Network pharmacology and molecular docking analysis on molecular targets and mechanisms of Buyang Huanwu Decoction in the treatment of Ischemic Stroke

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Research

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Abstract

Background and objective: With the exact clinical efficacy, Buyang Huanwu decoction (BHD) is a classical prescription for the treatment of ischemic stroke (IS). Here, we aimed to investigate the pharmacological mechanisms of BHD in treating IS using systems biology approaches.

Methods: The bioactive components and potential targets of BHD were screened by TCMSP, BATMAN-TCM, ETCM, and SymMap databases. Besides, compounds that failed to find the targets from the above databases were predicted through STITCH, SWISS, and SEA. Moreover, six databases were searched to mine targets of IS. The intersection targets were obtained, and analyzed by GO and KEGG enrichment. Furthermore, BHD-IS PPI network, compound-compound target-IS network and pathway of drug-compound target-IS network were constructed by Cytoscape 3.6.0. Finally, AutoDock was used for molecular docking verification.

Results: A total of 253 putative targets were obtained from 60 active compounds in BHD. Among them, 62 targets were related to IS. PPI network showed that the top ten key targets were IL6, TNF, VEGFA, and AKT1, etc. The enrichment analysis demonstrated candidate BHD targets were more frequently involved TNF, PI3K-Akt, and NF-kappa B signaling pathway. Network topology analysis showed that Radix Astragali was the main herb in BHD, and the key components were quercetin, beta-Sitosterol, kaempferol, and stigmasterol, etc. The results of molecular docking showed the active components in BHD had a good binding ability with the key targets.

Conclusions: This study firstly adopted the methods of network pharmacology and molecular docking to reveal the relationships among herbs in BHD, the putative targets and IS-related pathways.

Introduction

The prevalence of ischemic stroke (IS) is particularly high and survivors have more or less neurological function deficits, which brings about a large burden on society and patients’ families. With high mortality and morbidity rate, stroke has been the third most common cause of death following by coronary heart disease and cancer in the world [1]. IS accounts for 70%-80% of all stroke, which is the most common type of stroke in clinic [2]. At present, thrombolysis is the fastest and most effective treatment for IS, but the clinical effect of thrombolysis therapy is limited due to strict indications, a short time window, high risk of bleeding and reperfusion injury[3]. A large number of experimental studies and clinical observations have confirmed that Chinese medicine has unique advantages in treating IS. Various empirical prescriptions, single drugs and active ingredient extracts have been shown clear neuroprotective effects on IS [4–6].

Buyang Huanwu Decoction (BHD) is a classical prescription for the treatment of IS. This prescription is mainly composed of Huangqi(Radix Astragali), Honghua(Carthami Flos), Taoren(Persicae Semen), Chishao(Radix paeoniae Rubra), Danggui(Angelicae Sinensis Radix), Chuangxiong(Chuanxiong Rhizoma), and Dilong(Pheretima). Among them, Radix Astragali is the most widely used in the original
prescription, which is the monarch drug in BHD. A systematic review and meta-analysis of nineteen RCTs with 1580 individuals showed that BHD could significantly improve the neurological deficit score and the ability of self-care of patients with IS [7]. BHD could promote angiogenesis, attenuate infiltration of natural killer cells, and facilitate neurorehabilitation through an improvement of synaptic plasticity after cerebral ischemia/reperfusion injury. It could significantly decrease cerebral edema and rat neurological function scores, and reduce brain infarct volume [8–12]. Pharmacological researches have shown that *Radix Astragali* has the function of dilating blood vessels and improving microcirculation, which can significantly increase the brain's ability to withstand periods of severe hypoxia and/or ischemia [13, 14]. *Carthami Flos, Persicae Semen, Radix paeoniae Rubra, Angelicae Sinensis Radix*, and *Chuanxiong Rhizoma* can effectively improve the microcirculation of the body, significantly inhibit the proliferation of fibrous tissue, and reduce the inflammatory response [15–17].

However, the molecular mechanism of BHD has not been certainly clear. The clinical effect of the decoction is a comprehensive result of the complex biological process in human body. Network pharmacology, based on system biology and multi-directional pharmacology, integrates the contents of computer biology and network analysis [18]. It explains the integrity and systematization of drug, target and disease interaction from the perspective of multi-component, multi-target and multi-channel, which is consistent with the holistic view of Chinese medicine [19]. Therefore, it provides a method for the study of multi-component mechanism of Chinese medicine [20, 21]. In this paper, network pharmacology was used to explore the molecular mechanism of BHD against IS. The detailed workflow of the study is shown in Fig. 1.

### Materials And Methods

#### Chemical ingredients collection and active compounds screening

Traditional Chinese Medicine Systems Pharmacology Database (TCMSP, [http://lsp.nwsuaf.edu.cn](http://lsp.nwsuaf.edu.cn))[22], BATMAN-TCM ([http://bionet.ncpsb.org/batman-tcm/index.php/Home/Index/index](http://bionet.ncpsb.org/batman-tcm/index.php/Home/Index/index)) [24], ETCM ([http://www.tcmip.cn/ETCM/index.php/Home/index/index.html](http://www.tcmip.cn/ETCM/index.php/Home/index/index.html)) and SymMap ([https://www.symmap.org/](https://www.symmap.org/)) [25] was used to collect the compounds of BHD. In addition, those compounds which didn't meet the requirements but had significant pharmacological activities and high contents were also opt to the next research through text mining. Next, ADME analysis was carried out by collecting the main components of BHD according to the condition parameters (OB ≥ 30%, DL ≥ 0.18, Caco-2 ≥ 0.4) [23]. Through ADME analysis, the potential active components were screened out for further analysis.

#### Target identification

TCMSP, BATMAN-TCM, ETCM, and SymMap databases were used to screen the targets of all the components of BHD. If related targets of components of BHD could not be found from the above databases, further target prediction was carried out in the STITCH ([http://stitch.embl.de/](http://stitch.embl.de/)) [26], Swiss
Target Prediction (SWISS, http://www.swisstargetprediction.ch/) [27], and Similarity Ensemble Approach (SEA, http://sea.bkslab.org/) [28] database.

Targets related to IS were derived from six public databases, including DisGeNET (http://www.disgenet.org/) [29], OMIM (https://omim.org/) [28], TTD (http://bidd.nus.edu.sg/group/cjttd/), DrugBank (https://www.drugbank.ca/), PharmGKB (http://www.pharmgkb.org/) and MalaCards (https://www.malacards.org/) [30] with key words “ischemic stroke”.

The targets were normalized to the official gene symbols using UniProt database (https://www.uniprot.org/) [31] with the species limited to “Homo sapiens”. Finally, the intersection targets of BHD active component targets and IS targets were obtained and drawn using Venn Diagram.

Protein-protein interaction data

String 11.0 (https://string-db.org/) [32] is a database for storing known and predicted protein interactions, including direct and indirect protein interactions. It scores each protein interaction. A higher score means a higher confidence of protein interaction.

The selected intersection targets were imported into String for protein interaction analysis, and the protein interaction network was obtained with the species limited to “Homo sapiens” and a confidence score > 0.7. The protein interaction data were imported into Cytoscape 3.6.0 (https://cytoscape.org/) to construct the PPI network.

Gene Ontology (GO) and Pathway Enrichment

DAVID (https://david.ncifcrf.gov/) [33] database integrates various types of database resources, and uses the improved Fisher precision test algorithm to analyze the enrichment of gene sets, providing P and false discovery rate (FDR) of enrichment analysis results. GO annotation and KEGG pathway analysis were carried out for the intersection genes. Finally, we could get the pathway maps from KEGG PATHWAY Database (https://www.kegg.jp/) [34].

Network construction and cluster

Network construction

Network construction was performed as follows: (1) BHD-IS PPI network; (2) Compound-compound target-IS network; (3) Pathway of drug-compound target-IS network.

All networks can be constructed via utilizing the network visualization software Cytoscape, which displays network graphically. It supplies a basic set of features for data integration, analysis, and visualization for complicated network analysis. In the network diagram, "node" represents the active component and target in BHD, and "edge" represents the relationship between active component and target. The “Degree” parameter, presenting the number of connections between the nodes in the network, was used to evaluate important targets [30].
Cluster of BHD-IS PPI network

The closely related regions in protein-protein interaction networks is defined as topological modules or clusters. This cluster or functional modules can put nodes of similar or related function together in a same network. By MCODE, a plug-in of Cytoscape, we can get clusters.

Molecular docking verification

To validate the compound-target association, AutoDock software (version 4.2) was used to perform the molecular docking program[35]. RCSB PDB (http://www.rcsb.org/) [36] was used to retrieve and download the 3D structure files of key target proteins. 3D structure files of compounds were downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov/) [37]. Finally, AutoDock platform was used for molecular docking verification[38]. The binding energy was calculated to evaluate binding interactions between the compounds and their targets. The binding energy less than “-5” indicates a good binding interactions between the compounds and their targets[39].

Results

Active compounds

775 compounds were ultimately reserved: 87 in Radix Astragali, 189 in Chuanxiong Rhizoma, 119 in Radix paeoniae Rubra, 125 in Angelicae Sinensis Radix, 66 in Persicae Semen, 189 in Carthami Flos, and 4 in Pheretima. After ADME screening, 78 potential compounds (OB ≥ 30%, DL ≥ 0.18, Caco-2 ≥ 0.4) of seven herbal medicines in BHD were identified, including 16 from Radix Astragali, 6 from Chuanxiong Rhizoma, 20 from Radix Paeoniae Rubra, 2 from Angelicae Sinensis Radix, 13 from Persicae Semen, 21 from Carthami Flos, and 0 from Pheretima. The details of candidate ingredients are described in Table 1. Radix Astragali, Chuanxiong Rhizoma, Radix paeoniae Rubra, Angelicae Sinensis Radix, Persicae Semen and Carthami Flos are simplified as RA, CR, RPR, ASR, PS, and CF respectively.

Targets of BHD

As 18 compounds of BHD had no targets in TCMSP, SymMap and TCM-MESH, Canonical SMILES of these compounds were found in Pubchem. Based on chemical structural similarity, we used databases like STITCH, SWISS and SEA, to predict their targets. These compounds were excluded because of the targets score less than 50% eventually. These compounds were isoflavanone, senkyunone, paeoniflorin_qt, Albiflorin_qt, Paeo niflorgenone, 9-ethyl-neo-paeoniaflorinA_qt, evofolinB, 1-o-beta-d-glucopyranosylpaeonisuffrone_qt, 4-ethyl-paeoniflorin_qt, 4-o–methyl-pa eoniflorin_qt, GA122, Populoside_qt, Flavoxanthin, lupeol-palmitate, Phytoene, phytofluene, 6-Hydroxynaring enin, and 1-o-beta-d-glucopyranosyl-8-o–benzoylpaeo nisuffrone_qt. In brief, 235 targets were adopted in this research.

By means of the six available resources, namely, DisGeNET, OMIM, TTD, DrugBank, PharmGKB and MalaCards databases, we obtained 460 IS-related targets.
Based on targets of the candidate ingredients and IS intersection targets were got by R software. 62 intersection genes were found eventually, shown as Fig. 3A. The details of intersection targets are described in Table 2.

**Gene Ontology and Pathway Enrichment Analysis**

**Gene Ontology**

GO analysis of 62 candidate targets for BHD against IS was performed using the DAVID database to understand the relationship between functional units and their underlying significance in the biological system networks. The result was divided into three parts, biological processes, cellular component, and molecular function, as shown in Fig. 2 A, B, and C.

We found that biological processes were related to inflammatory response, negative regulation of apoptotic process, response to estradiol, lipopolysaccharide-mediated signaling pathway, positive regulation of nitric oxide biosynthetic process, positive regulation of protein phosphorylation, positive regulation of NF-kappa B transcription factor activity, response to hypoxia, immune response and MAPK cascade. The cellular component was related to extracellular space, extracellular region, plasma membrane, cell surface, extracellular exosome, cytosol, nucleus, nucleoplasm, cytoplasm and integral component of plasma membrane. Finally, molecular function was related to cytokine activity, enzyme binding, protein binding, heme-binding, steroid hormone receptor activity, peroxidase activity, transcription factor binding, calcium ion binding, metalloproteidase activity and kinase activity.

**Pathway Enrichment**

Through comprehensive analysis, we obtained an integrated IS pathway based on our current knowledge of IS pathogenesis to illuminate the integral role of BHD in treating IS. TOP 10 KEGG signaling pathways of BHD were obtained and constructed based on P-Value.

Based on this systems-level picture, we picked and constructed ten therapeutic pathways of TNF signaling pathway, PI3K-Akt signaling pathway, NF-kappa B signaling pathway, MAPK signaling pathway, Complement and coagulation cascades, T cell receptor signaling pathway, Toll-like receptor signaling pathway, HIF-1 signaling pathway, Estrogen signaling pathway and VEGF signaling pathway, as shown in Fig. 2D.

**BHD-IS PPI network analysis**

**BHD-IS PPI network**

62 intersection targets were imported into the String database, and TSV text showing the interaction relationship was obtained, as shown in Fig. 3B. Then, the network topology analysis was applied by the software of Cytoscape 3.6.0. By integrating IS network and compound-compound target network, we could get BHD-IS network, as shown in Fig. 3C. This network contained 59 nodes and 664 edges. In this
network, the rose red nodes had higher degrees. The number of those nodes’ edges was 36 in IL6, 31 in
TNF, 29 in VEGFA, 28 in AKT1, 27 in MMP9, 26 in IL1B, 23 in MAPK1, 22 in ICAM1, 22 in PTGS2 and 20 in
IL10 respectively. This suggested that these genes might be the key or central genes in IS development.
Bar graph of all protein nodes degree related to the targets is shown in Fig. 3D. The target proteins in the
PPI network were modularized and analyzed by using the plug-in of cluster maker of the software
Cytoscape 3.6.0. The results showed that 62 targets were divided into four modules, including 24 in
module one, 18 in module two, 11 in module three and 9 in module four, as shown in Fig. 3E.

**Compound-compound target-IS network analysis**

This network was composed of 284 nodes (253 compound target nodes and 49 compound nodes) and
1192 edges. In this network we could find that one target could be hit by several compounds (central
nodes, such as IL6, MMP9, TNF, AKT1, ICAM1, IL1B, PTGS2, IL-10, VEGFA, and MAPK1), but some were
modulated by only one compound in this network. Furthermore, one potential active compound could
correspond to multiple targets. Top ten compounds with high degree were Quercetin, beta-Sitosterol,
Kaempferol, Stigmasterol, Baicalein, Luteolin, Hederagenin, 7-O-methylis omicron ulatol, Formononetin,
Isorhamnetin, and Myricanone. It could be seen that the neuroprotective mechanism of BHD had the
characteristics of multi-component, multi-target and multi mechanism. The compound-compound target-IS
network is shown in Fig. 4.

**Pathway of drug-compound target-IS network**

By importing all targets into DAVID, we could get 20 IS-related pathways. Radix Astragali and Carthami
Flos had the highest degree, which means that the two herbs might be the main herbs in treating IS.
Meanwhile, TNF signaling pathway showed the highest degree, followed by PI3K-Akt signaling pathway,
MAPK signaling pathway, NF-kappa B signaling pathway, Toll-like receptor signaling pathway, and T cell
receptor signaling pathway respectively. The pathway of drug-compound target-IS network is shown in
Fig. 5.

**Molecular docking verification**

Compound-target interactions with binding energy less than -5.0 kcal/mol are shown in Fig. 6, including
IL6 with Luteolin(A), MMP9 with Luteolin(B), TNF with Kaempferol(C), AKT1 with Kaempferol(D), ICAM1
with Kaempferol(E), IL1B with Quercetin(F), PTGS2 with Quercetin(G), IL-10 with Quercetin(H), VEGFA with
Baicalein(I), and MAPK1 with 7-O-methylisomucronulatol(J).

**Target path analysis**

The pathway map of BHD in treating IS was obtained from KEGG PATHWAY Database, as shown in Fig. 7.
The related pathways were marked in red, and the targets of BHD in treating IS were marked in rose red.
The results showed that the main pathways of BHD in treating IS included TNF signaling pathway, MAPK
signaling pathway, NF-kB signaling pathway and PI3K/AKT signalling pathway.
Discussion

In our study, we found the molecular mechanism of BHD’s neuroprotection effect against IS using network pharmacology strategy. The network pharmacology strategy is helpful to clarify the mechanism of TCM’s function from a systematic viewpoint [40–41]. Furthermore, this method provide a multi-dimensional research strategy for a complicated decoction. At present, the application of network pharmacology to study the mechanism of Chinese medicine has become a research hotspot. In this study, we found that 60 active components of BHD could act on 62 targets related to IS. Further analysis showed that BHD could act on many biological processes of IS and had an influence on the outcome of stroke through TNF, PI3K-Akt, MAPK, and NF-kappa B signaling pathway. It further confirmed that BHD had the characteristics of multi-component, multi-channel and multi-target.

Core ingredients with the highest degree in compound-compound target-IS network were considered to be responsible for neuroprotection, including quercetin, beta-Sitosterol, kaempferol, stigmasterol, baicalein, luteolin, hederagenin, 7-O-methylisorcimontol, formononetin, isorhamnetin, and myricanone. Six of these components belong to Radix Astragali and Carthami Flos. The results of network topology analysis showed that the degree of Radix Astragali and Carthami Flos were the highest in BHD. As the core herb in BHD, the dosage of Radix Astragali is the highest, indicating that the results of network pharmacology are consistent with the clinical application of Chinese medicine. Quercetin and kaempferol are common components of Radix Astragali and Carthami Flos. It was found that quercetin could pass through the blood-brain barrier with the highest passage rate [42]. A research showed that quercetin had effects of antioxidant stress and promoting autophagy, which was helpful for the prevention and treatment of stroke [43, 44]. In addition, quercetin could also regulate protein phosphorase 2A subunit B (PP2A) to produce significant neuroprotective effects on rats with cerebral ischemia-reperfusion injury and HT22 cell model of glutamate injury [45]. Lu et al found that quercetin could inhibit the expression and release of many inflammatory factors such as TNF - α, IL-1 β and IL6 by reducing the production of NF – κB in elderly mice [46]. Kaempferol, a common flavonoid, has been widely concerned because of its anti-inflammatory, antioxidant, antibacterial and antiviral effects. It has been reported that kaempferol has neuroprotective effect in the acute phase of cerebral infarction [47, 48]. One study confirmed that kaempferol inhibited oxygen-glucose deprivation (OGD) induced cell viability decline, oxidative stress, mitochondrial dysfunction and apoptosis [49]. These findings suggested that kaempferol might be a promising choice for the intervention of IS. Baicalein is a common component of Carthami Flos and Radix paeoniae Rubra. As an important flavonoids, it has many pharmacological activities, such as antioxidant stress, anti-inflammatory, anti-excitatory toxicity, anti-apoptosis, stimulating neurogenesis and promoting the expression of neuroprotective factors [50–52]. Liu et al found that baicalein had protective effect on transient middle cerebral artery occlusion model rats, and could significantly reduce the apoptosis of ischemic penumbra cells around the ischemic infarct of middle cerebral artery occlusion (MCAO) model rats [53]. As an ingredient of Carthami Flos, luteolin could down regulate the expression of TLR4, TLR5, NF-κ B and P-P38MAPK, up regulate the expression of p-ERK, and protect cerebral ischemia in rats [54]. Experiments performed in vivo also demonstrated that luteolin reduced the infarct volume. It was suggested that luteolin had potential in the treatment of IS through inhibiting MMP9 and activating...
PI3K/Akt signaling pathway [55]. Beta-sitosterol and stigmasterol are the common components of *Carthami Flos, Persicae Semen, Radix paeoniae Rubra*, and *Angelicae Sinensis Radix*. They are both sterol compounds, mainly with the functions of reducing blood fat, anti-oxidation and anti-inflammation [56].

PPI analysis showed that IL6, TNF, VEGFA, AKT1, MMP9, IL1B, MAPK1, ICAM1, PTGS2 and IL10 were the top ten targets with high degree. Followed by cluster of the PPI network, the network could be divided into four modules, which were related to angiogenesis, inflammation, coagulation and blood brain barrier. Inflammation plays a critical role in the pathological process of stroke [57]. IL1B, IL10, TNF, IL6, and ICAM1 are closely related to the inflammatory response after stroke, among which IL-10 is an important anti-inflammatory factor, while L1B, TNF and IL6 are pro-inflammatory factors. ICAM1 is an important adhesion molecule mediating the adhesion reaction, which plays an important role in stabilizing the interaction between cells and promoting the migration of leukocytes and endothelial cells. Ischemic cascade reaction leads to microglial activation, which will promote the release of pro-inflammatory cytokines (TNF-α, IL1B, and IL6) and anti-inflammatory cytokines (IL10 and TGF-β) [58]. MMP-9 is a kind of matrix metalloproteinases (MMPs) closely related to the development of IS, which promotes embryo development, inflammation, atherosclerosis and other biological functions. Under the stimulation of cerebral ischemia and hypoxia, microglia and astrocytes produce part of MMP-9 under the guidance of inflammatory factors. By hydrolyzing the tight junction protein on the basement membrane of cerebrovascular, the integrity of blood-brain barrier is destroyed [59]. VEGF is a double-edged sword in the development of cerebral infarction. In the hyperacute stage of cerebral infarction, the increase of VEGF concentration will increase the permeability of blood-brain barrier, lead to brain edema and aggravate clinical symptoms. In the post infarction recovery stage, the high content of VEGF is conducive to the establishment of collateral circulation of ischemic focus and penumbra and the damage and repair of neurons [60]. AKT1 is one of serine/threonine-protein kinases (AKT1, AKT2 and AKT3), and it regulates many processes including metabolism, proliferation, cell survival, growth and angiogenesis. AKT1 gene deletion induces dysfunction of vascular endothelial cells, migration and survival of vascular smooth muscle cells [61].

Pathway enrichment analysis results showed that TNF signaling pathway, PI3K-Akt signaling pathway, MAPK signaling pathway, and NF-kappa B signaling pathway are the main pathways. TNF signaling pathway is an important inflammatory pathway. As an important cytokine, TNF can induce apoptosis, cell survival, inflammation, immunity and other intracellular signaling pathways. TNFR1 signal transduction can induce the activation of many genes, which are mainly controlled by NF-kappa B and MAPK cascade. TNFR2 signal activated PI3K and JNK pathway. In this present study, TNF, IL-1B, MYC and TGFB1 were potential targets of BHD, suggesting that BHD plays a neuroprotective role against ischemia-reperfusion injury through TNF signaling pathway. PI3K / Akt signaling pathway is one of the important pathways of cerebral ischemia and neuronal apoptosis. A study found that activating PI3K / Akt signal pathway could inhibit the apoptosis of nerve and reduce the occurrence of blood-borne brain edema. A series of studies have shown that many Chinese herbal extracts play a protective role in IS through this pathway [62, 63]. Another study found that Baicalein also decreased the LC3-II/LC3-I ratio and promoted phosphorylation of the PI3K/Akt/mTOR signaling pathway which implied inhibition of autophagy. The reduction of
phosphorylation Akt and glycogen synthase kinase-3beta (GSK3beta) induced by OGD was restored by Baicalein, which was associated with preserved levels of phosphorylation of PTEN, the phosphatase that negatively regulates Akt [64, 65]. It was reported that baicalein could activate PI3K/AKT pathway, inhibit caspase activation and reduce cerebral infarct volume in MCAO rats [66]. Besides, formononetin mediated neuroprotection against cerebral ischemia/reperfusion in rats via downregulation of the Bax/Bcl-2 ratio and upregulation PI3K/Akt signaling pathway [67]. MAPK signaling pathways may be a therapeutic targets for stroke[68]. Researches showed that suppressing the NF-κB and MAPK signaling pathways would down regulate the expression of proinflammatory factors. MAPK pathways could be a promising candidate for future applications in CNS injury treatment [69], BHD alleviated pressure overload induced cardiac remodeling by suppressing TGF-β/Smads and MAPKs signaling activated fibrosis [70].

However, our research also has some limitations. For example, the accuracy and integrity of existing databases need further verification. Higher quality databases of traditional Chinese medicine and more accurate background network databases are needed. Moreover, the results of network pharmacology needs experimental support.

The application of network pharmacology in the study of Chinese Medicine is just in its start-up step. We need to promote the interdisciplinary researches integrating network science, bioinformatics, computer science, mathematics, and pharmacology in the future.

**Conclusion**

In this study, we explored and discussed the characteristic of “multi-component, multi-target and multi-channel” of BHD-mediated IS treatment through the method of network pharmacology and molecular docking. In the future, we should provide experimental evidence for the neuroprotective effect of BHD against IS according to the results of network pharmacology research.

**Declarations**

**Authors' contributions**

Qiang Gao: Conceptualization, Writing - original draft. Zhenyun Han: Supervision, Writing - review & editing. Danfeng Tian: Software, Data curation. Jingfeng Lin: Methodology, Software. Ze Chang: Methodology, Software. Dandan Zhang: Supervision. Dayong Ma: Supervision, Funding acquisition, Writing - review & editing.

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Competing interests

All of authors declare no conflicts of interest.

Availability of data and materials

All data obtained or analyzed during this study are available from the published article and supplementary material.

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**Tables**

**Table1** Active compounds of BHD and their parameters
| Herb | Mol ID   | Molecule Name                                                                 | OB (%) | Caco-2 | DL   |
|------|----------|-------------------------------------------------------------------------------|--------|--------|------|
| RA   | MOL000211| Mairin                                                                         | 55.38  | 0.73   | 0.78 |
| RA   | MOL000239| Jaranol                                                                        | 50.83  | 0.61   | 0.29 |
| RA   | MOL000296| hederagenin                                                                   | 36.91  | 1.32   | 0.75 |
| RA   | MOL000333| (3S,8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-[[(2R,5S)-5-propan-2-yloctan-2-yl]-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol | 36.23  | 1.45   | 0.78 |
| RA   | MOL000354| isorhamnetin                                                                   | 49.6   | 0.31   | 0.31 |
| RA   | MOL000371| 3,9-di-O-methylnissolin                                                        | 53.74  | 1.18   | 0.48 |
| RA   | MOL000378| 7-O-methylisomucronulatol                                                       | 74.69  | 1.08   | 0.3  |
| RA   | MOL000380| (6aR,11aR)-9,10-dimethoxy-6a,11a-dihydro-6H-benzofurano[3,2-c]chromen-3-ol    | 64.26  | 0.93   | 0.42 |
| RA   | MOL000387| Bifendate                                                                      | 31.1   | 0.15   | 0.67 |
| RA   | MOL000392| formononetin                                                                  | 69.67  | 0.78   | 0.21 |
| RA   | MOL000398| isoflavanone                                                                   | 109.99 | 0.53   | 0.3  |
| RA   | MOL000417| Calycosin                                                                     | 47.75  | 0.52   | 0.24 |
| RA   | MOL000422| kaempferol                                                                    | 41.88  | 0.26   | 0.24 |
| RA   | MOL000438| (3R)-3-(2-hydroxy-3,4-dimethoxyphenyl)chroman-7-ol                            | 67.67  | 0.96   | 0.26 |
| RA   | MOL000442| 1,7-Dihydroxy-3,9-dimethoxy pterocarpene                                      | 39.05  | 0.89   | 0.48 |
| RA   | MOL000098| quercetin                                                                      | 46.43  | 0.05   | 0.28 |
| CR   | MOL001494| Mandenol                                                                       | 42     | 1.46   | 0.19 |
| CR   | MOL002135| Myricanone                                                                     | 40.6   | 0.67   | 0.51 |
| CR   | MOL002140| Perlolyrine                                                                    | 65.95  | 0.88   | 0.27 |
| CR   | MOL002151| senkyunone                                                                    | 47.66  | 1.15   | 0.24 |
| CR   | MOL002157| wallichilide                                                                   | 42.31  | 0.82   | 0.71 |
| CR   | MOL000359| sitosterol                                                                     | 36.91  | 1.32   | 0.75 |
| RPR  | MOL001918| paeoniflororgenone                                                            | 87.59  | -0.09  | 0.37 |
| RPR     | MOL001925  | paeoniflorin_qt          | 68.18 | -0.34 | 0.4  |
|---------|------------|--------------------------|-------|-------|------|
| RPR     | MOL002714  | baicalein                | 33.52 | 0.63  | 0.21 |
| RPR     | MOL000358  | beta-sitosterol          | 36.91 | 1.32  | 0.75 |
| RPR     | MOL000359  | sitosterol               | 36.91 | 1.32  | 0.75 |
| RPR     | MOL004355  | Spinasterol              | 42.98 | 1.44  | 0.76 |
| RPR     | MOL000449  | Stigmasterol             | 43.83 | 1.44  | 0.76 |
| RPR     | MOL000492  | (+)-catechin             | 54.83 | 0.03  | 0.24 |
| RPR     | MOL006992  | (2R,3R)-4-methoxyl-distylin | 59.98 | 0.17  | 0.3  |
| RPR     | MOL006994  | 1-o-beta-d-glucopyranosyl-8-o-benzoypaeonisuffrone_qt | 36.01 | -0.03 | 0.3  |
| RPR     | MOL006996  | 1-o-beta-d-glucopyranosylpaeonisuffrone_qt | 65.08 | -0.05 | 0.35 |
| RPR     | MOL006999  | stigmast-7-en-3-ol       | 37.42 | 1.32  | 0.75 |
| RPR     | MOL007005  | Albiflorin_qt            | 48.7  | -0.38 | 0.33 |
| RPR     | MOL007008  | 4-ethyl-paeoniflorin_qt  | 56.87 | -0.17 | 0.44 |
| RPR     | MOL007012  | 4-o-methyl-paeoniflorin_qt | 56.7  | 0.4   | 0.43 |
| RPR     | MOL007016  | Paeoniflorigenone        | 65.33 | -0.13 | 0.37 |
| RPR     | MOL007018  | 9-ethyl-neo-paeoniaflorin A_qt | 64.42 | -0.01 | 0.3  |
| RPR     | MOL007022  | evofolinB                | 64.74 | 0     | 0.22 |
| RPR     | MOL002883  | Ethyl oleate (NF)        | 32.4  | 1.4   | 0.19 |
| RPR     | MOL005043  | campest-5-en-3beta-ol    | 37.58 | 1.32  | 0.71 |
| ASR     | MOL000358  | beta-sitosterol          | 36.91 | 1.32  | 0.75 |
| ASR     | MOL000449  | Stigmasterol             | 43.83 | 1.44  | 0.76 |
| PS      | MOL001323  | Sitosterol alpha1        | 43.28 | 1.41  | 0.78 |
| PS      | MOL001328  | 2,3-didehydro GA70       | 63.29 | -0.27 | 0.5  |
| PS      | MOL001339  | GA119                    | 76.36 | -0.12 | 0.49 |
| PS      | MOL001340  | GA120                    | 84.85 | 0.38  | 0.45 |
| PS      | MOL001342  | GA121-isolactone         | 72.7  | -0.26 | 0.54 |
| PS      | MOL001343  | GA122                    | 64.79 | -0.17 | 0.5  |
| PS      | MOL001344  | GA122-isolactone         | 88.11 | -0.18 | 0.54 |
| PS      | MOL001351  | Gibberellin A44          | 101.61| -0.13 | 0.54 |
|    | MOL001358 | gibberellin 7 |    |    |
|    | PS       | 73.8         | -0.18 | 0.5 |
|    | MOL001371 | Populoside_qt | 108.89 | 0.49 | 0.2 |
|    | PS       | hederagenin | 36.91 | 1.32 | 0.75 |
|    | PS       | beta-sitosterol | 36.91 | 1.32 | 0.75 |
|    | PS       | campesterol | 37.58 | 1.31 | 0.71 |
|    | CF       | poriferast-5-en-3beta-ol | 36.91 | 1.45 | 0.75 |
|    | CF       | Flavoxanthin | 60.41 | 0.97 | 0.56 |
|    | CF       | 4-[(E)-4-(3,5-dimethoxy-4-oxo-1-cyclohexa-2,5-dienylidene)-but-2-enylidene]-2,6-dimethoxy-2,5-dien-1-one | 48.47 | 0.81 | 0.36 |
|    | CF       | lignan | 43.32 | 0.42 | 0.65 |
|    | CF       | lupeol-palmitate | 33.98 | 1.52 | 0.32 |
|    | CF       | Phytoene | 39.56 | 2.22 | 0.5 |
|    | CF       | phytofluene | 43.18 | 2.29 | 0.5 |
|    | CF       | Pyrethrin II | 48.36 | 0.53 | 0.35 |
|    | CF       | 6-Hydroxykaempferol | 62.13 | 0.16 | 0.27 |
|    | CF       | baicalein | 33.52 | 0.63 | 0.21 |
|    | CF       | qt_carthamone | 51.03 | -0.31 | 0.2 |
|    | CF       | 6-Hydroxynaringenin | 33.23 | 0.27 | 0.24 |
|    | CF       | quercetagetin | 45.01 | -0.06 | 0.31 |
|    | CF       | 7,8-dimethyl-1H-pyrimido[5,6-g]quinoxaline-2,4-dione | 45.75 | 0.06 | 0.19 |
|    | CF       | beta-carotene | 37.18 | 2.25 | 0.58 |
|    | CF       | beta-sitosterol | 36.91 | 1.32 | 0.75 |
|    | CF       | kaempferol | 41.88 | 0.26 | 0.24 |
|    | CF       | Stigmasterol | 43.83 | 1.44 | 0.76 |
|    | CF       | luteolin | 36.16 | 0.19 | 0.25 |
|    | CF       | CLR | 37.87 | 1.43 | 0.68 |
|    | CF       | quercetin | 46.43 | 0.05 | 0.28 |
| Target Information | BHD Related to IS |
|-------------------|------------------|
|                   |                  |
| UniProt ID | Protein name | Gene name |
|------------|--------------|-----------|
| Q9UNQ0     | ABCG2        | ATP-binding cassette sub-family G member 2 |
| P35348     | ADRA1A       | Alpha-1A adrenergic receptor |
| P07550     | ADRB2        | Beta-2 adrenergic receptor |
| P31749     | AKT1         | RAC-alpha serine/threonine-protein kinase |
| P18054     | ALOX12       | Arachidonate 12-lipoxygenase, 12S-type |
| P09917     | ALOX5        | Arachidonate 5-lipoxygenase |
| P10275     | AR           | Androgen receptor |
| O15392     | BIRC5        | Baculoviral IAP repeat-containing protein 5 |
| P42574     | CASP3        | Caspase-3 |
| P24385     | CCND1        | G1/S-specific cyclin-D1 |
| P29965     | CD40LG       | CD40 ligand |
| P42771     | CDKN2A       | Cyclin-dependent kinase inhibitor 2A |
| P02741     | CRP          | C-reactive protein |
| P99999     | CYCS         | Cytochrome c |
| Q9NRD8     | DUOX2        | Dual oxidase 2 |
| P03372     | ESR1         | Estrogen receptor 1 |
| Q92731     | ESR2         | Estrogen receptor 2 |
| P00742     | F10          | Coagulation factor Xa |
| P00734     | F2           | Thrombin |
| P13726     | F3           | Tissue factor |
| P08709     | F7           | Coagulation factor VII |
| P42262     | GRIA2        | Glutamate receptor 2 |
| P09601     | HMOX1        | Heme oxygenase 1 |
| P05362     | ICAM1        | Intercellular adhesion molecule 1 |
| O14920     | IKBKB        | Inhibitor of nuclear factor kappa-B kinase subunit beta |
| P22301     | IL10         | Interleukin-10 |
| P01583     | IL1A         | Interleukin-1A |
| P01584     | IL1B         | Interleukin-1B |
| P05112 | IL4  | Interleukin-4 |
|--------|------|--------------|
| P05231 | IL6  | Interleukin-6|
| P35968 | KDR  | Vascular endothelial growth factor receptor 2 |
| P09960 | LTA4H| Leukotriene A-4 hydrolase |
| P11137 | MAP2 | Microtubule-associated protein 2 |
| P28482 | MAPK1| Mitogen-activated protein kinase 1 |
| P03956 | MMP1 | Matrix metalloproteinase-1 |
| P09238 | MMP10| Matrix metalloproteinase-10 |
| P08253 | MMP2 | Matrix metalloproteinase-2 |
| P08254 | MMP3 | Matrix metalloproteinase-3 |
| P14780 | MMP9 | Matrix metalloproteinase-9 |
| P05164 | MPO  | Myeloperoxidase |
| P01106 | MYC  | Myc proto-oncogene protein |
| Q16236 | NFE2L2| Nuclear factor erythroid 2-related factor 2 |
| P25963 | NFKBIA| NF-kappa-B inhibitor alpha |
| P29474 | NOS3 | Nitric oxide synthase, endothelial |
| P15559 | NQO1 | NAD(P)H dehydrogenase [quinone] 1 |
| P08235 | NR3C2| Mineralocorticoid receptor |
| P78380 | OLR1 | Oxidized low-density lipoprotein receptor 1 |
| P12004 | PCNA | Proliferating cell nuclear antigen |
| P00750 | PLAT| Tissue-type plasminogen activator |
| P00749 | PLAU | Urokinase-type plasminogen activator |
| P27169 | PON1 | Serum paraoxonase/arylesterase 1 |
| P37231 | PPARG| Peroxisome proliferator activated receptor gamma |
| P23219 | PTGS1| Prostaglandin G/H synthase 1 |
| P35354 | PTGS2| Prostaglandin G/H synthase 2 |
| P16581 | SELE | E-selectin |
| P05121 | SERPINE1| Plasminogen activator inhibitor 1 |
| P10451 | SPP1 | Osteopontin |
Figures

Figure 1

The flowchart of the whole study design.
Figure 2

GO and KEGG pathway enrichment analysis for targets of BHD related to IS. Notes: A: Biological processes, B: Cellular component, C: Molecular function, D: Bubble chart of KEGG pathway analysis. The order of importance was ranked by -Log10 (P-Value) and gene number.
Figure 3

Topological analysis of the target proteins of BHD related to IS. Notes: A: Venn diagram showing shared and unique targets of IS and BHD. B: The protein-protein interaction (PPI) network diagram constructed by the String database. C: BHD-IS PPI network constructed by Cytoscape. D: Bar graph of all protein nodes degree related to the targets. E: Cluster of PPI network.
Figure 4

Compound-compound target-IS network. Notes: Lilac circles stand for IS genes and rose red one stand for genes related to BHD. The red, yellow, green, navy blue, light blue, purple circle stand for compounds of Radix Atragali, Chuanxiong Rhizoma, Radix paeoniae Rubra, Persicae Semen, Angelicae Sinensis Radix, Carthami Flos.
Figure 5

Pathway of drug-compound target-IS network.
Figure 6

The conformations of some important compounds and key targets. Notes: A: IL6 with Luteolin (Binding energy=-8.12), B: MMP9 with Luteolin (Binding energy=-6.9), C: TNF with Kaempferol (Binding energy=-7.67), D: AKT1 with Kaempferol (Binding energy=-7.96), E: ICAM1 with Kaempferol (Binding energy=-8.5), F: IL1B with Quercetin (Binding energy=-8.32), G: PTGS2 with Quercetin (Binding energy=-9.29), H: IL-10 with Quercetin (Binding energy=-7.55), I: VEGFA with Baicalein (Binding energy=-6.67), J: MAPK1 with 7-O-methylisomucronulatol (Binding energy=-6.7).
Figure 7

Pathway map of BHD against IS. Notes: The key targets of BHD in the treatment of IS are shown rose red in the TNF signal pathway.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTable2.xlsx
- SupplementaryTable1.xlsx