieved through regulation of $ERK_{1/2}$, NF-κB signaling pathways, and GSK-3β/β-catenin signaling pathway. Furthermore, the network pharmacology technology is a feasible drug repurposing strategy to reposition new clinical use of approved TCM and explore the mechanism of action. The study lays a foundation for the subsequent in-depth study of BZBS in the treatment of AD and provides a basis for its application in the clinical treatment of AD.

1. Introduction

Drug development is a challenging process that requires huge cost and long-term investment with high-risk of failure. One type of drug repurposing (also known as drug repositioning) refers to the development of marketed drugs for new indications, which is an effective strategy to reduce time-to-market costs and risks [1]. Traditional Chinese medicine (TCM) originated in ancient China has

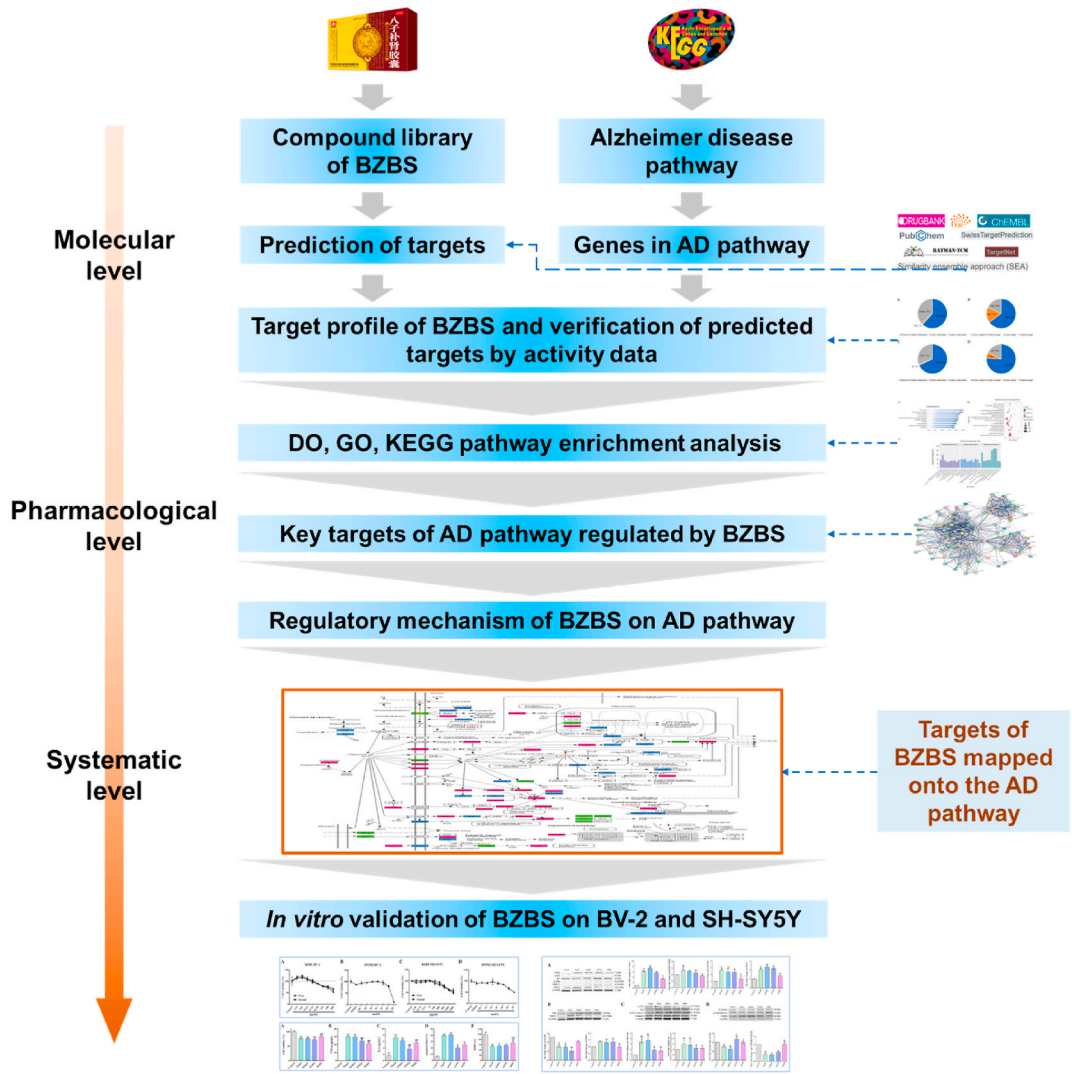


Fig. 1. Schematic diagram describing the concept of enrichment-based drug repositioning of BZBS applied in this study.

been practiced over thousands of years for treating various symptoms and diseases. However, TCM is a very complex system with multiple components, multiple targets and synergistic or antagonistic interactions among its components [2]. Unlike western medicine of "one target, one drug," the concept of the integrity of the whole human body is emphasized in the theory of TCM. Recently, network pharmacology, combining with pharmacokinetic evaluation, as well as pathway and network analysis, has been be widely accepted as an efficient research strategy to explore TCM from the perspective of biological network balance [3,4]. Its systematic thought coincides with the holistic view and syndrome differentiation of TCM [5]. This method is very suitable for the research and development of TCM. In recent years, Chinese scholars have made some important achievements and progress in the establishment of network pharmacology research methods and the application of them to study the scientific connotation of TCM, such as identification of new targets, discovery of potential active compounds, elucidation of mechanism of action, etc. [6–11]. There are also researchers using drug repositioning strategies in network pharmacology to discover new indications for marketed TCM, thereby accelerating drug innovation [12,13]. Therapeutic idea of "*Homotherapy for heteropathy*" is the typical characteristics of TCM [14]. Therefore, it is the high possibility of discovery new indications for existing herbal formulas with drug repurposing strategies of network pharmacology.

Bazi Bushen capsule (BZBS), a Chinese patented drug approved by the CFDA (No. B20020585), consists of 16 herbs, including *Cuscuta chinensis* Lam. (Tu-Si-Zi), *Lycium harharum* L. (Gou-Qi-Zi), *Schisandra chinensis* (Turcz.) Baill (Wu-Wei-Zi), *Cnidium monnieri* (L.). Cusson (She-Chuang-Zi), *Rosa Laevigata* Michx. (Jin-Ying-Zi), *Rubus chingii* Hu (Fu-Pen-Zi), *Allium tuberosum* Rottler ex Spreng. (Jiu-Cai-Zi), *Toosendan fructus* (Chuan-Lian-Zi), *Epimedium brevicornu* Maxim. (Yin-Yang-Huo), *Morindae officinalis* radix (Ba-Ji-Tian), *Cistanche deserticola* Ma (Rou-Cong-Rong), *Rehmannia root* (Di-Huang), *Cyathula officinalis* K·C.Kuan (Chuan-Niu-Xi), *Panax ginseng* C. A.Mey. (Ren-Shen), *Cervus nippon* Temminck (Lu-Rong), and *Hippocampus Kelloggi* (Hai-Ma) (). In clinical practice, it has been officially approved to counteract the waist and knee pains, dizziness and tinnitus, attenuate mental fatigue. Recent studies have been conducted to examine efficacy of BZBS on new indications, such as improvement of sexual function [15], promotion of anaerobic exercise endurance [16], combat of various aging-related declines [15,17–19], improvement of lipid metabolism [17], alleviation of post-menopausal atherosclerosis [18]. In addition, BZBS intervention ameliorated reduced brain performances in aging mice, including memory, cognitive, and motor functions [19]. It is reasonably expected that BZBS may have some other indications. The bioactive compounds, the potential targets, mode of action, and new indications of BZBS are far from being explored.

In the current study, we carried out a systematic and comprehensive study on the components, targets, new indications, and key mechanism of BZBS through network pharmacology (Fig. 1). Based on this, *in vitro* experiments were further conducted to validate the new indication and key mechanisms. This study lays a foundation for the subsequent in-depth study of BZBS and provides a basis for its application in the clinical treatment of Alzheimer's disease (AD).

2. Materials and methods

2.1. Collection of the ingredients in BZBS

Ingredients of BZBS were manually collected as comprehensively as possible from TCM_ID [20], TCM_Mesh [21], TCMGeneDIT [22], TCMID [23,24], ETCM [25], TM_MC [26], TcmSP [27], BATMAN-TCM database [28]. Finally, the ingredients information was normalized through the CID number in PubChem database [29].

2.2. Acquisition of target profiles of BZBS and verification of data reliability

A comprehensive target spectrum of BZBS was the base to discovery new indication and to explore mechanism of action [30]. In order to explore the potential target of components as fully as possible, several target prediction tools such as TargetNet [31], SwissTargetPrediction [32], ChEMBL_prediction tool [33], BATMAN-TCM [28], and STITCH [34] were used to obtain the putative targets. To improve reliability, the targets from (1) marketed drug databases such as DrugBank [35] and TTD [36], (2) activity assay databases such as ChEMBL [37] and PubChem [38] were also got as the known targets. Only the putative targets which can be predicted in at least two prediction models were preserved. All the known targets were kept (see Table 1). All the targets were normalized by UniProt database (http://www.uniprot.org) [39] and only the targets belonging to "*Homo sapiens*" were kept. In this study, the known target data were used as the validation of the prediction target to verify the accuracy of the prediction methods and improve the integrity and reliability of the target profiles.

Table 1
Databases used to obtain target profiles of BZBS.

Target source	Interaction type	Target type	Reference
TargetNet	Putative interaction	Putative target	[31]
SwissTargetPrediction	Putative interaction	Putative target	[32]
CHEMBL prediction	Putative interaction	Putative target	[33]
BATMAN-TCM	Putative interaction	Putative target	[28]
STITCH	Putative interaction	Putative target	[34]
DrugBank	Known interaction	Known target	[35]
TTD	Known interaction	Known target	[36]
CHEMBL	Known interaction	Known target	[37]
PubChem	Known interaction	Known target	[38]

2.3. Enrichment analysis for targets of BZBS

For the sake of repositioning BZBS for potential new indications, disease ontology (DO) (http://disease-ontology.org/) [40] enrichment analysis was conducted. Further, gene ontology (GO), and KEGG pathway enrichment analysis were performed [41]. On the one hand, the results of DO can be preliminarily verified, and on the other hand, the mechanism of action of BZBS against new indications can be interpreted from a systematic perspective. All the above enrichment analyses were carried out using clusterProfiler Version 4.0.3, an R Bioconductor package [42].

2.4. Networks construction for anti-AD targets of BZBS

Protein-protein interaction (PPI) network were constructed by using BZBS acting on the targets of the AD pathway through STRING database Version 11.5 (https://string-db.org/) [43]. The anti-AD compound-target (*C*-T) network were constructed by using Cytoscape v3.7.1 [44]. In these networks, the compounds or proteins were represented as nodes, and the relationship between compounds and targets were showed as edges. NetworkAalyzer plugin [45] of Cytoscape was used to compute the degree. The degree of a node is the number of neighbor nodes.

The network topology information of target in PPI network and anti-AD *C*-T network was considered comprehensively. The targets with degree greater than the average degree of the PPI network or the anti-AD *C*-T network were selected as candidate key targets.

2.5. In vitro cell experiments

2.5.1. Preparation of BZBS and its compounds

The BZBS stock solution was prepared with DMEM medium, and donepezil (DNPQ) was dissolved with dimethyl sulfoxide (DMSO). We diluted them to the required concentration (DMSO $\leq$0.1%) with DMEM or MEM before the experiment.

2.5.2. Cell culture and treatment

BV-2 mouse microglia cells were purchased from Zhongqiao Xinzhou (ZQ0397). SH-SY5Y human neuroblastoma cells were purchased from ATCC (CRL-2266). BV-2 cells were cultured in MEM medium supplemented with 10% FBS, 1% sodium pyruvate, 1‰ streptomycin, penicillin, and gentamicin. SH-SY5Y cells were cultured in DMEM medium supplemented with 10% FBS, 1‰ streptomycin, penicillin and gentamicin. It was incubated at 37 °C and 5% CO_2. The culture medium was changed every two days.

Logarithmic growth phase cells were planted on plates at a density of 2×10^5/mL and grown for 24 h before treatment. SH-SY5Y cells were divided into control group (without $A\beta_{25\text{-}35}$), model group (40 μM $A\beta_{25\text{-}35}$, 24 h), DMSO group (40 μM $A\beta_{25\text{-}35}$ + 1‰ DMSO, 24 h), DNPQ group (40 μM $A\beta_{25\text{-}35} + 10^{-6}$ M DNPQ, 24 h), BZBS group (40 μM $A\beta_{25\text{-}35}$+ 60 μg/mL BZBS, 24 h). BV-2 cells were divided into control group (without $A\beta_{25\text{-}35}$), model group (30 μM $A\beta_{25\text{-}35}$, 24 h), DMSO group (30 μM $A\beta_{25\text{-}35}$ + 1‰ DMSO, 24 h), DNPQ group (30 μM $A\beta_{25\text{-}35} + 10^{-6}$ M DNPQ, 24 h), BZBS group (30 μM $A\beta_{25\text{-}35}$ + 60 μg/mL BZBS, 24 h).

2.5.3. Cell viability assay

BV-2 cells and SH-SY5Y cells were seeded at a density of 2×10^4 cells per well into 96 well microplates. Drug intervention was carried out according to the above groups. CellTiter 96® AQueous One Solution Cell Proliferation Assay kit (G3581, Promega, USA) was used to determine the viability of cells. Each group contained triplicate wells in every independent experiment to ensure data reliability. The absorbance of the samples was measured at 490 nm/630 nm.

2.5.4. Enzyme linked immunosorbent assay (ELISA)

BV-2 cells were seeded at a density of 2×10^4 cells per well into 96 well microplates. Drug intervention was carried out according to the above groups. After 24 h of drug intervention, the culture supernatant was collected and stored at −80 °C. IL-6 and TNFα were quantified by ELISA Kit (70-EK206/3–96, 70-EK282/4–96, MultiSciences, China). The absorbance of the samples was measured at 450 nm.

2.5.5. Apoptosis was detected by flow cytometry

SH-SY5Y cells were seeded at a density of 2×10^5 cells per well into 6-well plates. Drug intervention was carried out according to the above groups. After 24 h of culture, the supernatant was discarded and cells was collected after digesting with trypsin. The cells with density of 2×10^5 cells/mL were resuspended at 200 μL in binding buffer. Annexin V-FITC (5 μL) and PI (10 μL) (70-AP101-100, MultiSciences, China) were gently mixed and incubated at room temperature in dark for 15 min. Next, 400 μL 1 × binding buffer was added and SH-SY5Y were harvested and analyzed using the BD-FACSAria-III flow cytometry.

2.5.6. Mitochondrial membrane potential detection

SH-SY5Y cells were seeded at a density of 2×10^4 cells per well into 96 well microplates. Drug intervention was carried out according to the above groups. After 24 h of incubation, we discarded the supernatant, added 100 μl of JC-1 (10,009,172, Cayman, USA) staining solution, and fully mixed it. They were incubated at 37 °C for 20 min. After incubation, the supernatant was aspirated and washed twice with JC-1 staining buffer. 100 μL of cell culture medium was added and the absorbance was measured by using a microplate reader (Bio Tek Synergy4). The excitation and emission light wavelength were set to 490 nm/525 nm and 530 nm/590 nm respectively.

2.5.7. Western blotting

SH-SY5Y cells and BV-2 cells were treated as described above. The cells were washed twice with pre-cooled PBS, and the total protein extract was prepared in RIPA lysis buffer containing protease inhibitor. We performed ultrasonic crushing (ultrasonic 2 s, pause 5 s, 80% power, 30 times) and centrifugation at 4 °C (12,000×*g*) for 30 min. The supernatant was finally taken. Protein concentrations were measured with the BCA assay (P0012, Beyotime Biotechnology, China). The same amount of protein was subjected to SDS polyacrylamide gel electrophoresis (SDS-PAGE) in 10–15% gel, electrophoresis at 120 v for 1.5 h, and then transferred to nitrocellulose (NC) membrane, and the membrane was transferred at 0.1 A for 45 min. Then the membranes were blocked with blocking solution at 37 °C for 1 h. Membranes were then incubated with primary antibodies overnight at 4 °C. After incubation with primary antibodies, membranes were washed three times and incubated with appropriate secondary antibodies (1:5000 dilution) at 37 °C for 1 h. GAPDH was used to control protein quality and ensure equal loading. Finally, Odyssey protein scanner was used to scan the image. The antibodies used for BV-2 cells are: p-p65 (#3033, CST), p65 (ab32536, Abcam), COX2 (ab62331, Abcam), *p*-ERK1/2 (#4370, CST), ERK1/2 (ab17942, Abcam), PSEN1 (#5643, CST), GAPDH (#2118, CST). The antibodies used for SH-SY5Y cells are: β-catenin (ab32572, Abcam), *p*-GSK3β (#9322, CST), GSK3β (ab131356, Abcam), IDE (ab32216, Abcam), p-Tau (ab109390, Abcam), Tau (ab76128, Abcam), Caspase3 (#9664, CST), PSEN1 (#5643, CST).

2.5.8. Statistics

Experimental data were expressed as the mean ± standard deviation ($\bar{x} \pm s$). SPSS19.0 software was used for statistical analysis. One-way analysis of variance (ANOVA) was used for comparison among groups, the least significant difference (LSD) method was used for pairwise comparison between groups, and $P < 0.05$ was considered statistically significant. All graphs are done through GraphPad Prism (V.5.01).

3. Results

3.1. Compound library of BZBS

By comprehensively retrieve the ingredients from public TCM databases, a total of 1498 unique compounds after excluding repeated ones were obtained as the compound library of BZBS (Table S1). The number of ingredients corresponding to each herb was shown in Table 2.

3.2. Target profile of BZBS and verification of predicted targets

The target spectrum of BZBS with high reliability was screened by considering the source of the targets and the node degree in *C*-T network. After eliminating the overlaps, 1320 protein targets were kept for the subsequent analysis (Table S2). The number of potential targets corresponding to each herb was shown in Table 2.

Fig. 2A showed that 62% of the predicted interaction relationships can be verified by the reported activity data. 65% of the predicted targets were validated by known targets, which, combined with the other 17% known targets, accounted for 82% of the total (Fig. 2B). The above data proved the reliability of the target prediction methods and ensured the accuracy of subsequent analysis. The target spectrum of BZBS square was constructed by integrating the putative targets with the known targets for subsequent analysis.

3.3. Enrichment analysis suggests that BZBS may have therapeutic potential for new indications of Alzheimer's disease

DO enrichment analysis of 1320 BZBS targets showed that the top three significantly enriched diseases were: tauopathy (pvalue =

Table 2
The number of ingredients and targets contained in each herb in BZBS capsule.

#	Latin name	Chinese pinyin	# of ingredients	# of targets
1	*Cuscuta chinensis* Lam.	Tu-Si-Zi	49	636
2	*Lycium harharum* L.	Gou-Qi-Zi	245	1035
3	*Schisandra chinensis* (Turcz.) Baill.	Wu-Wei-Zi	309	912
4	*Cnidium monnieri* (L.) Cusson	She-Chuang-Zi	137	626
5	*Rosa Laevigata* Michx.	Jin-Ying-Zi	54	833
6	*Rubus chingii* Hu	Fu-Pen-Zi	132	887
7	*Allium tuberosum* Rottler ex Spreng.	Jiu-Cai-Zi	43	673
8	*Toosendan fructus*	Chuan-Lian-Zi	76	755
9	*Epimedium brevicornu* Maxim.	Yin-Yang-Huo	148	866
10	*Morindae officinalis* radix	Ba-Ji-Tian	152	765
11	*Cistanche deserticola* Ma	Rou-Cong-Rong	106	846
12	*Rehmannia root*	Di-Huang	100	842
13	*Cyathula officinalis* K·C.Kuan	Chuan-Niu-Xi	28	586
14	*Panax ginseng* C.A.Mey.	Ren-Shen	445	1113
15	*Cervus nippon* Temminck	Lu-Rong	48	915
16	*Hippocampus Kelloggi*	Hai-Ma	4	117

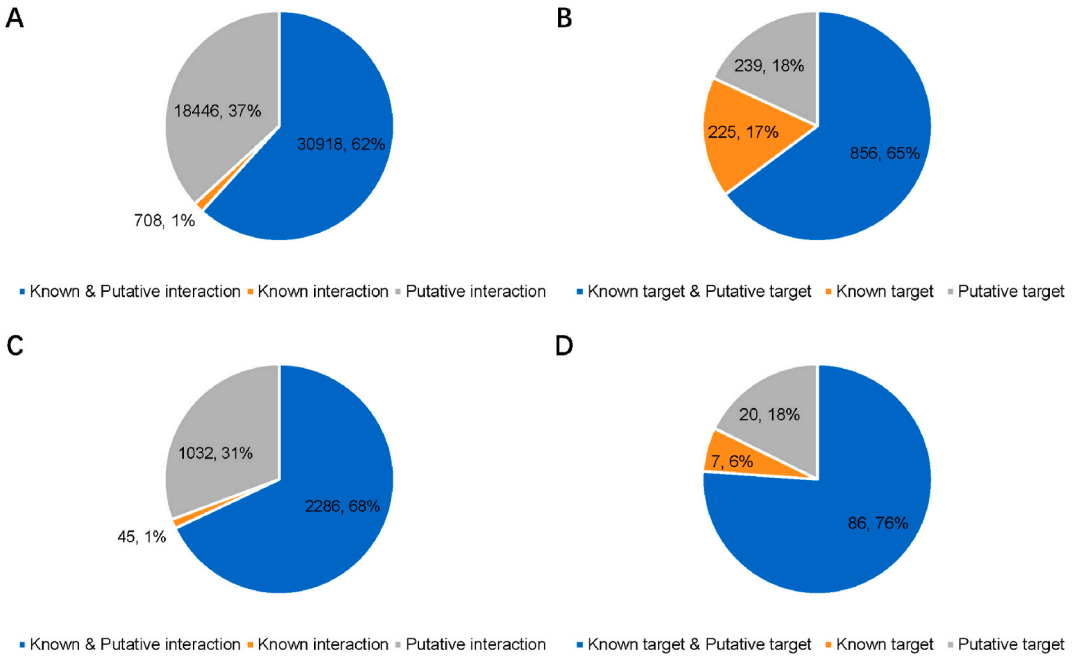


Fig. 2. Statistics of the target type and interaction type in compound-target network based on target data sources. (A) Types of interactions in compound-target network and **(B)** types of 1320 targets with high confidence; **(C)** Types of interactions in anti-AD compound-target network and **(D)** types of 113 anti-AD targets.

4.62e-26), Alzheimer's disease (pvalue = 5.74e-26) and brain disease (pvalue = 6.38e-24) (Fig. 3A). These results strongly suggest that BZBS has therapeutic potential for neurodegenerative diseases.

Further, the KEGG pathway and GO enrichment analysis results were used to preliminarily verify the newly discovered indications of BZBS. KEGG pathway enrichment analysis showed that there were two pathways most related to neurodegenerative diseases: Alzheimer's disease (pvalue = 3.06e-18) and pathways of neurodegeneration (pvalue = 5.31e-17) were significantly enriched. Other highly ranked signaling pathways, such as neuroactive ligand-receptor interaction (pvalue = 8.86e-46), cAMP signaling pathway (pvalue = 1.02e-28), serotonergic synapse (pvalue = 1.06e-20), are also closely related to neurodegenerative diseases such as AD (Fig. 3B). GO enrichment analysis results showed that the first two significantly enriched biological processes (regulation of membrane potential (pvalue = 6.67e-78), regulation of postsynaptic membrane potential (pvalue = 4.34e-60)), the first five significantly enriched cell components (transmembrane transporter complex (pvalue = 4.34e-70), transporter complex (pvalue = 1.54e-68), ion channel complex (pvalue = 1.50e-64), Synaptic membrane (pvalue = 3.31e-48), postsynaptic membrane (pvalue = 7.96e-46), and the first three significantly enriched molecular functions (neurotransmitter receptor activity (pvalue = 6.80e-68), channel activity (pvalue = 2.83e-60), and passive transmembrane transporter activity (pvalue = 3.92e-60)) are all closely related to neurodegenerative diseases (Fig. 3C). The above KEGG pathway and GO enrichment analysis results preliminarily proved the reliability of the new indications for BZBS predicted by DO enrichment analysis. In this study, we focused on Alzheimer's disease for subsequent analysis.

3.4. Screening of key targets of alzheimer disease pathway regulated by BZBS

In total, 113 targets of BZBS acting on AD signaling pathway were identified by intersection analysis. In the corresponding anti-AD *C*-T subnetwork (Table S3), the known interaction relationship accounted for 69% (Fig. 2C) and the known target accounted for 83.6% (Fig. 2D).

A PPI network containing 113 nodes and 1051 edges was constructed based on the STRING database (Fig. 4). The network. The average node degree and the average local clustering coefficient were 18.6 and 0.66, respectively. PPI interaction enrichment result indicated that this PPI sub-network has significantly more interactions than what would be expected for a random set of proteins of similar size, drawn from the genome (P = 1.0e-16). This means that these proteins are closely connected biologically as a group (Table S4).

By comprehensively considering the network topology information of targets in PPI network and anti-AD component-target network, a total of 65 targets with degree greater than the average degree of the PPI network or anti-AD *C*-T network were selected as candidate key targets. Among them, the degree of 19 targets met the above two conditions (Table 3).

113 therapeutic targets enriched in the Alzheimer disease pathway (hsa05010) were displayed (Fig. 5). We found that targets were widely distributed in this pathway and were involved in regulating multiple biological processes, such as APP processing, energy production, neuronal injury, apoptosis, mitochondrial dysfunction, Tau protein phosphorylation, synaptic dysfunction, reactive

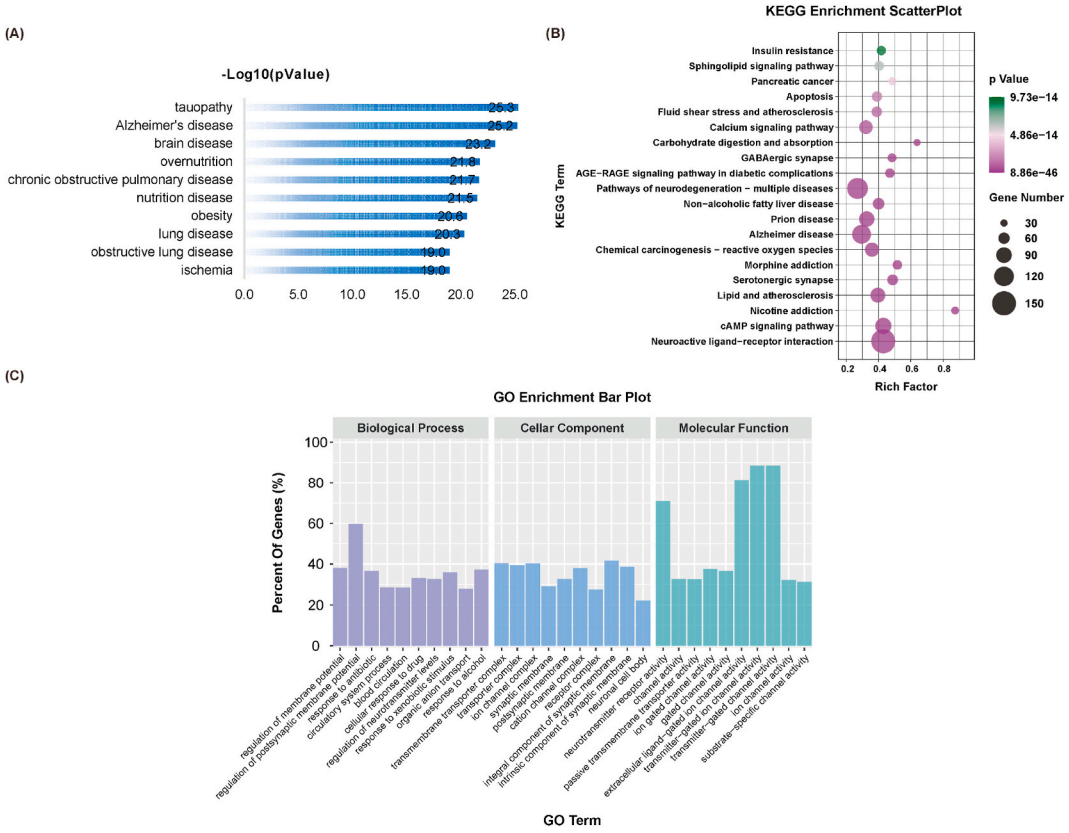


Fig. 3. Enrichment analysis of 1320 BZBS targets. (A) Disease ontology (DO) enrichment analysis results; **(B)** KEGG pathway enrichment analysis results. Rich Factor refers to the ratio of the number of annotated GO entries in 1320 targets to the total number of genes in the GO entry. The greater the Rich Factor, the greater the degree of enrichment. The size of the dot is proportional to the number of genes enriched in the term; **(C)** Gene ontology (GO) enrichment analysis results. GO categories include "Molecular Function" and "Cellular Component", "Biological Process" terms.

oxygen species (ROS), neurofibrillary tangles, neuroinflammation, long-term potentiation (LTP), autophagy, and axonal transport defects, ultimately affecting the survival of neurons. In addition, we also found the cross-talk effect between this pathway and other pathways, such as calcium signaling pathway, apoptosis, Wnt signaling pathway, AGE-RAGE signaling pathway in diabetic complications, insulin signaling pathway, and autophagy.

3.5. *Effects of BZBS on the viability of BV-2 and SH-SY5Y cells*

The experimental results are shown. Compared with the control group, the viability of BV-2 cells in the BZBS group with the concentration below 62.5 μg/mL has no significant difference (Fig. 6A), and viability of SH-SY5Y cells in the BZBS group with the concentration below 300 μg/mL has no significant difference (Fig. 6C). The viability of BV-2 and SH-SY5Y cells in DNPQ group with concentrations below 10^{-6} M has no significant difference (Fig. 6B and D).

3.6. *Anti-inflammation effects of BZBS in BV-2 cells*

The pathophysiology of AD is closely related to inflammation of the central nervous system [46]. The anti-inflammatory activity of BZBS was evaluated on BV-2 cells induced by $A\beta_{25\text{-}35}$. Compared with the control group, the cell viability in model group was significantly decreased ($p < 0.01$), and the secretion of TNF-α and IL-6 were significantly increased ($p < 0.01$). Compared with the model group, the cell viability of BZBS group was significantly increased ($p < 0.01$), and the secretion of TNF-α and IL-6 in BZBS group were significantly decreased ($p < 0.01$). These results suggested that BZBS can inhibit the release of cytokines in BV-2 glial cells (Fig. 7A–C).

3.7. *Effects of BZBS on the apoptosis and mitochondrial membrane potential of SH-SY5Y cells induced by $A\beta_{25\text{-}35}$*

Compared with the control group, the apoptosis rate of the model group was significantly increased ($P < 0.01$), and the mitochondrial membrane potential (MMP) was significantly decreased ($P < 0.01$). Compared with the model group, the apoptosis rate of

Fig. 4. PPI network of 113 anti-AD functional targets of BZBS. PPI, Protein-protein interaction; AD, Alzheimer's disease; BZBS, Bazi Bushen capsule.

the drug treatment group was decreased, and the apoptosis rate of DNPQ group was significantly decreased ($P < 0.05$). The MMP of BZBS group was significantly increased ($P < 0.01$), and BZBS group was significantly higher than DNPQ group ($P < 0.01$). These results suggested that BZBS can decrease the apoptosis rate and significantly increase the MMP of SH-SY5Y cells induced by $A\beta_{25\text{-}35}$, which is better than DNPQ (Fig. 7D and E).

3.8. Effects of BZBS on the expression of COX-2, p-p65, p-ERK1/2, PSEN1 in BV-2 cells induced by $A\beta_{25\text{-}35}$

Compared with the control group, the protein expressions of COX-2, p-p65, p- $ERK_{1/2}$ and PSEN1 in the model group were increased. Compared with the model group, the protein expression of COX-2, p-p65, *p*-$ERK_{1/2}$ and PSEN1 in BZBS group were decreased. These results indicated that BZBS can inhibit the expression of COX-2 and p-p65, the hallmark proteins of glial inflammation, which may be related to $ERK_{1/2}$ and NF-κB signaling pathways (Fig. 8A).

3.9. Effects of BZBS on the expression of IDE, PSEN1, p-Tau and active caspase3 in SH-SY5Y cells induced by $A\beta_{25\text{-}35}$

Compared with the control group, the expression of PSEN1, p-Tau and active caspase-3 in the model group were increased, but there was no significant difference in insulin degrading enzyme (IDE). Compared with the model group, the expression of IDE in BZBS

Table 3
Key targets of Alzheimer disease pathway regulated by BZBS.

Gene	Degree in PPI net	Degree in anti-AD *C*-T net	Target type
APP	36	196	Known & Putative target
RELA	33	182	Known & Putative target
CASP9	24	176	Known & Putative target
NOS1	27	150	Known & Putative target
PTGS2	26	129	Known & Putative target
NOS2	23	104	Known & Putative target
COX4I1	28	54	Putative target
COX2	24	54	Putative target
COX5B	20	54	Putative target
COX1	20	54	Putative target
COX5A	19	54	Putative target
COX6B1	19	54	Putative target
TNF	37	53	Known & Putative target
GRIN2B	30	43	Known & Putative target
GRIN1	23	41	Known & Putative target
SDHC	27	39	Known & Putative target
SDHB	20	39	Known & Putative target
IL1B	37	38	Known & Putative target
PIK3CA	23	35	Known & Putative target
MAPT	48	26	Known & Putative target
AKT3	20	26	Putative target
GSK3B	36	24	Known & Putative target
NFKB1	26	23	Known & Putative target
PIK3R1	24	23	Known & Putative target
AKT1	58	16	Known & Putative target
CASP3	42	12	Known & Putative target
INS	41	12	Known & Putative target
MAPK1	38	8	Known & Putative target
CASP8	29	8	Known & Putative target
CALM1	30	6	Known & Putative target
MAPK8	30	6	Known & Putative target
CALM2	21	6	Known & Putative target
CALM3	21	5	Known & Putative target
CDK5	21	4	Known & Putative target
TUBB	19	4	Known & Putative target
CTNNB1	44	3	Known & Putative target
MAPK9	20	3	Known & Putative target
GAPDH	69	2	Known & Putative target
MAPK3	43	2	Known & Putative target
MTOR	35	2	Known & Putative target
SNCA	30	2	Known & Putative target
NRAS	25	2	Known & Putative target
ATP5F1 B	22	2	Known & Putative target
TUBB4A	22	2	Known & Putative target
ATP5F1 A	19	2	Known & Putative target
CHRM5	4	146	Known & Putative target
PIK3CD	7	124	Putative target
CHRM1	9	65	Known & Putative target
CHRNA7	9	64	Known & Putative target
CSNK2A1	11	57	Known & Putative target
COX6C	18	54	Putative target
COX6A2	17	54	Putative target
COX7C	17	54	Putative target
COX8A	17	54	Putative target
COX7B	14	54	Putative target
COX7A1	13	54	Putative target
COX3	9	54	Putative target
WNT4	4	54	Putative target
GRIN2A	18	49	Known & Putative target
GRIN2C	7	46	Known & Putative target
SDHA	16	39	Known & Putative target
SDHD	15	39	Known & Putative target
GRIN2D	8	39	Putative target
CAPN1	16	38	Putative target
CACNA1C	14	34	Known & Putative target

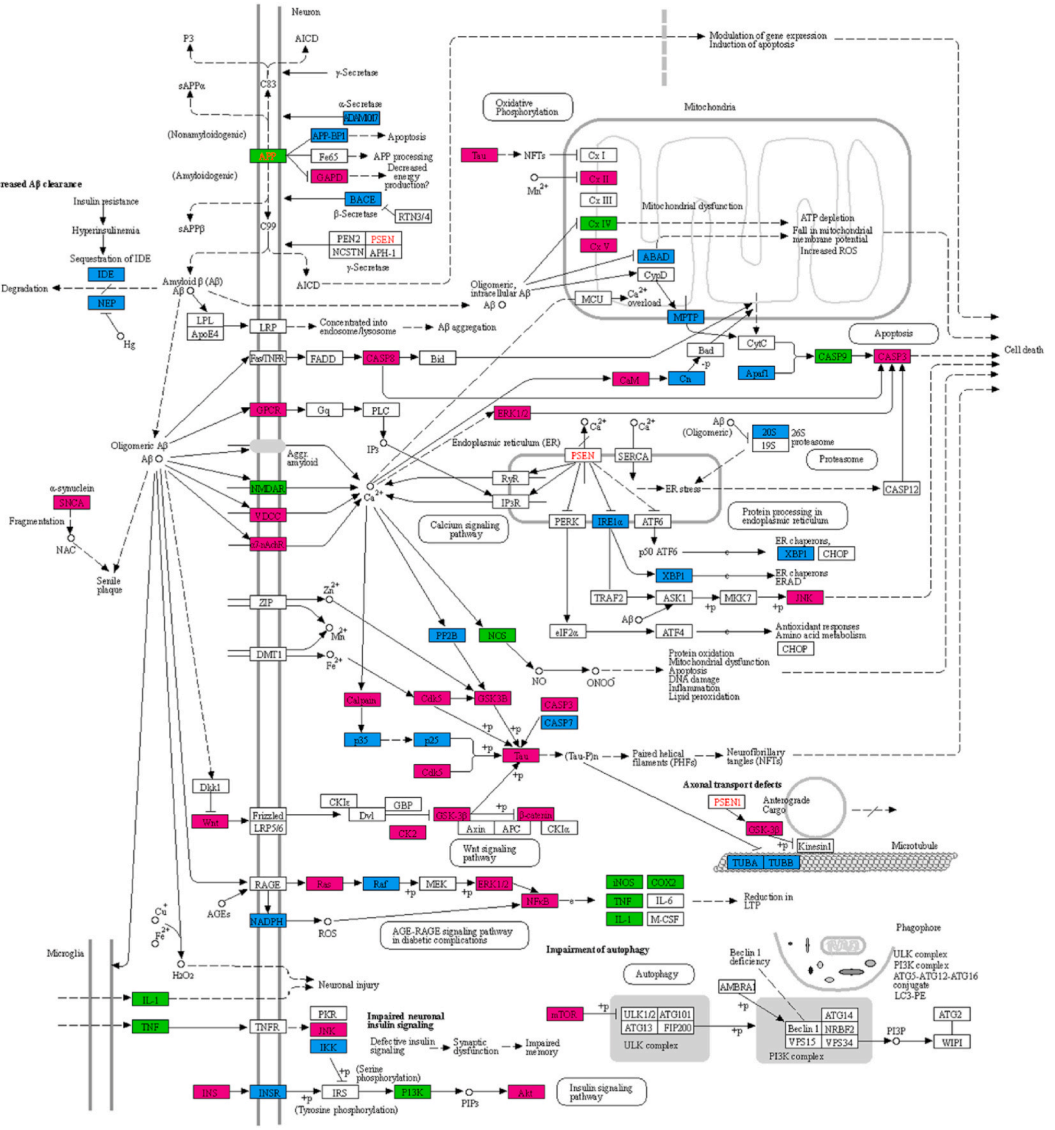


Fig. 5. Regulatory mechanism of BZBS on Alzheimer disease pathway. Colored nodes are targets regulated by BZBS. Deep pink and green nodes are the key targets regulated by BZBS meeting one and two screening conditions, respectively. Blue nodes are the rest targets regulated by BZBS. The solid line represents the direct regulatory effect, and the dotted line indicates the indirect effect. The line with arrow indicates activation. The line with small line segments represents inhibition.

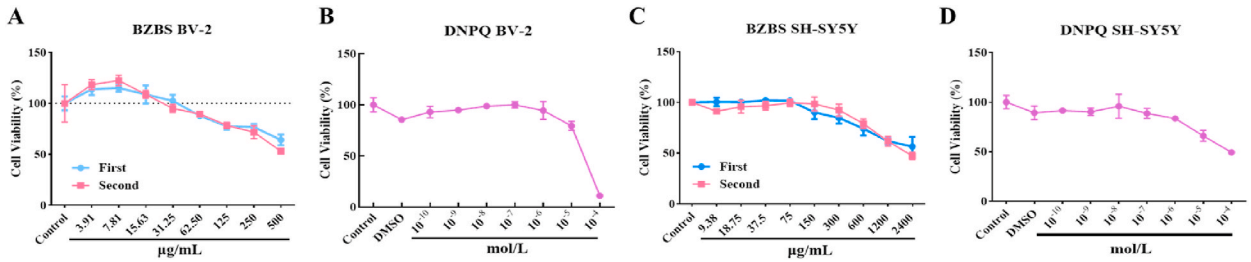


Fig. 6. Effects of BZBS and DNPQ on cell viability. (A) Effects of BZBS and **(B)** DNPQ on BV-2 cell viability; **(C)** Effects of BZBS and **(D)** DNPQ on SH-SY5Y cell viability. The results represent the mean ± SD (n = 3). BZBS, Bazi Bushen; DNPQ, Donepezil; DMSO, Dimethyl sulfoxide.

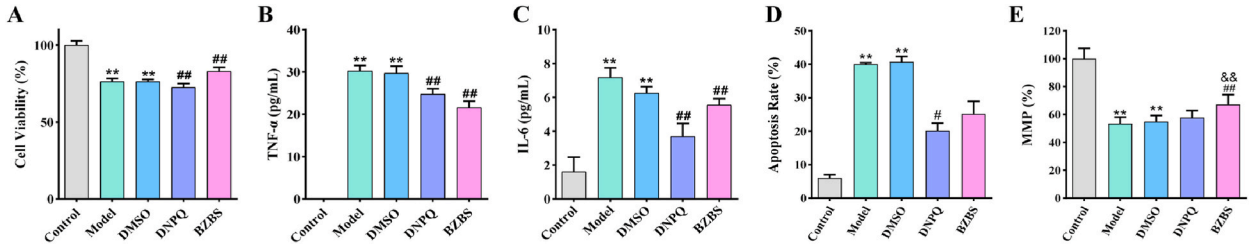


Fig. 7. ***In vitro*** **validation of BZBS against AD. (A)** Effects of BZBS on cell viability of $A\beta_{25\text{-}35}$ induced BV-2 cells; **(B–C)** Inhibitory effects of BZBS on TNF-α and IL-6 in $A\beta_{25\text{-}35}$ induced BV-2 cells; **(D–E)** Effects of BZBS on apoptosis rate and mitochondrial membrane potential of $A\beta_{25\text{-}35}$ induced SH-SY5Y cells. The results represent the mean ± SD (n = 3), vs. Control, $^{**}p < 0.01$, vs. Model $^{\#}p < 0.05$, $^{\#\#}p < 0.01$. vs. DNPQ, $^{\&\&}$ $p < 0.01$. BZBS, Bazi Bushen; AD, Alzheimer's disease; DNPQ, Donepezil; DMSO, Dimethyl sulfoxide; MMP, Mitochondrial membrane potential.

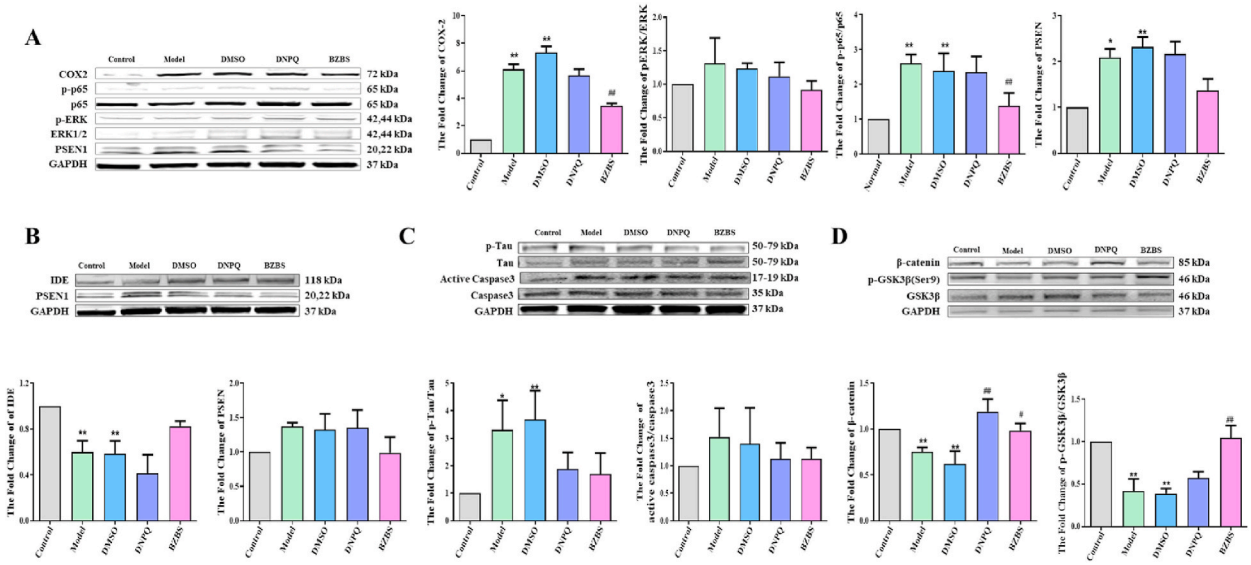


Fig. 8. ***In vitro*** **validation of BZBS anti-AD signaling pathway mechanism. (A)** Effects of BZBS on the expression of COX-2, p-p65, *p*-ERK1/2, PSEN1 in BV-2 cells induced by $A\beta_{25\text{-}35}$; **(B–C)** Effects of BZBS on the expression of IDE, PSEN1, p-Tau and active caspase3 in SH-SY5Y cells induced by $A\beta_{25\text{-}35}$; **(D)** Effects of BZBS on the expression of β-catenin and *p*-GSK3β in SH-SY5Y cells induced by $A\beta_{25\text{-}35}$. The results represent the mean ± SEM (n = 3). IDE, Insulin degrading enzyme; BZBS, Bazi Bushen; AD, Alzheimer's disease; DNPQ, Donepezil; DMSO, Dimethyl sulfoxide.

group was increased, while the expression of PSEN1 and active caspase-3 were decreased. These results suggested that BZBS can increase the expression of IDE, promote the decomposition of $A\beta_{25\text{-}35}$, inhibit the production of PSEN1 and apoptotic protein active fragments, but has no significant decrease on the expression of p-Tau protein (Fig. 8B and C).

3.10. Effects of BZBS on the expression of β-catenin and p-GSK3β in SH-SY5Y cells induced by $A\beta_{25\text{-}35}$

Compared with the control group, the expression of *p*-GSK-3β (Ser9) and β-catenin in the model group were decreased. Compared with the model group, the expression of *p*-GSK-3β (Ser9) and β-catenin were increased in each drug group. It demonstrated that BZBS ameliorated the injury of SH-SY5Y cells induced by $A\beta_{25\text{-}35}$, which may be related to inhibition the activation of GSK-3β/β-catenin signaling pathway (Fig. 8D).

4. Discussion

Drug repurposing is an effective strategy to reduce time-to-market costs and risks. TCM has unique advantages in the treatment of complex diseases with therapeutic idea of "homotherapy for heteropathy". It is highly possible and feasible to find new indications from the drugs already on the marketed herbal formulas through drug repurposing strategies of network pharmacology. In this study, we aimed to explore the new indications and key mechanism of BZBS by network pharmacology and *in vitro* experiment.

High reliability of target spectrum data is an important basis to ensure the reliability of conclusions obtained in this study. During the process of obtaining the therapeutic target profile of BZBS formula, multiple sources were used to predict the potential targets, and strict screening criteria were adopted for the data of predicted sources before integration. In general, this strategy is more reliable than

a single target prediction approach [47]. Further, the known target data were used as the validation of the prediction target to verify the accuracy of the prediction methods and improve the integrity and reliability of the target profiles. In the final target spectrum, the known target data accounted for up to 82%, which proved that the target spectrum acquisition strategy used in this study was reliable, and ensured the reliability of subsequent analysis results. These data lay a foundation for further research on the material basis and mechanism of action in BZBS formula.

DO enrichment analysis showed that BZBS had therapeutic potential for AD. Previous studies have also shown that BZBS intervention could ameliorate reduced brain performances in aging mice, including memory, cognitive, and motor functions [48]. In this study, two AD cell models were used to validate the new indication of BZBS, which lays the foundation for further research. In addition, there still have some other significantly enriched diseases: (1) Metabolic diseases, such as overnutrition (pvalue = 1.68e-22), nutrition disease (pvalue = 1.68e-22), obesity (pvalue = 2.46e-21), inherited metabolic disorder (pvalue = 2.55e-15); (2) Neurological and psychiatric diseases, for example, mood disorder (pvalue = 2.89e-16), motor neuron disease (pvalue = 2.42e-15), epilepsy syndrome (pvalue = 2.79e-15), amyotrophic lateral sclerosis (pvalue = 2.66e-14); (3) Circulatory diseases, including arteriosclerosis (pvalue = 4.99e-14) and arteriosclerotic cardiovascular disease (pvalue = 4.47e-14); (4) Respiratory diseases, for example, chronic obstructive pulmonary disease (pvalue = 1.87e-22), lung disease (pvalue = 4.47e-21), obstructive lung disease (pvalue = 1.03e-19); (5) Endocrine system disease (pvalue = 2.95e-15); (6) Skin system diseases, such as skin disease (pvalue = 5.46e-15) and integumentary system disease (pvalue = 1.56e-13); (7) Urinary system disease (pvalue = 8.82e-14); (8) Musculoskeletal system disease, such as osteoarthritis (pvalue = 5.68e-11) and muscle tissue disease (pvalue = 4.62e-06); (9) As well as neoplasms involving multiple systems, such as cell type benign neoplasm (pvalue = 4.72e-19), connective tissue by cancer (pvalue = 2.33e-17), lymphoblastic leukemia (pvalue = 7.37e-17), female reproductive organ cancer (pvalue = 9.60e-17), bone cancer (pvalue = 8.48e-16), musculoskeletal system cancer (pvalue = 4.85e-15), etc. *In vivo* experiments showed that BZBS treatment reduced serum lipid levels and improved fatty acid metabolism in high-fat diet-fed, surgically induced menopausal ApoE$^{-/-}$ mice [17]. Huang, D. et al. explored the potential theoretical mechanism of the prevention and treatment of postmenopausal atherogenesis by the BZBS. And further study revealed that BZBS exhibited a clear effect against atherogenesis via GPER1-dependent anti-inflammatory and anti-apoptotic mechanisms [18]. In addition, from the perspective of aging, the diseases mentioned above are closely related to the aging mechanism [49,50]. The decline of body function and senile diseases caused by aging seriously affect human health and life span [49,51]. Based on collateral disease theory, "*Deficiency of kidney essence*" is the foundation of aging, "*Deficiency of promordial Qi*" is the key to aging, and "*Physical and spiritual loss*" is the manifestation of aging [52]. BZBS was successfully developed by absorbing the essence of clinical tonifying kidney medicine experience in the past two thousand years. It has the anti-aging effect of "*Nourishing kidney essence*", "*Coordinating Yin and Yang*", "*Supplementing primordial Qi*", and "*Nourishing body and spirit*" [51]. The results in this study lay a foundation for a systematic study of BZBS from a new perspective of aging mechanism. Experimental researches have demonstrated that BZBS can improve overall aging and systemic aging, as well as prevent and treat aging related diseases. Li, L. et al. [19] found that BZBS attenuates premature senescence possibly via the preservation of redox homeostasis and telomere integrity, and inhibition of apoptosis in rapid aging mouse through the activation of Sirt6/NRF2/HO-1 and Sirt6/P53-PGC-1alpha-TERT signaling pathways. The results suggest that BZBS may provide a novel strategy for confronting aging and age-associated diseases. This embodies the therapeutic idea of "*Homotherapy for heteropathy*" of traditional Chinese medicine [14,53,54].

The strategy adopted in the process of identifying the core target takes into account not only the importance of the target in the molecular network of AD pathology, but also the importance of the target in the anti-AD role of BZBS. Most of the selected core targets are the reported active targets corresponding to the components of BZBS, such as APP, RELA, CASP9, NOS1, PTGS2, and NOS2, which occupy important topological positions in the AD pathway and participate in many important biological processes [55–61]. In addition, by searching DrugBank database, it was found that APP, NOS1, CHRM1, CHRNA7, GRIN2A, GRIN2B, GRIN1, GRIN2D, IL1B, CHRM3, and NFKB1 were all therapeutic targets of marketed drugs [35]. COX2 is a newly predicted target, and *in vitro* validation showed that BZBS formula had a clear and significant regulatory effect on this target. All the above indicate that the target spectrum construction strategy and key target mining strategy adopted in this study have high efficiency and reliability, which might provide important reference for other researchers. In this study, through preliminary *in vitro* experiments, BZBS formula has significant regulatory effects on multiple biological processes in AD pathway. For example, APP processing, neuronal injury, apoptosis, mitochondrial dysfunction, Tau protein phosphorylation and neuroinflammation. Follow-up studies could focus on other biological processes, for example, synaptic dysfunction, ROS, neurofibrillary tangles, LTP, autophagy, and axonal transport defects for systematically verification. However, it is worth noting that this study is based on the ingredients data in herbs of BZBS formula, rather than the that obtained from the direct detection of BZBS whole formula. Although Song, G. S. et al. have determined 14 components in BZBS by UPLC-ESI-MS/MS [62], the main purpose is for quality control. Therefore, in the future study, the overall components of BZBS formula, the components absorbed into blood, and metabolites would be systematically detected and identified by HPLC-MS/MS [63,64]. In turn, the strategies in this study can be used again for in-depth analysis, mining the material basis that exert key therapeutic effects in BZBS formula, and providing a reference for finding new therapeutic strategies for complex diseases.

5. Conclusions

In this study, the therapeutic potential and mechanism of action of BZBS in the treatment of AD were investigated by network pharmacology approach and *in vitro* experimental validation. On the basis of obtaining high quality therapeutic target profiles of BZBS and enrichment analysis, the therapeutic potential of BZBS against AD was systematically repositioned. *In vitro* experimental validations were conducted to explore the efficacy and mechanism of action of BZBS against AD. BZBS could significantly reduce the release of TNF-α and IL-6 and the expression of COX-2 and PSEN1 in BV-2 cells induced by $A\beta_{25\text{-}35}$, which might be related to the

regulation of ERK1/2 and NF-κB signaling pathways. In addition, BZBS can reduce the apoptosis rate of SH-SY5Y cells induced by $A\beta_{25-35}$, significantly increase mitochondrial membrane potential, reduce the expression of Caspase3 activity fragment and PSEN1, and increase the expression level of IDE, the main degradation enzyme of $A\beta_{25-35}$. This may be related to the regulation of GSK-3β/β-catenin signaling pathway. This study demonstrated that network pharmacology method can be an efficient strategy to reposition new clinical use of approved TCM and explore the mechanism of action. BZBS formula has a potential use in the treatment of AD, and it is achieved by "multi-compound, multi-target, multi-pathway". The study lays a foundation for the subsequent in-depth study of BZBS in the treatment of AD and provides a basis for its application in the clinical treatment of AD. More importantly, it will provide research paradigms for the development of new indications of other approved TCM.

Author contribution statement

Tongxing Wang; Meng Chen: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Huixin Li; Guoyuan Ding; Yanfei Song; Bin Hou; Bing Yao; Zhixin Wang; Yunlong Hou: Performed the experiments; Contributed reagents, materials, analysis tools or data.

Junqing Liang; Cong Wei; Zhenhua Jia: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Data availability statement

Data included in article/supplementary material/referenced in article.

Funding statement

This work was supported by the Chinese Academy of Engineering Strategic Consulting Project [grant number 2022-XY-45]; S&T Program of Hebei [grant number 22372502D]; and the Scientific Research Project of Hebei Provincial Administration of Traditional Chinese Medicine [grant number 2023172 and 2021273].

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We wish to extend our thanks to any individual for their assistance in the conduction of this study.

Supplementary materials

The data used to support the findings of this study are included within the supplementary information file(s): Table S1. 1499 unique compounds information of BZBS; Table S2. 1320 potential target profiles with high reliability of BZBS capsule; Table S3. Compound-target network corresponding to 1320 targets with high confidence; Table S4. PPI network statistical parameters of 113 anti-AD targets. Fig. S1. Raw images for Western blot assay results in Fig. 8.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e17603.

References

[1] M. Abdelsayed, E.J. Kort, S. Jovinge, M. Mercola, Repurposing drugs to treat cardiovascular disease in the era of precision medicine, Nat. Rev. Cardiol. 19 (2022) 751–764, https://doi.org/10.1038/s41569-022-00717-6.

[2] W.U. Jiashuo, Z. Fangqing, L.I. Zhuangzhuang, J. Weiyi, S. Yue, Integration strategy of network pharmacology in Traditional Chinese Medicine: a narrative review, J. Tradit. Chin. Med. 42 (2022) 479–486, https://doi.org/10.19852/j.cnki.jtcm.20220408.003.

[3] A.L. Hopkins, Network pharmacology, Nat. Biotechnol. 25 (2007) 1110–1111, https://doi.org/10.1038/nbt1007-1110.

[4] A.L. Hopkins, Network pharmacology: the next paradigm in drug discovery, Nat. Chem. Biol. 4 (2008) 682–690, https://doi.org/10.1038/nchembio.118.

[5] T.T. Zhang, R. Xue, X. Wang, S.W. Zhao, L. An, Y.F. Li, Y.Z. Zhang, S. Li, Network-based drug repositioning: a novel strategy for discovering potential antidepressants and their mode of action, Eur. Neuropsychopharmacol 28 (2018) 1137–1150, https://doi.org/10.1016/j.euroneuro.2018.07.096.

[6] S. Li, B. Zhang, Traditional Chinese medicine network pharmacology: theory, methodology and application, Chin. J. Nat. Med. 11 (2013) 110–120, https://doi.org/10.1016/S1875-5364(13)60037-0.

[7] S. Zhao, S. Li, A co-module approach for elucidating drug-disease associations and revealing their molecular basis, Bioinformatics 28 (2012) 955–961, https://doi.org/10.1093/bioinformatics/bts057.

[8] S. Li, B. Zhang, N. Zhang, Network target for screening synergistic drug combinations with application to traditional Chinese medicine, BMC Syst. Biol. 5 (Suppl 1) (2011) S10, https://doi.org/10.1186/1752-0509-5-S1-S10.

[9] S. Zhao, S. Li, Network-based relating pharmacological and genomic spaces for drug target identification, PLoS One 5 (2010), e11764, https://doi.org/10.1371/journal.pone.0011764.

[10] X. Liang, H. Li, S. Li, A novel network pharmacology approach to analyse traditional herbal formulae: the Liu-Wei-Di-Huang pill as a case study, Mol. Biosyst. 10 (2014) 1014–1022, https://doi.org/10.1039/c3mb70507b.

[11] J. Zheng, M. Wu, H. Wang, S. Li, X. Wang, Y. Li, D. Wang, S. Li, Network pharmacology to unveil the biological basis of health-strengthening herbal medicine in cancer treatment, Cancers 10 (2018), https://doi.org/10.3390/cancers10110461.

[12] D.W. Lim, H. Kim, Y.M. Kim, Y.W. Chin, W.H. Park, J.E. Kim, Drug repurposing in alternative medicine: herbal digestive Sochehwan exerts multifaceted effects against metabolic syndrome, Sci. Rep. 9 (2019) 9055, https://doi.org/10.1038/s41598-019-45099-x.

[13] T. Wang, L. Liang, C. Zhao, J. Sun, H. Wang, W. Wang, J. Lin, Y. Hu, Elucidating direct kinase targets of compound Danshen dropping pills employing archived data and prediction models, Sci. Rep. 11 (2021) 9541, https://doi.org/10.1038/s41598-021-89035-4.

[14] Y.Y. Shi, Y.Q. Li, X. Xie, Y.T. Zhou, Q. Zhang, J.L. Yu, P. Li, N. Mi, F. Li, Homotherapy for heteropathy active components and mechanisms of Qiang-Huo-Sheng-Shi decoction for treatment of rheumatoid arthritis and osteoarthritis, Comput. Biol. Chem. 89 (2020), 107397, https://doi.org/10.1016/j.compbiolchem.2020.107397.

[15] L. Li, B. Chen, T. An, H. Zhang, B. Xia, R. Li, R. Zhu, Y. Tian, L. Wang, D. Zhao, et al., BaZiBuShen alleviates altered testicular morphology and spermatogenesis and modulates Sirt6/P53 and Sirt6/NF-kappaB pathways in aging mice induced by D-galactose and NaNO(2), J. Ethnopharmacol. 271 (2021), 113810, https://doi.org/10.1016/j.jep.2021.113810.

[16] Q. Wang, Z. Fang, Y. Zheng, X. Chen, J. Li, Y. Zhou, H. Gao, B. Xu, j. Fu, [Chinese tonifying formula "Bazi bushen capsules" benefits the performance of soccer players during preparation and competition period], Chinese Journal of Sports Medicine 31 (2012) 1054–1058, https://doi.org/10.16038/j.1000-6710.2012.12.005.

[17] D. Huang, H. Hu, L. Chang, S. Liu, J. Liang, Y. Song, X. Wang, H. Zhang, C. Wei, Y. Wu, Chinese medicine Bazi Bushen capsule improves lipid metabolism in ovariectomized female ApoE-/- mice, Ann. Palliat. Med. 9 (2020) 1073–1083, https://doi.org/10.21037/apm-20-906.

[18] D. Huang, X. Wang, Y. Zhu, J. Gong, J. Liang, Y. Song, Y. Zhang, L. Liu, C. Wei, Bazi bushen capsule alleviates post-menopausal atherosclerosis via GPER1-dependent anti-inflammatory and anti-apoptotic effects, Front. Pharmacol. 12 (2021), 658998, https://doi.org/10.3389/fphar.2021.658998.

[19] L. Li, H. Zhang, B. Chen, B. Xia, R. Zhu, Y. Liu, X. Dai, Z. Ye, D. Zhao, F. Mo, et al., BaZiBuShen alleviates cognitive deficits and regulates Sirt6/NRF2/HO-1 and Sirt6/P53-PGC-1alpha-TERT signaling pathways in aging mice, J. Ethnopharmacol. 282 (2022), 114653, https://doi.org/10.1016/j.jep.2021.114653.

[20] X. Chen, H. Zhou, Y.B. Liu, J.F. Wang, H. Li, C.Y. Ung, L.Y. Han, Z.W. Cao, Y.Z. Chen, Database of traditional Chinese medicine and its application to studies of mechanism and to prescription validation, Br. J. Pharmacol. 149 (2006) 1092–1103, https://doi.org/10.1038/sj.bjp.0706945.

[21] R.Z. Zhang, S.J. Yu, H. Bai, K. Ning, TCM-Mesh: the database and analytical system for network pharmacology analysis for TCM preparations, Sci. Rep. 7 (2017) 2821, https://doi.org/10.1038/s41598-017-03039-7.

[22] Y.C. Fang, H.C. Huang, H.H. Chen, H.F. Juan, TCMGeneDIT: a database for associated traditional Chinese medicine, gene and disease information using text mining, BMC Compl. Alternative Med. 8 (2008) 58, https://doi.org/10.1186/1472-6882-8-58.

[23] R. Xue, Z. Fang, M. Zhang, Z. Yi, C. Wen, T. Shi, TCMID: traditional Chinese Medicine integrative database for herb molecular mechanism analysis, Nucleic Acids Res. 41 (2013) D1089–D1095, https://doi.org/10.1093/nar/gks1100.

[24] L. Huang, D. Xie, Y. Yu, H. Liu, Y. Shi, T. Shi, C. Wen, Tcmid 2.0: a comprehensive resource for TCM, Nucleic Acids Res. 46 (2018) D1117–d1120, https://doi.org/10.1093/nar/gkx1028.

[25] H.Y. Xu, Y.Q. Zhang, Z.M. Liu, T. Chen, C.Y. Lv, S.H. Tang, X.B. Zhang, W. Zhang, Z.Y. Li, R.R. Zhou, et al., ETCM: an encyclopaedia of traditional Chinese medicine, Nucleic Acids Res. 47 (2019) D976–d982, https://doi.org/10.1093/nar/gky987.

[26] S.K. Kim, S. Nam, H. Jang, A. Kim, J.J. Lee, TM-MC: a database of medicinal materials and chemical compounds in Northeast Asian traditional medicine, BMC Compl. Alternative Med. 15 (2015) 218, https://doi.org/10.1186/s12906-015-0758-5.

[27] J. Ru, P. Li, J. Wang, W. Zhou, B. Li, C. Huang, P. Li, Z. Guo, W. Tao, Y. Yang, et al., TCMSP: a database of systems pharmacology for drug discovery from herbal medicines, J. Cheminf. 6 (2014) 13, https://doi.org/10.1186/1758-2946-6-13.

[28] Z. Liu, F. Guo, Y. Wang, C. Li, X. Zhang, H. Li, L. Diao, J. Gu, W. Wang, D. Li, et al., BATMAN-TCM: a bioinformatics analysis tool for molecular mechANism of traditional Chinese medicine, Sci. Rep. 6 (2016), 21146, https://doi.org/10.1038/srep21146.

[29] S. Kim, J. Chen, T. Cheng, A. Gindulyte, J. He, S. He, Q. Li, B.A. Shoemaker, P.A. Thiessen, B. Yu, et al., PubChem 2019 update: improved access to chemical data, Nucleic Acids Res. 47 (2019) D1102–D1109, https://doi.org/10.1093/nar/gky1033.

[30] U. Rix, G. Superti-Furga, Target profiling of small molecules by chemical proteomics, Nat. Chem. Biol. 5 (2009) 616–624, https://doi.org/10.1038/nchembio.216.

[31] Z.J. Yao, J. Dong, Y.J. Che, M.F. Zhu, M. Wen, N.N. Wang, S. Wang, A.P. Lu, D.S. Cao, TargetNet: a web service for predicting potential drug-target interaction profiling via multi-target SAR models, J. Comput. Aided Mol. Des. 30 (2016) 413–424, https://doi.org/10.1007/s10822-016-9915-2.

[32] D. Gfeller, A. Grosdidier, M. Wirth, A. Daina, O. Michielin, V. Zoete, SwissTargetPrediction: a web server for target prediction of bioactive small molecules, Nucleic Acids Res. 42 (2014) W32–W38, https://doi.org/10.1093/nar/gku293.

[33] N. Bosc, F. Atkinson, E. Felix, A. Gaulton, A. Hersey, A.R. Leach, Large scale comparison of QSAR and conformal prediction methods and their applications in drug discovery, J. Cheminf. 11 (2019) 4, https://doi.org/10.1186/s13321-018-0325-4.

[34] D. Szklarczyk, A. Santos, C. von Mering, L.J. Jensen, P. Bork, M. Kuhn, Stitch 5: augmenting protein-chemical interaction networks with tissue and affinity data, Nucleic Acids Res. 44 (2016) D380–D384, https://doi.org/10.1093/nar/gkv1277.

[35] D.S. Wishart, Y.D. Feunang, A.C. Guo, E.J. Lo, A. Marcu, J.R. Grant, T. Sajed, D. Johnson, C. Li, Z. Sayeeda, et al., DrugBank 5.0: a major update to the DrugBank database for 2018, Nucleic Acids Res. 46 (2018) D1074–D1082, https://doi.org/10.1093/nar/gkx1037.

[36] Y. Wang, S. Zhang, F. Li, Y. Zhou, Y. Zhang, Z. Wang, R. Zhang, J. Zhu, Y. Ren, Y. Tan, et al., Therapeutic target database 2020: enriched resource for facilitating research and early development of targeted therapeutics, Nucleic Acids Res. 48 (2020) D1031–D1041, https://doi.org/10.1093/nar/gkz981.

[37] A. Gaulton, A. Hersey, M. Nowotka, A.P. Bento, J. Chambers, D. Mendez, P. Mutowo, F. Atkinson, L.J. Bellis, E. Cibrian-Uhalte, et al., The ChEMBL database in 2017, Nucleic Acids Res. 45 (2017) D945–D954, https://doi.org/10.1093/nar/gkw1074.

[38] S. Kim, P.A. Thiessen, E.E. Bolton, J. Chen, G. Fu, A. Gindulyte, L. Han, J. He, S. He, B.A. Shoemaker, et al., PubChem substance and compound databases, Nucleic Acids Res. 44 (2016) D1202–D1213, https://doi.org/10.1093/nar/gkv951.

[39] C. Iwasawa, N. Kuzumaki, Y. Suda, R. Kagawa, Y. Oka, N. Hattori, H. Okano, M. Narita, Reduced expression of somatostatin in GABAergic interneurons derived from induced pluripotent stem cells of patients with parkin mutations, Mol. Brain 12 (2019) 5, https://doi.org/10.1186/s13041-019-0426-7.

[40] L.M. Schriml, E. Mitraka, J. Munro, B. Tauber, M. Schor, L. Nickle, V. Felix, L. Jeng, C. Bearer, R. Lichenstein, et al., Human Disease Ontology 2018 update: classification, content and workflow expansion, Nucleic Acids Res. 47 (2019) D955–D962, https://doi.org/10.1093/nar/gky1032.

[41] M. Kanehisa, M. Furumichi, M. Tanabe, Y. Sato, K. Morishima, KEGG: new perspectives on genomes, pathways, diseases and drugs, Nucleic Acids Res. 45 (2017) D353–D361, https://doi.org/10.1093/nar/gkw1092.

[42] G. Yu, L.G. Wang, Y. Han, Q.Y. He, clusterProfiler: an R package for comparing biological themes among gene clusters, OMICS 16 (2012) 284–287, https://doi.org/10.1089/omi.2011.0118.

[43] D. Szklarczyk, J.H. Morris, H. Cook, M. Kuhn, S. Wyder, M. Simonovic, A. Santos, N.T. Doncheva, A. Roth, P. Bork, et al., The STRING database in 2017: quality-controlled protein-protein association networks, made broadly accessible, Nucleic Acids Res. 45 (2017) D362–D368, https://doi.org/10.1093/nar/gkw937.

[44] P. Shannon, A. Markiel, O. Ozier, N.S. Baliga, J.T. Wang, D. Ramage, N. Amin, B. Schwikowski, T. Ideker, Cytoscape: a software environment for integrated models of biomolecular interaction networks, Genome Res. 13 (2003) 2498–2504, https://doi.org/10.1101/gr.1239303.

[45] Y. Assenov, F. Ramirez, S.E. Schelhorn, T. Lengauer, M. Albrecht, Computing topological parameters of biological networks, Bioinformatics 24 (2008) 282–284, https://doi.org/10.1093/bioinformatics/btm554.

[46] T. Ozben, S. Ozben, Neuro-inflammation and anti-inflammatory treatment options for Alzheimer's disease, Clin. Biochem. 72 (2019) 87–89, https://doi.org/10.1016/j.clinbiochem.2019.04.001.
[47] P. Charoenkwan, N. Schaduangrat, P. Lio, M.A. Moni, W. Shoombuatong, B. Manavalan, Computational prediction and interpretation of druggable proteins using a stacked ensemble-learning framework, iScience 25 (2022), 104883, https://doi.org/10.1016/j.isci.2022.104883.
[48] L. Li, H. Zhang, B. Chen, B. Xia, R. Zhu, Y. Liu, X. Dai, Z. Ye, D. Zhao, F. Mo, et al., BaZiBuShen alleviates cognitive deficits and regulates Sirt6/NRF2/HO-1 and Sirt6/P53-PGC-1α-TERT signaling pathways in aging mice, J. Ethnopharmacol. 282 (2022), 114653, https://doi.org/10.1016/j.jep.2021.114653.
[49] N. Kubben, T. Misteli, Shared molecular and cellular mechanisms of premature ageing and ageing-associated diseases, Nat. Rev. Mol. Cell Biol. 18 (2017) 595–609, https://doi.org/10.1038/nrm.2017.68.
[50] J.R. Aunan, M.M. Watson, H.R. Hagland, K. Soreide, Molecular and biological hallmarks of ageing, Br. J. Surg. 103 (2016) e29–e46, https://doi.org/10.1002/bjs.10053.
[51] H. Li, C. Wei, C. Ji, X. Mao, D. Wang, K. Ma, J. Ai, Y. Hou, Z. Jia, [Bazi bushen capsules on anti-aging: a review], Chin. J. Exp. Tradit. Med. Formulae 28 (2022) 239–246, https://doi.org/10.13422/j.cnki.syfjx.20220349.
[52] H. Li, C. Wei, Z. Jia, C. Ji, X. Mao, D. Wang, K. Ma, D. Huang, Y. Wu, [Research on anti-aging guided by qiluo doctrine], Chin. J. Exp. Tradit. Med. Formulae 28 (2022) 7–15, https://doi.org/10.13422/j.cnki.syfjx.20220151.
[53] B. Zhang, K. Liu, H. Yang, Z. Jin, Q. Ding, L. Zhao, Gut microbiota: the potential key target of TCM's therapeutic effect of treating different diseases using the same method-UC and T2DM as examples, Front. Cell. Infect. Microbiol. 12 (2022), 855075, https://doi.org/10.3389/fcimb.2022.855075.
[54] C. Zhao, W. Wang, K. Yan, H. Sun, J. Han, Y. Hu, The therapeutic effect and mechanism of qishen yiqi dripping pills on cardiovascular and cerebrovascular diseases and diabetic complications, Curr. Mol. Pharmacol. 15 (2022) 547–556, https://doi.org/10.2174/1874467214666210811153610.
[55] S. Mekala, G. Nelson, Y.M. Li, Recent developments of small molecule gamma-secretase modulators for Alzheimer's disease, RSC Med Chem 11 (2020) 1003–1022, https://doi.org/10.1039/d0md00196a.
[56] S. Chen, H. Liu, S. Wang, H. Jiang, L. Gao, L. Wang, L. Teng, C. Wang, D. Wang, The neuroprotection of verbascoside in Alzheimer's disease mediated through mitigation of neuroinflammation via blocking NF-kappaB-p65 signaling, Nutrients 14 (2022), https://doi.org/10.3390/nu14071417.
[57] M.I. Avrutsky, C.C. Ortiz, K.V. Johnson, A.M. Potenski, C.W. Chen, J.M. Lawson, A.J. White, S.K. Yuen, F.N. Morales, E. Canepa, et al., Endothelial activation of caspase-9 promotes neurovascular injury in retinal vein occlusion, Nat. Commun. 11 (2020) 3173, https://doi.org/10.1038/s41467-020-16902-5.
[58] F. Blandini, E. Sinforiani, C. Pacchetti, A. Samuele, E. Bazzini, R. Zangaglia, G. Nappi, E. Martignoni, Peripheral proteasome and caspase activity in Parkinson disease and Alzheimer disease, Neurology 66 (2006) 529–534, https://doi.org/10.1212/01.wnl.0000198511.09968.b3.
[59] L. Viejo, A. Noori, E. Merrill, S. Das, B.T. Hyman, A. Serrano-Pozo, Systematic review of human post-mortem immunohistochemical studies and bioinformatics analyses unveil the complexity of astrocyte reaction in Alzheimer's disease, Neuropathol. Appl. Neurobiol. 48 (2022), e12753, https://doi.org/10.1111/nan.12753.
[60] Y. Zhang, Z. Zhu, H.Y. Liang, L. Zhang, Q.G. Zhou, H.Y. Ni, C.X. Luo, D.Y. Zhu, nNOS-CAPON interaction mediates amyloid-beta-induced neurotoxicity, especially in the early stages, Aging Cell 17 (2018), e12754, https://doi.org/10.1111/acel.12754.
[61] A.B. Niculescu, H. Le-Niculescu, K. Roseberry, S. Wang, J. Hart, A. Kaur, H. Robertson, T. Jones, A. Strasburger, A. Williams, et al., Blood biomarkers for memory: toward early detection of risk for Alzheimer disease, pharmacogenomics, and repurposed drugs, Mol. Psychiatr. 25 (2020) 1651–1672, https://doi.org/10.1038/s41380-019-0602-2.
[62] G.S. Song, L. Zhou, Y.M. Xu, X.R. Zhang, L.T. Zhang, [Determination of 14 components in Bazibushen capsule by UPLC-ESI-MS/MS], Yao Xue Xue Bao 51 (2016) 792–796.
[63] Y. He, H. Zheng, L. Zhong, N. Zhong, G. Wen, L. Wang, Y. Zhang, Identification of active ingredients of huangqi guizhi wuwu decoction for promoting nerve function recovery after ischemic stroke using HT22 live-cell-based affinity chromatography combined with HPLC-MS/MS, Drug Des. Dev. Ther. 15 (2021) 5165–5178, https://doi.org/10.2147/DDDT.S333418.
[64] Y. Rong, S. Feng, C. Wu, S. Wang, S. Liang, D. Liu, LC-high-resolution-MS/MS analysis of chemical compounds in rat plasma after oral administration of Nao-Mai-Tong and its individual herbs, Biomed. Chromatogr. 31 (2017), https://doi.org/10.1002/bmc.3920.