Original Article

Identification and verification of effective components of Huanghuai for dysfunctional uterine bleeding based on network pharmacology and molecular docking

Yun-xiu Yao [a,b,1], Yu-ling Xu [a,c,1], Bo-wen Liu [c], Hui Yang [a], Song-yang Li [a], Ling-li Zhazo [a], Tao Liu [a,b,*]

[a] School of Food and Biological Engineering, Chengdu University, Chengdu 610106, China
[b] Guangxi University of Chinese Medicine, Nanning 530200, China
[c] College of Pharmacy, Chengdu University, Chengdu 610106, China

ARTICLE INFO

ABSTRACT

Objective: The Huanghuai (HH), which is made from the dried roots of *Scutellaria baicalensis* (Huangqin in Chinese) and the dried flowers and buds of *Sophora japonica* (Huaihua in Chinese), is a traditional Chinese formula used to treat dysfunctional uterine bleeding (DUB) (Benglou in Chinese) and proven to treat hemostasis effectively in our previous study. Network pharmacology and molecule docking were performed to study the underlying mechanism of Huanghuai (HH), and pharmacodynamic experiments were conducted to verify its curative effect.
Methods: TCMSP, UniProt, GeneCards, STRING, DAVID databases, and Cytoscape 3.7.2 were utilized for the construction of a compound-target-pathway network. Docking the potential effective components with potential targets. The HPLC analysis of the potential effective components was performed. *In vivo*, the hot plate test model was used to study the analgesic activity, the egg white was used to study the swollen reaction in the sole in mice, and the hemostasis effect was studied by the capillary method, tail-breaking method and abortion uterus test.
Results: The results showed that six compounds (acacetin, beta-sitosterol, wogonin, baicalein, kaempferol and quercetin) and four potential targets (PTGS2, AKT1, TP53 and TNF) in the compound-target-pathway network were the potential material basis for HH to treat DUB. It can be seen that the binding energy of the acacetin, wogonin, baicalein, beta-sitosterol, kaempferol and quercetin in HH docked with the receptor proteins PTGS2, AKT1, TP53, and TNF were far less than −5.0 kJ/mol, which means the molecules have low conformational energy, stable structure and high binding activity. And the result of HPLC analysis showed that acacetin, wogonin, baicalein, kaempferol and quercetin were the potential effective components of the hemostasis mechanism of HH, beta-sitosterol was removed due to low content. *In vivo* testing of the potential effective components, it revealed that the group of potential effective components identified by HPLC could increase the pain threshold, inhibit the swelling hind paws of mice induced by egg white, reduce the bleeding time and clotting time, reduce uterine bleeding, decrease the uterine weight, increase the content of Ca and ET-1, and reduce the content of NO in uterine homogenate tissue, and decrease of E_2 and P content in uterine serum in aborted rats, whose efficacy was equal to HH.
Conclusion: The results indicated that HH and potential active ingredient groups obtained from network pharmacology can treat DUB and play a hemostatic effect. The results obtained by network pharmacology have certain reliability. This study provides new indications for further mechanism research of HH on DUB and the development of HH or its components as an alternative therapy for patients with DUB. At the same time, the application of network pharmacology strategy may provide a powerful tool for exploring the mechanism of traditional Chinese medicine and discovering new biologically active ingredients.

1. Introduction

Dysfunctional uterine bleeding (DUB) (Benglou in Chinese) is irregular uterine bleeding that occurs in the absence of pathology or medical illness (Abdellah & Elsaman, 2012) and it is a common

gynecologic problem, occurring most frequently at the extremes of a woman's reproductive life (Keene & Payne, 2010), that accounts of >70% of all gynecologic consults (Tam, 2018). DUB can be treated medically or surgically. Medical treatment of DUB includes anti-fibrinolytic tranexamic acid, non-steroidal anti-inflammatory drugs, the combined contraception pill, progestogen, danazol, or analogues of gonadotrophin releasing hormone (Bongers et al., 2004). Surgical treatment includes hysteroscopic surgery and hysterectomy. General medical therapy is not as effective as endometrial resection in terms of patient satisfaction and recurrence rate after discontinuation of the treatment. However, hysterectomy is associated with the increased risk of morbidity and mortality (Feitoza et al., 2003). On the other hand, hysteroscopic surgery is effective and less morbidity and mortality when compared with hysterectomy. However, not only does it require additional surgical expertise, but serious complications can also occur, including excessive fluid, uterine perforation, infection, hemorrhage, and even death (Gurtcheff, 2003). Traditional Chinese formulas have a long history of treating DUB, with precise curative effects, no obvious side effects, and wide clinical application. The commonly used drugs are Motherwort Granules, Wuji Baifeng Pills, Yunnan Baiyao, Gongxuening Capsule (He et al., 2019), Ziyin Tiaojing Granule (Tang et al., 2018), etc.

Huanghuai (HH), which is made from the dried roots of *Scutellaria baicalensis* Georgi (Huangqin in Chinese) and the dried flowers and buds of *Sophora japonica* L. (Huaihua in Chinese), comes from "*Detailed Outline for Benefiting Female*" (*Jiyin Gangmu* in Chinese) (Wu, 2006), which was a traditional Chinese formula for the treatment of DUB in traditional Chinese medicine. It was proposed that HH can achieve the efficacy of cooling the blood, stanching bleeding, purging fire, detoxifying toxicosis (Chinese Pharmacopoeia Commission, 2015). Previous research has confirmed that HH has a hemostatic effect (Li et al., 2017). However, the hemostatic mechanism of HH has not been systematically elucidated, and active compounds and potential targets have not been fully identified. Therefore, network pharmacology and molecular docking were used in this study to explore the hemostatic components and mechanism of HH.

Network pharmacology was first proposed by Hopkins in 2007 in *Nature Biotechnology* (Hopkins, 2007). Network pharmacology is a new discipline based on the disease-gene-drug multi-level network to predict drug targets as a whole and improve the efficiency of drug discovery. Network pharmacology has been widely used to screen active ingredients, elucidate the mechanism of drug action, and study the pathogenesis of diseases (Wei et al., 2019; Qin et al., 2020; Jiang et al., 2019; Qu et al., 2019; Niu et al., 2019). Molecular docking is a theoretical simulation method to predict the binding mode and affinity of the receptor by the characteristics of the receptor and the way of interaction between the receptor and the drug molecule. However, the current network pharmacology research has only studied the mechanism of drug treatment for diseases and has not verified its efficacy.

Therefore, in this study, network pharmacology and molecular docking were used to predict the hemostatic components and hemostatic mechanisms of HH. Then, the content of each potential ingredient that exerts the hemostatic effect in the prescription was determined according to the HPLC method in the reference. Finally, the medicinal ingredient group of HH was determined, and the extracts of each medicinal ingredient were combined to perform an *in vivo* medicinal effect test for verification. It will provide new ideas for traditional Chinese medicine to treat the DUB disease.

2. Materials and methods

2.1. Discovery of potential active constituents of HH

2.1.1. Construction of chemical library

The main chemical composition of HH was searched by Traditional Chinese Medicines Systems Pharmacology (TCMSP) Database and Analysis Platform (http://lsp.nwu.edu.cn/tcmsp.php) (Yao et al., 2020), and the active compounds were screened by absorption, distribution, metabolism, excretion (ADME) to oral bioavailability (OB) ≥ 30% and drug-likeness (DL) ≥ 0.18. The primary screening results were again compared by CAS number and Pubchem database (https://pubchem.ncbi.nlm.nih.gov/) to confirm the only valid active compound database. Finally, the database of all active compounds in HH was established.

2.1.2. Screening targets

The target prediction function in the TCMSP database was used to collect the targets of known chemical constituents of HH. The predicted targets of the compounds were screened and searched in the UniProt database (https://www.uniprot.org/). Import the protein name in the Uniprot database. And select the "species" as "Homo sapiens", and the "repeated", "non-human" and "non-standard" targets were eliminated. The gene targets of the active components of HH were finally obtained through retrieval and transformation.

2.1.3. Collection of DUB targets

DUB-related targets were searched in the GeneCards database (https://www.genecards.org), and the DUB target database was established, and the top 300 targets with the highest correlation score were used as DUB targets.

2.1.4. Acquisition of DUB-related chemically potential targets

The screened chemical targets and the DUB targets were uploaded to Venny 2.1.0 (http://bioinfogp.cnb.csic.es/tools/venny/index.html). Ultimately, DUB-related chemically potential targets of HH were obtained.

2.1.5. Construction and analysis of protein-protein interaction (PPI) network

The genes from "2.1.4" were uploaded to the STRING database (https://string-db.org/), designated the "species" as "humans", and the PPI network was obtained. Node 1, node 2, and the combined score were exported from the database, the higher the score is, the higher the confidence degree of protein interaction is. The results were imported to Cytoscape 3.7.2 software for visual analysis, and the network analysis was obtained. The color, degree value, and size of the node reflected the comprehensive score, and the PPI network graph was obtained.

2.1.6. Gene ontology (GO) analysis

GO is a bioinformatics program initiated primarily to unify the nature of genes and gene products of all species (Wu et al., 2018). GO classification enrichment analysis includes molecular function (MF) analysis, biological process (BP) analysis, and cellular component (CC) analysis. To elucidate the biological function of HH in the treatment of DUB, the GO classification and enrichment analysis of DUB-related chemically potential targets gene were performed using the STRING database (https://string-db.org/). The top 20 results of molecular function, biological process, and cell component analysis were selected, and the histogram was drawn using GraphPad Prism 6.0.

2.1.7. KEGG pathway enrichment analysis

The genes from "2.1.4" were imported into David 6.8 database (https://david.ncifcrf.gov/) in the format of "Gene Symbol". The identifier was set as "OFFICIAL Gene Symbol", and "List Type" was set as "Gene List", designated the "species" as "humans" and the results were saved. Differences between the pathways were considered as significant at $P < 0.05$, the data were ranked from low to high. Then thetop 20 pathway information was selected with $P < 0.05$, Omicshare database (http://www.omicshare.com), and GraphPad Prism 6.0 software were used for plotting.

2.1.8. Construction of component-target-pathway (CTP) network

Organizing the components, targets, and pathways information contained in the 20 pathways selected by "2.1.7" and the component-target-pathway (CTP) network was constructed by using the "merge" function of Cytoscape software (http://www.cy-toscape.org). Nodes in the network represent components, targets, and pathways. A potential target of a component was connected by edges. The effect of HH on DUB was studied by constructing the component-target-pathway (CTP) network. Network analyzer in Cytoscape 3.7.2 software was utilized for computing the topological parameters of the network. If there is an interaction between the nodes in the network, they were connected by the edges. In this study, the nodes were evaluated based on the degree value. Degree in the component-target-pathway (CTP) network refers to the number of interacting proteins with a certain protein. Generally, in a network, the highest-ranked target protein of degree plays an important role in the hemostasis of HH. In the CTP network, a network analysis method with a degree greater than or equal to twice the median of all nodes-degree was used to determine potential components, targets and pathways.

2.1.9. Molecular docking of potential components with potential proteins

The 3D structured PDB file of PTGS2, AKT1, TP53, and TNF was download from the RSCB PDB database (Wu et al., 2020) (https://www.rcsb.org/), and Discovery Studio 2020 Client software was used to remove ligands from the protein and non-protein molecules (such as water molecules), and then saved as a PDB file. The 2D structured SDF file of the main active hemostasis components of HH was download from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). We used PyRx software to upload the dehydrated protein file first, converted it to a pdbqt format file, uploaded the compound file to minimize its energy, and converted it to a pdbqt format file. Finally, vina was used for docking. The binding energy of <0 indicates that the ligand and the receptor can spontaneously bind. The binding energy ≤ -5.0 kJ/mol (Zong et al., 2020) was used as a standard to evaluate the possibility of the interaction between the compound and the protein.

2.2. Determination of potential active constituents of HH

According to the results of the CTP network screening, acacetin, wogonin, baicalein, beta-sitosterol, kaempferol, and quercetin were considered to be the main active hemostasis components of HH. However, baicalin is the major flavonoid present in Huangqin and has been considered as the main effective constituent. Also, baicalin has not been screened out in the network pharmacology screening part. Therefore, to investigate the accuracy of network pharmacology screening results, baicalin was included in the main active hemostasis components in this study. In this section, the HPLC-UV analysis method for determining the content of seven active ingredients in HH will be established. On this basis, based on the results of the HH component determination, the actual active components were screened out, which were the pharmacodynamic effect of the hemostasis mechanism of HH.

2.2.1. Reagents and instruments

The dried roots of *S. baicalensis* as identified by the authors were purchased from the Pharmacy of Chengdu Tongrentang (Sichuan, China). And the dried flowers and buds of *S. japonica* were purchased from the Lotus Pond Chinese herbal medicine market (Sichuan, China). The reference substances of acacetin, baicalin, wogonin, baicalein, beta-sitosterol, kaempferol, and quercetin were purchased from the Sichuan Weikeqi Biological Technology Co., Ltd (Sichuan, China). SQP electronic balance Sartorius was purchased from Sartorius Scientific Instruments (Beijing) Co., Ltd., TGL-16G high-speed centrifuge was purchased from Shanghai Anting Scientific Instrument Factory, electronic analysis balance BS-6KH was purchased from Shanghai Yousheng Weighing Apparatus Co., Ltd., DZTW thermostat (the electric heating jacket) was purchased from Beijing Yongguangming Medical Instrument Co., Ltd., and the SHZ-D(III) circulating water vacuum pump was from Zhengzhou Du Fu Instrument Factory and Waters 2695 high-performance liquid chromatography from the United States.

2.2.2. Preparation of HH sample solutions and standard solutions (Li et al., 2017)

A total of 40 g dry *S. baicalensis* and 10 g dry *S. japonica* were weighed and boiled three times, two hours each time with a six-fold 40% volume fraction of ethanol in the electric heating jacket. Combine the filter liquids and cool to measure the volume of the filtrate. The filtrate was centrifuged at a speed of 12,000 r/min for 10 min in a high-speed centrifuge, and the supernatant was filtered through a 0.22 μm membrane filter and injected into the HPLC system for analysis. When determining the content of baicalin, quercetin, and kaempferol, the sample solutions were diluted ten times.

The following standard stock solutions were prepared in methanol, stored at 4 °C, and diluted as required for their use: baicalin (1209.60 mg/L), baicalein (528.00 mg/L), wogonin (103.20 mg/L), quercetin (33.43 mg/L), kaempferol (824.00 mg/L), acacetin (8.784 mg/L) and beta-sitosterol (193.60 mg/L).

2.2.3. Chromatographic conditions for HPLC analysis

HPLC analysis was performed on waters 2695 Alliance HT system equipped with a UV detector. The analytes were separated on a Supersil ODS-B C_{18} column (250 mm × 4.6 mm, 5 μm). The flow rate was 1.0 mL/min for the quantitative analysis. The analytical methods (Table 1) in the references had been successfully applied for the determination of seven compounds of HH (Li et al., 2018; Liu et al., 2015; Zhou et al., 2019; Chen, 2019; Bi et al., 2014).

2.3. Pharmacological studies

HH demonstrated markedly anti-inflammatory, hemostasis, and analgesic efficacy in the previous study. Therefore, this study used the hot plate test to study the analgesic activity; The egg white was used to study the swollen reaction in the sole in mice, and the hemostasis effect was studied by the capillary method, tail-breaking method and abortion uterus test. To provide a reference for verifying the application of network pharmacology in the hemostasis mechanism of HH. The research was conducted by the internationally accepted principles for laboratory animal use and care as found in the US guidelines (NIH publication #85-23, revised in 1985).

The HH is made from the dried roots of *S. baicalensis* and the dried flowers and buds of *S. japonica*. It is used clinically to treat DUB and has a good curative effect. The research group used the fibrinogen plate method to perform *in vitro* coagulation activity measurement. The results showed that baicalin in HH had strong *in vitro* coagulation activity. The effective components were

Table 1
Analytical methods of potential active constituents of HH.

Constituents	Mobile phase(A:B)	Column temperature/°C	Gradient conditions	Wavelength/nm
Wogonin Baicalein	Acetonitrile: 0.1% phosphoric acid	30	0–15 min, 15%–22% A; 15–31 min, 22%–55% A; 31–50 min, 55%–75% A	280
Quercetin Kaempferol	Methanol: 0.4% phosphoric acid	20	0–40 min, 50% A	260
Acacetin	Acetonitrile: 0.1% phosphoric acid	30	0–16 min, 25% A; 16–20 min, 25%–30% A; 20–25 min, 30%–33% A; 25–35 min, 33%–43% A; 35–40 min, 43%–50% A; 40–45 min, 50%–25% A	365
Baicalin	Methanol: 0.2% phosphoric acid	30	A:B = 47:53	280
Beta-sitosterol	Methanol	25	100% methanol	210

screened and it was found to be related to five kinds of ingredients, such as acacetin, wogonin, baicalein, kaempferol, and quercetin, which did not include baicalin. To investigate whether baicalin has hemostatic activity in the body, we designed two groups of trials compared the efficacy of five extract mixtures (FEM) and baicalin + FEM. The baicalin content in the compound had been determined in Section 3.7.

2.3.1. Reagents and instruments

Baicalin extract ($\geq$85%) and baicalein extract ($\geq$98%) were purchased from Xi'an Chuang' en Biotechnology Co., Ltd. (Shanxi, China). Acacetin extract ($\geq$99%), kaempferol, quercetin extract ($\geq$98%) was purchased from Xi'an Rongzhen Biotechnology Co., Ltd. (Shanxi, China). The sodium chloride (0.9%) injection was from Sichuan Kelun Pharmaceutical Co., Ltd. (Sichuan China). Aspirin effervescent tablets were purchased from AstraZeneca. Tranexamic Acid Tablets were from CMIC CMO Co., Ltd. Shizuoka Plant. Baogongzhixue (BGZX) Granules were purchased from Tianjin Zhongsheng Haitian Pharmaceutical Co., Ltd. Mifepristone Tablets and Misoprostol Tablets were from Hubei Gedian Renfu Pharmaceutical Co., Ltd. Chloral hydrate was from Chengdu Cologne Chemical Co., Ltd. Calcium (Ca) kit, nitric oxide (NO) assay kit, estradiol (E_2) kit, endothelin-1 (et-1) kit and progesterone (PROG) test kit were purchased from Nanjing Jiancheng Bioengineering Research Institute (Nanjing, China). FA2004 analytical electronic balance was purchased from Shanghai Liangping Instrument Co., Ltd. RE-501 rotary evaporator was purchased from Chengdu Kangyu Technology Co., Ltd. Vacuum drying oven was purchased from Beijing Zhongxing Weiye Instrument Co., Ltd. PS-40 ultrasonic cleaner purchased from Shenzhen Dekang Cleaning Equipment Co., Ltd. Optical microscope from Haoyu Optical Technology Co., Ltd. YLS-6B intelligent hot plate the instrument was purchased from the equipment station of Shandong Academy of Medical Sciences. Measurement data were compared with a single-factor analysis of variance. Other reagents and instruments were the same as 2.2.1.

2.3.2. Animals

Female Kunming mice, aged 6–8 weeks (30–45 g, SPF grade, Certificate No. SCXK(Chuan) 2015–030), 6–8 weeks old female SD rat (200–300 g, SPF grade) and 6–8 weeks old male SD rat (250–350 g, SPF grade) were provided from Dashuo Experimental Animals Co., Ltd. (Chengdu, China) and maintained under specific pathogen-free conditions. Animals were kept under a 12 h light/dark cycle at the animal care facility, acclimatized for at least 3 d before the experiments, and given a fresh diet with free access to water. All *in vivo* experiments were carried out under the guidelines approved by the Institutional Animal Care and Use Committee (IACUC) of Chengdu University.

2.3.3. Data and statistical analysis

All data processed by one-factor analysis of variance using IBM SPSS Statistics 21. Data are presented as the mean ± standard error.

2.3.4. Preparation of HH extract

A total of 2000 g dry *S. baicalensis* and 500 g dry *S. japonica* were weighed and boiled for three times, two hours each time with a six-fold 40% volume fraction of ethanol in the electric heating jacket. Combine the filter liquids and concentrate the filtrate until its relative density was about 1.30 (70 °C), and vacuum drying technological parameters were –0.08 MPa in combination with 70 °C.

2.3.5. Hot plate method induced mice pain test

Adult female Kunming mice (30–45 g) (n = 60) were divided randomly into six groups: model group (20 mL/kg), aspirin group (0.3 g/kg), Baogongzhixue (BGZX) group (6 g/kg), Huanghuai (HH) group (3 g/kg), five extract mixtures (FEM) group (0.12 g/kg), and baicalin + FEM group (0.40 g/kg). After continuous gastric administration for 7 d, the reaction of pain perception was detected with the hot-plate method at 30, 60, 90, and 120 min. Measurement data were compared with a single-factor analysis of variance.

2.3.6. Swelling hind paws of mice induced by egg white

Adult female mice (30–45 g) (n = 60) were divided randomly into six groups, with the same animal groups as 2.3.5. Then, a clear marker line was drawn with a marker on the same part of the right hind paws of mice, and the normal thickness was measured with a vernier caliper. After the measurement, all rats were administered with gavage once a day for 7 d. After 15 min of the last administration, 0.05 mL of 10% egg white was injected intramuscularly from the marker. The thickness of the hind paws of mice was measured at 0.5, 1, 1.5 h after sensitization. Measurement data were compared with a single-factor analysis of variance.

2.3.7. Hemostasis in mice

i. Clotting time study in mice

A total of 54 adult female mice were randomly divided into six groups, with the same animal groups as 2.3.5. After continuous gastric administration for 7 d, 1 h after the last administration, the blood was taken from the posterior venous plexus of the mouse with a capillary tube, and the blood was collected from mice by retrobulbar puncture with capillary glass pipettes. After the blood was filled with the capillary glass tube, the capillary was placed flat on the table, and the capillary was broken 0.5 cm every 10 s, and slowly pull it left and right until the blood streak appeared, the

time was the blood coagulation time. Measurement data were compared with a single-factor analysis of variance.

ii. Determination of hemorrhagic time in mice with broken tail

After continuous gastric administration for 7 d, 1 h after the last administration, the tip of the tail was cut by scissors from the tip of the mouse 3 mm, then began timing when the blood flowed out of the tail. Drawing the blood with a filter paper every 30 s until there was no blood outflowing. Measurement data were compared with a single-factor analysis of variance.

2.3.7. Medicine spontaneous abortion uterus of rats

Female rats and male rats were raised in a cage at a ratio of 2: 1 at 20:00, and the rats were separated by male and female at 8:00 the next morning. The vaginal smear method was used to ascertain the characteristics of each stage of the estrous cycle in rats. When preparing a vaginal smear, 80 μL of physiological saline was sucked into the rat's vagina by a pipette, blown repeatedly five times, 10–20 μL of the liquid was dropped on the glass slide, then spread evenly, and smear dried naturally and fixed with 95% ethanol. Finally, we observed the changes in vaginal smear through a microscope to determine whether the rat was pregnant. Vaginal smear photographs of 64 female SD rats were observed. The rats were given mifepristone 0.83 mg/kg in the morning and misoprostol 10 μg/kg in the evening by gavage on the 7th d of pregnancy. At the same time, a quantitative cotton ball was inserted into the vagina (the cotton ball weighs 85 to 90 mg). A total of 54 rats with successful modeling were randomly divided into six groups: model group (10 mL/kg), tranexamic acid group (0.15 g/kg), Baogongzhixue (BGZX) group (3 g/kg), Huanghuai (HH) group (1.5 g/kg), Five extract mixtures (FEM) group (0.06 g/kg), and baicalin + FEM group (0.20 g/kg). And continuous gastric administration for 7 d was done. The cotton balls were taken out at 8:00 and 20:00 every day, placed in a plastic bag, and kept in cold storage, and a new cotton ball was replaced in the vagina to observe the vaginal bleeding for 7 d. The vaginal cotton balls containing uterine bleeding from each rat were placed in EP, and an appropriate amount of 5% NaOH was added according to the actual situation of the bleeding volume, generally 4–7 mL. After immersion extraction for some time, the cotton blood stains were squeezed and squeezed until the eluent was colorless. Using a 5% NaOH solution as a blank control, the absorbance (A) of the extract of the rats was measured at 546 nm. We narcotized all rats on the last day, then we draw blood from abdominal aorta after surgery, and serum was isolated; E_2 and P level were measured. Simultaneously, the uterus was weighed and the proportion coefficient of the uterus was calculated. The content of Ca, ET, and NO were measured in the homogenized uterus.

3. Results

3.1. Screening of chemical constituents of HH

Based on literature and databases, OB $\geq$ 30% and DL $\geq$ 0.18 were selected as the screening parameters. A total of 34 compounds were selected as active compounds

3.2. Acquisition of DUB-related chemically potential targets

In the GeneCards database, 1558 candidate genes were initially screened with DUB, and the top 300 results with the highest correlation score were obtained. A total of 61 DUB-related chemically potential targets were obtained by Venny as a potential target gene for the treatment of DUB as an active ingredient of HH (Fig. 1).
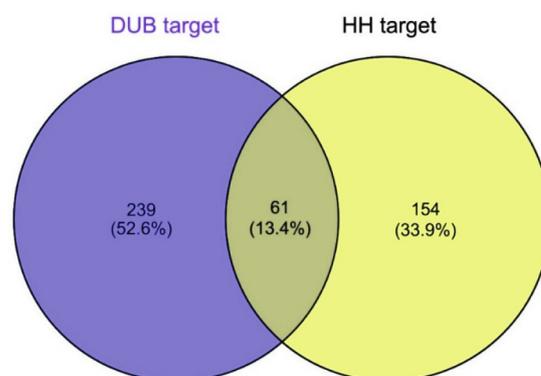

Fig. 1. DUB-related chemically potential targets.

3.3. Construction of PPI network of HH

A total of 61 targets were imported into the STRING database. The PPI network graph was obtained (Fig. 2). There were interactions between the 61 targets, suggesting that at some level these targets were interrelated and played a role in DUB through multiple pathways and coordination. And the greater the degree value of a node is, the redder its color is, and the larger its shape is, the more important it is in the network.

3.4. Bioinformatics of DUB targets of HH

The results of GO and KEGG analysis were shown in Fig. 3. The response to an organic substance, response to oxygen-containing compound, cellular response to chemical stimulus, and cellular response to organic substance ranked first in BP (Fig. 3A). Extracellular space, extracellular region part, extracellular region, and endomembrane system ranked first in CC (Fig. 3B). Signaling receptor binding, protein binding, cytokine receptor binding, and identical protein binding were the top ones in MF (Fig. 3C). The p-value of each pathway in the graph gradually decreased from top to bottom. The larger the dot in Fig. 3E, the redder the color, indicating the smaller the p-value. KEGG (Fig. 3D and E) showed that the main pathways for DUB were pathways in cancer, hepatitis B, proteoglycans in cancer, and Chagas disease (American trypanosomiasis).

3.5. Construction of CTP network

The "Merge" function of Cytoscape software was used for constructing the CTP network for studying the DUB targets of HH. As shown in Fig. 4, the main active hemostasis components of HH were distributed in different pathways and coordinated with each other to regulate the hemostasis mechanism of HH. The results of the CTP network were shown in Table 2. The greater the degree value of a node is, the larger its shape, the more important it is in the network. It was shown that acacetin, wogonin, baicalein, beta-sitosterol, kaempferol, and quercetin were the main active hemostasis components of HH. PTGS2, AKT1, TP53, and TNF were key nodes in the network. The main hemostasis mechanism of HH was related to the pathways in cancer.

3.6. Molecular docking of potential compounds in HH acting on potential proteins

It is generally believed that when the conformation of the ligand binding with the receptor is stable, the lower the energy, the more likely it will work. The molecular docking results showed that six potential active compounds of HH in the CTP network,
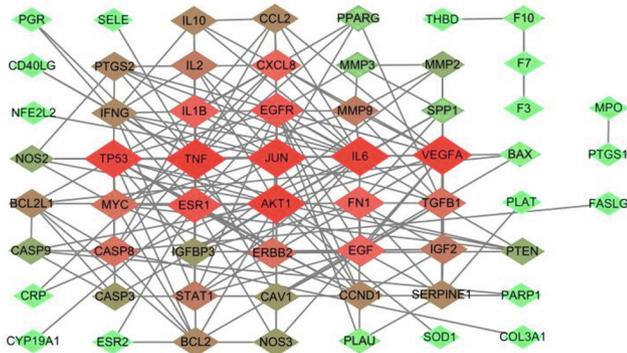
Fig. 2. PPI network of DUB target proteins of HH.

whose degree value was greater than or equal to twice the median of the degree of the component, were molecularly docked with PTGS2, AKT1, TP53 and TNF. Taking the binding energy of less than

or equal to −5.0 kJ/mol as the criterion, it can be seen that the binding energies of potential compounds in HH docked with PTGS2, AKT1, TP53 and TNF were far less than −5.0 kJ/mol. It can be seen that the acacetin, wogonin, baicalein, beta-sitosterol, kaempferol, and quercetin in HH docked with the receptor proteins PTGS2, AKT1, TP53, and TNF had low conformational energy, stable structure, and high binding activity. The results were shown in Table 3.

3.7. HPLC analysis results

The results of HPLC analysis showed that acacetin, wogonin, baicalein, kaempferol, and quercetin were the potential medical components of the hemostasis mechanism of HH, beta-sitosterol was removed due to low content. Among them, the ratio of wogonin, baicalein, quercetin, kaempferol, acacetin and baicalin in HH was 0.13%, 0.45%, 0.11%, 4.15%, 0.02% and 9.23%, respectively. The chromatograms were as follows (Fig. 5).
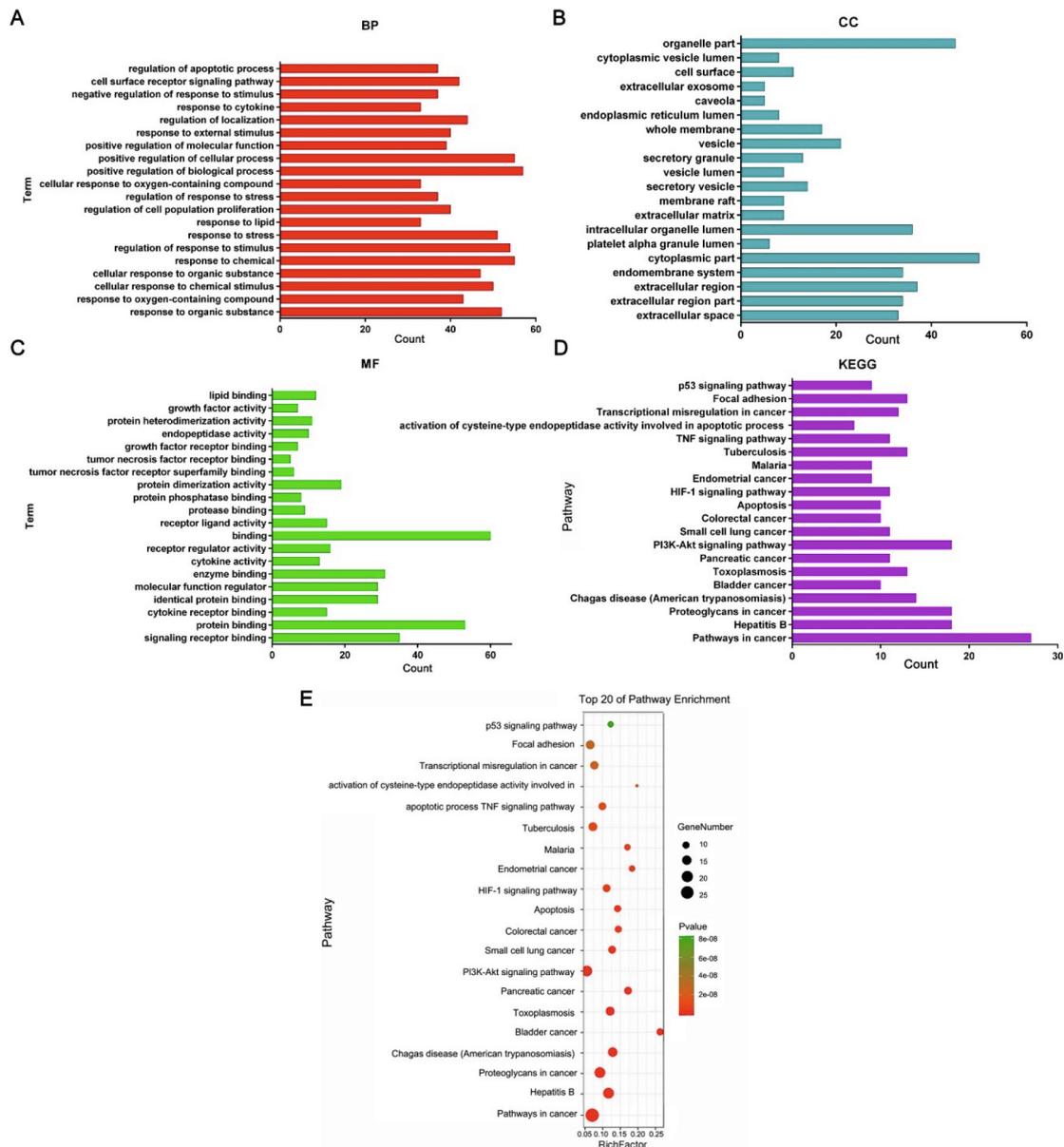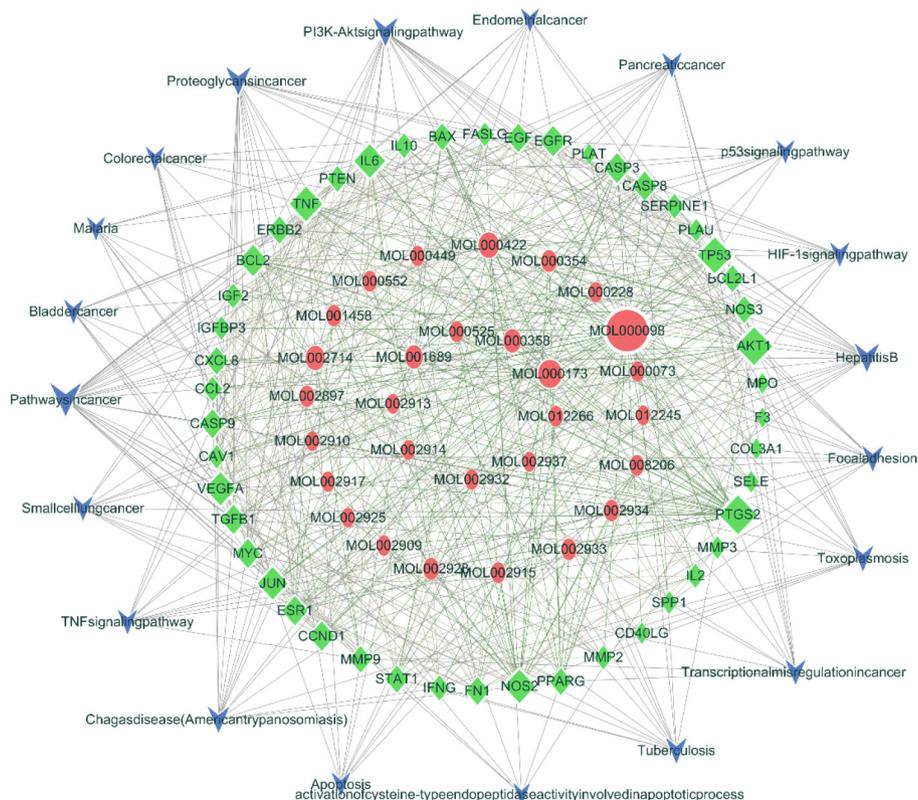

Fig. 3. GO analysis and KEGG pathways analysis by DAVID and STRING databases. GO analysis of candidate targets. The database showed the four remarkably enriched items in biological processes (BP, A), cell component (CC, B), and molecular function (MF, C). KEGG pathways of target genes (D and E).

Fig. 4. CTP network of 20 pathways and 29 compounds in HH, predicted to 47 candidate protein targets. The blue arrows nodes represent the pathways, green diamonds nodes represent the protein targets, while red ellipses nodes represent compounds.

Table 2
CTP network topology parameter information.

Categories	ID/Name	Average shortest path length (ASPL)	Betweenness centrality (BC)	Closeness centrality (CC)	Degree	Median
Compound	MOL000098 (quercetin)	1.547368	0.156145	0.646259	43	3
Compound	MOL000173 (wogonin)	1.894737	0.010513	0.527778	17	
Compound	MOL000422 (kaempferol)	2.021053	0.007964	0.494792	12	
Compound	MOL002714 (baicalein)	2.021053	0.00818	0.494792	10	
Compound	MOL001689 (acacetin)	2.157895	0.004732	0.463415	8	
Compound	MOL000358 (beta-sitosterol)	2.094737	0.003407	0.477387	8	
Target	PTGS2	1.6	0.263805	0.625	38	15
Target	AKT1	1.694737	0.04948	0.590062	36	
Target	TP53	1.663158	0.056702	0.601266	33	
Target	TNF	1.684211	0.053633	0.59375	30	
Pathway	Pathways in cancer	1.757895	0.031817	0.568862	27	11

Table 3
Binding affinities of potential compounds in HH with potential proteins.

| Compounds	Binding energy with PTGS2/ (kJ·mol⁻¹)	Binding energy with TNF/ (kJ·mol⁻¹)	Binding energy with TP53/ (kJ·mol⁻¹)	Binding energy with AKT1/ (kJ·mol⁻¹)
MOL000098 (quercetin)	−35.98	−30.54	−29.29	−40.17
MOL000173 (wogonin)	−31.80	−39.33	−26.78	−38.49
MOL000422 (kaempferol)	−33.89	−28.87	−27.61	−39.33
MOL002714 (baicalein)	−34.73	−29.71	−27.61	−40.17
MOL001689 (acacetin)	−34.31	−28.03	−28.03	−39.33
MOL000358 (beta–sitosterol)	−46.44	−45.19	−36.82	−58.99
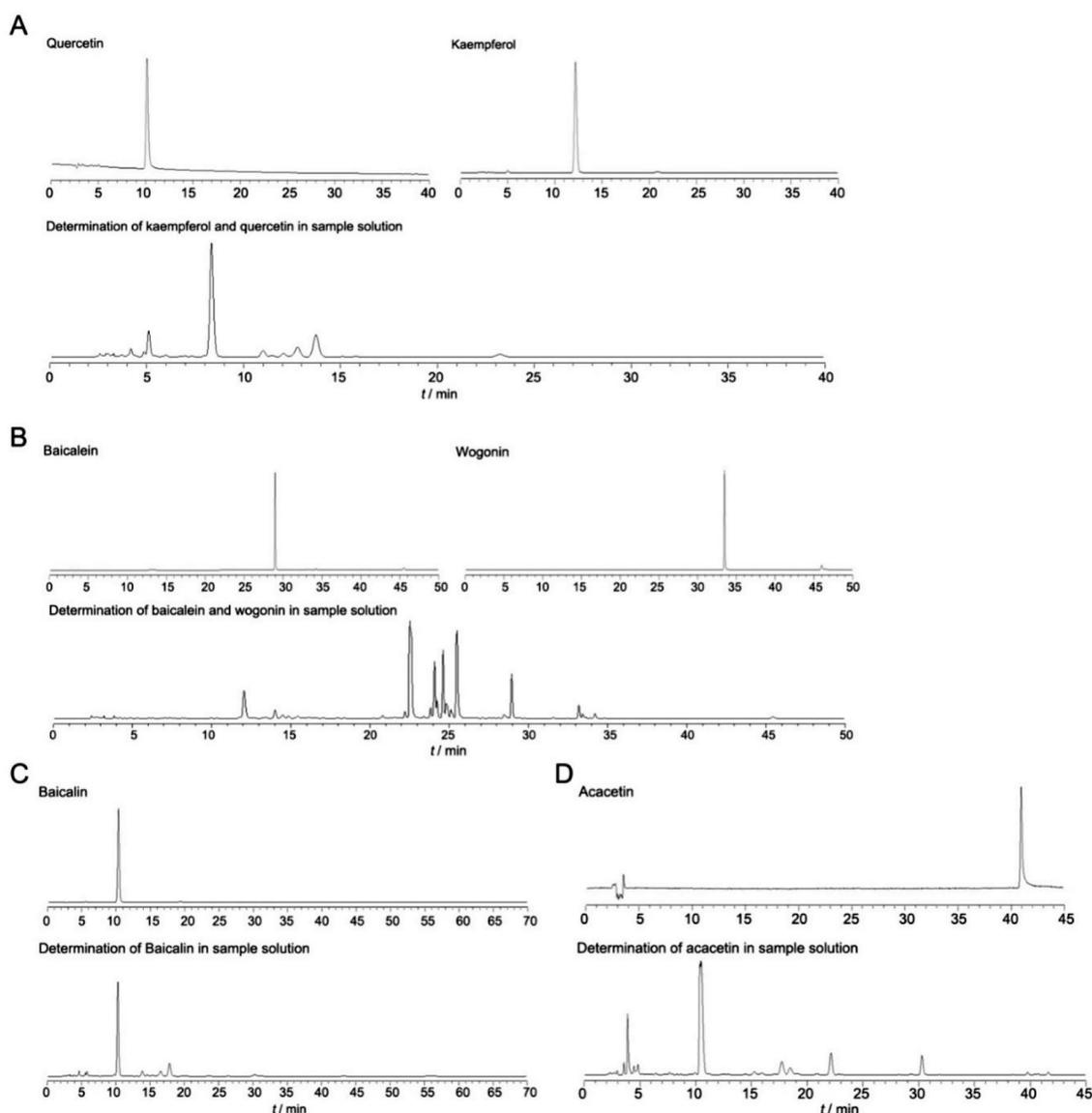
Fig. 5. Chromatograms of HPLC analysis. Determination of kaempferol and quercetin (A), baicalein and wogonin (B), baicalin (C) and acacetin (D) in sample solution.

3.8. Analgesic effect in mice

As the result shown (Fig. 6), the pain threshold was higher in the HH group and FEM group at 60 min after administration than in the model group, which had a significant difference (29.65 ± 13.33) s, (24.147 ± 16.54) s, (16.65 ± 7.15) s, $P < 0.05$. There was no significant difference between the baicalin + FEM group and model group. FEM group showed no significant differences at 30, 60, 90, 120 min in the pain threshold, compared with the HH group ($P > 0.05$). And FEM group had a statistically significant increase at 30 min in the pain threshold, compared with baicalin + FEM group ($P < 0.01$). This study indicateed that the HH and FEM had a significant analgesic effect, and there was no significant difference between them. Furthermore, the baicalin + FEM had no significant analgesic effect. In other words, in terms of analgesia, the FEM was equal to HH, which was superior to the baicalin + FEM.
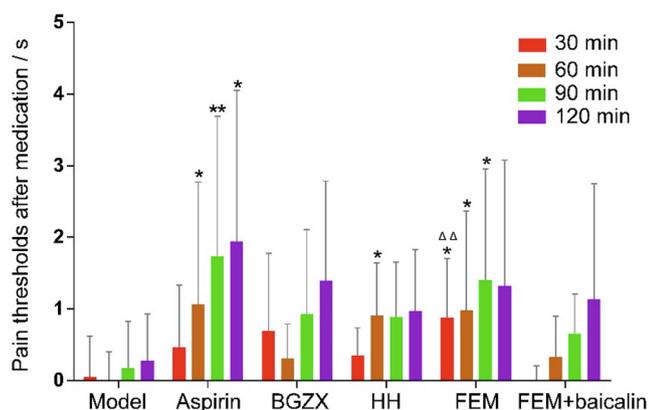
3.9. Anti-inflammatory effects in mice

As the result shown (Fig. 7), the swollen reaction in the sole in mice induced by egg white could be significantly inhibited with the treatment of the HH, FEM, and baicalin + FEM, compared with the model group ($P < 0.05$). Especially at 1.5 h, they had extremely significant inhibition ($P < 0.01$). Compared with the HH group, there was no significant difference in the FEM group at 0.5, 1, and 1.5 h ($P > 0.05$), and there was a significant difference in the baicalin + FEM group between 1 and 1.5 h ($P < 0.05$). And compared with the baicalin + FEM group, the FEM group had significant differences between 1 and 1.5 h ($P < 0.05$). This study indicates that the FEM was equal to HH, while the anti-inflammatory effect of the baicalin + FEM was more pronounced.

3.10. Clotting time study in mice

The results (Fig. 8A) showed that the coagulation time measured with capillary was (2.79 ± 1.78) min, (1.80 ± 1.34) min ($P < 0.05$), (1.46 ± 0.31) min ($P < 0.01$), (0.87 ± 0.25) min ($P < 0.01$), (1.30 ± 0.54) min ($P < 0.01$) and (0.88 ± 0.17) min ($P < 0.01$) in different groups. The BGZX, HH, FEM, and baicalin + FEM had extremely significant coagulation ($P < 0.01$). And there were no significant differences between the FEM and the baicalin + FEM compared with the HH on blood coagulation

Fig. 6. Analgesic effect in mice. $^*P < 0.05$, $^{**}P < 0.01$ vs model group; $^{\triangle\triangle}P < 0.01$ vs FEM + baicalin group. Data are presented as the mean ± standard error (n = 10).
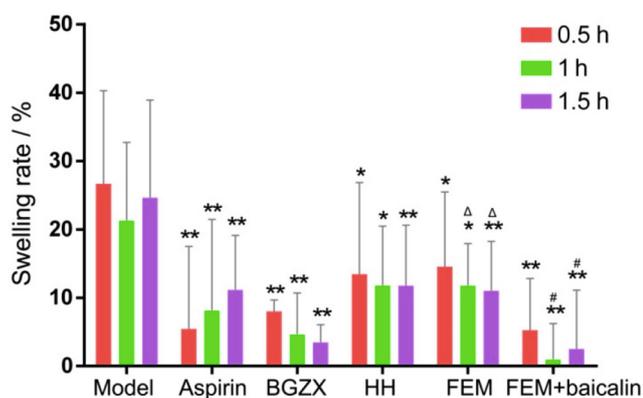
Fig. 7. Anti-inflammatory effects in mice. $^*P < 0.05$, $^{**}P < 0.01$ vs model group; $^{\#}P < 0.05$ vs HH group; $^{\triangle}P < 0.05$ vs FEM + baicalin group. Data are presented as the mean ± standard error (n = 10).

time ($P > 0.05$). And no significant difference between the FEM and the baicalin + FEM ($P > 0.05$). This study indicates that the FEM and the baicalin + FEM were equal to HH, and the coagulation effect of the baicalin + FEM was equal to the FEM.

3.11. Bleeding time study in mice

The results (Fig. 8B) showed that the bleeding time measured with tail breaking method was (5.88 ± 4.39) min, (3.67 ± 1.30) min ($P < 0.05$)、(3.22 ± 1.63) min ($P < 0.05$), (2.77 ± 1.37) min ($P < 0.01$), (3.46 ± 1.81) min ($P < 0.05$) and (2.91 ± 1.33) min ($P < 0.01$) in different groups. The HH, FEM, and baicalin + FEM remarkably shortened blooding time. And there were no significant differences between the FEM and the baicalin + FEM compared with the HH on blooding time ($P > 0.05$). And no significant difference between the FEM and the baicalin + FEM ($P > 0.05$). This study indicated that the FEM, baicalin + FEM, and HH all had obvious hemostasis effects, and the FEM and the baicalin + FEM were equal to HH, while the hemostasis effect of the baicalin + FEM was better than the FEM.

3.12. Abortion uterus test

3.12.1. Effects on abortion uterine bleeding and uterine weight in rats

Compared with the model group, the experimental groups reduced uterine bleeding quantity in drug abortion model rats

and decreased the uterine weight, but there was no statistical significance (Fig. 9).

3.12.2. Effects on contents of Ca, NO and ET-1 in aborted uterine tissue of rats

The calcium content of uterine tissue in experimental groups was higher than that in the model group (Fig. 10A). And only the baicalin + FEM group was statistically significant ($P < 0.01$). Compared with the model group, the HH, FEM, and baicalin + FEM groups could reduce the content of NO in the uterus homogenate (Fig. 10B) and increase the content of ET-1 (Fig. 10C), but there was no statistical significance ($P > 0.05$).

3.12.3. Effects on levels of E_2 and P in serum of aborted rats

Compared with the model group, the experimental groups could reduce the content of E_2 (Fig. 11A) and significantly reduce the content of PROG ($P < 0.01$) (Fig. 11B). And no significant difference between HH, FEM, and baicalin + FEM groups ($P > 0.05$).

4. Discussion

DUB, a traditional Chinese medicine disease called "benglou", refers to menstrual diseases. The main manifestation is the sudden heavy bleeding or continuous bleeding from the vagina during non-menstrual periods. Hemostasis is necessary to treat this disease. The HH is made from the dried roots of *S. baicalensis* and the dried flowers and buds of *S. japonica*. *S. baicalensis* has the effect of clearing heat and stopping bleeding, and *S. japonica* has the function of stopping bleeding. "Jiyin Gangmu" (Wu, 2006) records that they can be combined to achieve the effect of purging fire and detoxifying, cooling blood, and stopping bleeding. It is used clinically to treat DUB and has a good curative effect, but the mechanism of action is still unclear.

In this study, network pharmacology was used to predict the mechanism of action of HH in the treatment of DUB. The core nodes were screened by constructing a component-target-pathway network. The results showed that HH mainly exerted its effects on the targets of PTGS2, AKT1, TP53, and TNF through the six components of accepting, wogonin, baicalein, beta-sitosterol, kaempferol, and quercetin. The results of molecular docking showed that AKT1 and PTGS2 have lower docking binding energy, indicating a better binding ability with HH. Among them, the binding energy of TP53 and TNF is far less than −5.0 kJ/mol, and also have strong binding activity, indicating that HH can stably bind to the DUB receptor protein and play a significant role. Studies have found that PTGS2 is one of the key enzymes that cause an inflammatory response and can promote the inflammatory response. When cells are affected by stimulating factors such as growth factors, cytokines, and inflammatory transmitters, the concentration of prostaglandin E_2 (PGE$_2$) increases, causing contraction of uterine smooth muscle and increasing uterine smooth muscle tension and contraction frequency (Yang, 2009; Li et al., 2020). Therefore, the mechanism of HH treatment for DUB may be related to the regulation of PTGS2 expression. The regulation of PTGS2 is specifically manifested in regulating platelet aggregation and increasing uterine contractility, which may play an important role in the treatment of uterine bleeding. PTGS2, as a key enzyme for prostaglandin synthesis, can catalyze the synthesis of various prostaglandins by arachidonic acid, which acts on the hypothalamus and regulates the secretion of gonadotropin-releasing hormones, leading to the occurrence of uterine bleeding (Li et al., 2020). The mechanism of HH's hemostatic effect may be related to inhibiting the expression of PTGS2, reducing the synthesis of prostaglandins, and then reducing the secretion of single estrogen to prevent bleeding. At the same time, researchers have
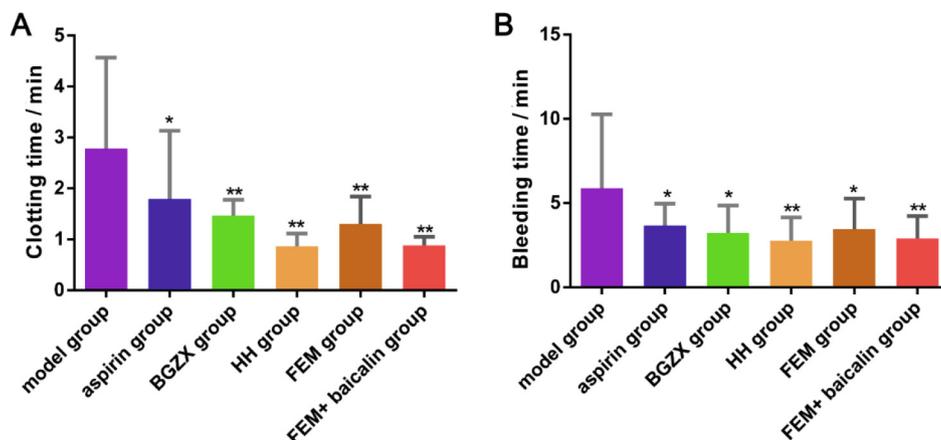
Fig. 8. Clotting time (A) and bleeding time (B) study in mice. *$P < 0.05$, **$P < 0.01$ *vs* model group. Data are presented as the mean ± standard error ($n = 10$).
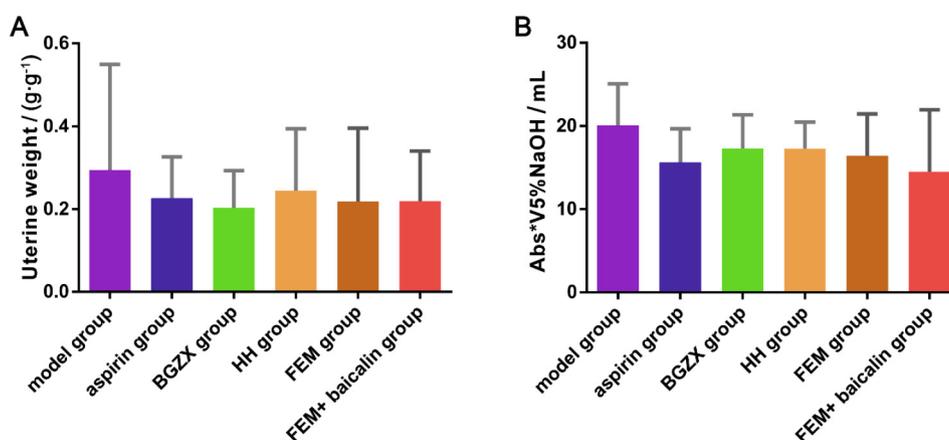

Fig. 9. Effects on uterine weight (A) and abortion uterine bleeding (B) in rats. Data are presented as the mean ± standard error ($n = 10$).
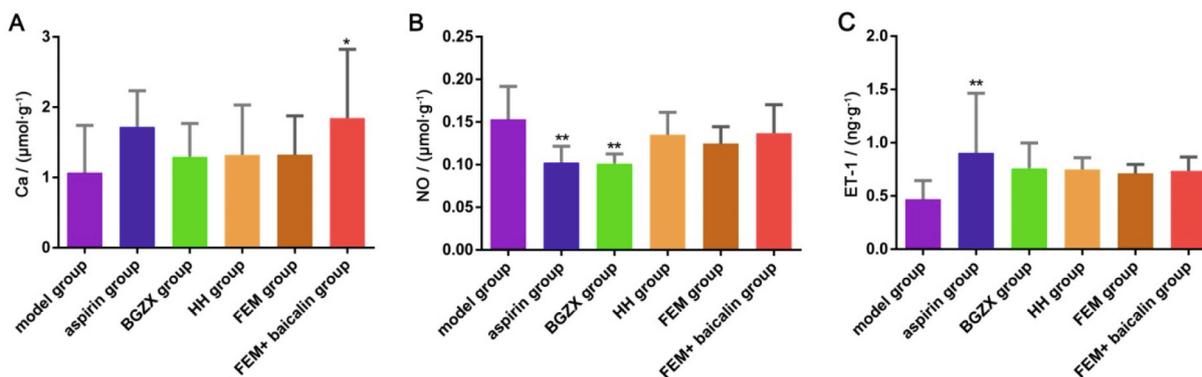

Fig. 10. Effects on contents of Ca (A), NO (B) and ET-1 (C) in aborted uterine tissue of rats. *$P < 0.05$, **$P < 0.01$ *vs* model group. Data are presented as the mean ± standard error ($n = 10$).

shown that (Nie et al., 2019) when oxytocin is used to treat postpartum hemorrhage, the TNF-α index of patients in the observation group is higher than that of the control group, and oxytocin can effectively promote intrauterine smooth muscle contraction and reduce the amount of postpartum hemorrhage. This suggests that TNF may play an important role in the treatment of uterine bleeding by HH. Studies have found that (Yu et al., 2017) AKT1 expression is increased in gastric cancer, and abnormal expression of AKT1 is also found in breast cancer, which plays an important role in the metastatic process of breast cancer. At present, a large

number of studies have confirmed that TP53 is closely related to endometrial cancer, but the mechanism of AKT1 and TP53 in uterine bleeding is unclear.

The GO functional enrichment analysis results showed that HH in the treatment of bleeding-related diseases may mainly be regulated by the response to an organic substance, response to oxygen-containing compound, cellular response to chemical stimulus, cellular response to organic substance, and response to the chemical. KEGG pathway enrichment analysis results found that most of the pathways were enriched for AKT1 and TP53 genes, and there was a
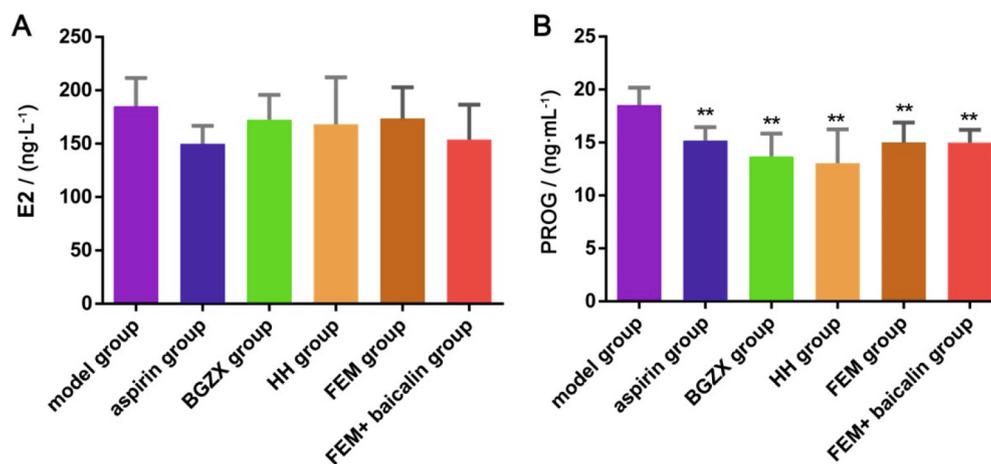
Fig. 11. Effects on levels of E_2 (A) and PROG (B) in serum of aborted rats. $^*P < 0.05$, $^{**}P < 0.01$ vs model group. Data are presented as the mean ± standard error ($n = 10$).

strong correlation between these pathways. This indicates that one gene may be involved in multiple pathways of bleeding-related diseases, and the treatment of DUB by HH may be achieved through the combined action of multiple pathways. Therefore, it is speculated that the active ingredients of HH may affect the signaling pathway by acting on the above-mentioned targets, thereby achieving the purpose of treating diseases.

The research group used the fibrinogen plate method to perform *in vitro* coagulation activity measurement (Li et al., 2017). The results showed that baicalin in HH has strong *in vitro* coagulation activity. The effective components were screened and it was found to be related to five kinds of ingredients, such as acacetin, wogonin, baicalein, kaempferol, and quercetin, which did not include baicalin. To investigate whether baicalin has hemostatic activity in the body, we designed two groups of trials compared the efficacy of FEM and baicalin + FEM. The results showed that the baicalin + FEM group was superior to the FEM group in anti-inflammatory, controlling bleeding time and increasing calcium content in the uterus of aborted rats. In terms of controlling coagulation time, uterine bleeding volume, uterine coefficient, decrease of NO content in uterine homogenate tissue, increase of ET-1 content, decrease of E_2 and PROG content in uterine serum in aborted rats, the efficacy was equal. In terms of analgesia, the FEM group has a significant analgesic effect, but the baicalin + FEM group has not, probably because baicalin will reduce the analgesic effect, and this finding can provide new directions for subsequent research. Also, compared with the original prescription (HH), a mixture of six ingredients such as acacetin, baicalin, wogonin, baicalein, kaempferol, and quercetin is equivalent to a new prescription (baicalin + FEM prescription) and the mixture of five ingredients such as acacetin, wogonin, baicalein, kaempferol, and quercetin is equivalent to a new prescription (FEM prescription). In the baicalin + FEM prescription, the content of baicalin is as high as 65%, while compared to a prescription without baicalin (FEM prescription), the efficacy between them is almost the same. This finding conflicts with the current research of traditional Chinese medicine, which mostly uses the main components of the drug as indicators of efficacy, and provides new research ideas for the development of traditional Chinese medicine.

Moreover, "Medicine spontaneous abortion uterus of Rats" is a key method for the *in vivo* test which mimics DUB. However, in such a crucial model, all test substances, including HH, did not exert a significantly hemostatic effect. The reason causing this result may be that the rats had to take cotton balls twice a day after the miscarriage of misoprostol and mifepristone drugs, so they may be excessively painful, which resulted in a large number of

rats dying after five days in each group. Hence, in such a crucial model, all test substances, including HH, did not exert a significantly hemostatic effect. Therefore, the cause of death can be explored in later studies, and research on its hemostatic effect will continue in the future.

5. Conclusion

In summary, this study uses network pharmacology and molecular docking to predict the drug-target-disease interaction and multi-target mechanism of HH in DUB treatment and validates its efficacy based on this. The results show that the use of network pharmacology predicting the mechanism of disease action has certain reliability, and the bottleneck of TCM research needs to be broken. Therefore, it provides new indications for further mechanism research of HH on DUB and the development of HH or its components as an alternative therapy for patients with DUB. At the same time, the application of network pharmacology strategies may provide a powerful tool for exploring the mechanism of traditional Chinese medicine and discovering new biologically active ingredients.

Author contributions

Y. X. Yao carried out the laboratory experiments. T. Liu designed the project and edited the manuscript. B. W. Liu and H. Yang performed data analysis. S. Y. Li designed the experiment. L. L. Zhao and Y. L. Xu wrote the manuscript.

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.

Acknowledgment

References

Abdellah, M. S., & Elsaman, A. M. M. M. (2012). Trichloroacetic acid for the treatment of dysfunctional uterine bleeding: A pilot prospective clinical trial. *European Journal of Obstetrics & Gynecology and Reproductive Biology, 165*(2), 280–283. https://doi.org/10.1016/j.ejogrb.2012.07.003.

Bi, R., Sun, R., Li, D., & Liu, X. (2014). Determination of β-sitosterol in *Gastrodia elata* by HPLC from different origin. *Journal of West China Forestry Science, 43*(6), 116–119.

Bongers, M. Y., Mol, B. W. J., & Brölmann, H. A. M. (2004). Current treatment of dysfunctional uterine bleeding. *Maturitas, 47*(3), 159–174. https://doi.org/10.1016/j.maturitas.2003.08.002.

Chen, Y. (2019). Determination of baicalin in Shufeng Qingre Mixture by HPLC. *Liaoning Journal of Traditional Chinese Medicine, 46*(7), 1483–1485.

Chinese Pharmacopoeia Commission (2015). *Pharmacopoeia of the People's Republic of China* Ⅰ (pp. 292–293). Beijing: China Medical Science Press.

Feitoza, S. S., Gebhart, J. B., Gostout, B. S., Wilson, T. O., & Cliby, W. A. (2003). Efficacy of thermal balloon ablation in patients with abnormal uterine bleeding. *American Journal of Obstetrics and Gynecology, 189*(2), 453–457. https://doi.org/10.1067/S0002-9378(03)00403-4.

Gurtcheff, S. E., & Sharp, H. T. (2003). Complications associated with global endometrial ablation: the utility of the mAUDE database. *Obstetrics & Gynecology, 102*(6), 1278–1282. https://doi.org/10.1097/00006250-200312000-00012.

He, K., Li, J., & Wu, X. (2019). The Efficacy of Gongxuening Capsule in the treatment of dysfunctional uterine bleeding: A Meta-analysis. *China Journal of Pharmaceutical Economics, 14*(11), 42–48.

Hopkins, A. L. (2007). Network pharmacology. *Nat Biotechnol, 25*(10), 1110–1111. https://doi.org/10.1038/nbt1007-1110.

Jiang, Y., Liu, N., Zhu, S., Hu, X., Chang, D., & Liu, J. (2019). Elucidation of the mechanisms and molecular targets of Yiqi Shexue Formula for treatment of primary immune thrombocytopenia based on network pharmacology. *Frontiers in Pharmacology, 10*, 1136.

Keene, F. E., & Payne, F. L. (2010). Dysfunctional uterine bleeding. *Journal of the American Association of Nurse Practitioners, 5*(4), 159–165.

Li, E., Liu, Y., Liu, Y., Jiang, S., & Wang, Y. (2018). Simultaneous determination of six components in *Scutellaria baicalensis* Georgi from different districts by HPLC. *Chinese Journal of Hospital Pharmacy, 38*(9), 946–948.

Li, J., Wu, J., Jia, S., Liu, S., Liu, X., Ni, M., et al. (2020). Mechanism of Motherwort Injection for hemostasis and regulating menstruation based on network pharmacology. *Evaluation and Analysis of Drug-Use in Hospitals of China, 20*(1), 1–6.

Li, P. C., Liu, T., Wu, Y., Zhang, Q., Fu, C. M., & Liu, C. (2017). Optimization of extraction technology from compound Huanghuai based on coagulation activity and central composite design-response surface methodology. *China Journal of Chinese Materia Medica, 42*(2), 290–297.

Liu, Y., Liu, H., Wang, J., Gou, L., Wang, Z., Fu, S., et al. (2015). Investigation of the contents of main flavonoid aglycones in *Flos Sophorae* Immaturus. *Journal of Hebei Normal University (Natural Science), 39*(3), 252–256.

Nie, S., Zhang, R., & Wang, Y. (2019). Effect of Leonurus biochemical mixture treatment on hemostatic effect, hemorheology, and inflammatory reaction of

patients with postpartum hemorrhage. *Maternal and Child Health Care of China, 34*(22), 5106–5109.

Niu, B., Zhang, H., Li, C., Yan, F., Song, Y., Hai, G., et al. (2019). Network pharmacology study on the active components of *Pterocypsela elata* and the mechanism of their effect against cerebral ischemia. *Drug Design, Development and Therapy, 13*, 3009–3019.

Qin, T., Wu, L., Hua, Q., Song, Z., Pan, Y., & Liu, T. (2020). Prediction of the mechanisms of action of Shenkang in chronic kidney disease: A network pharmacology study and experimental validation. *Journal of Ethnopharmacology, 246*, 112128. https://doi.org/10.1016/j.jep.2019.112128.

Qu, Y., Zhang, Z., Lu, Y., Zheng, D., & Wei, Y. (2019). Network pharmacology reveals the molecular mechanism of Cuyuxunxi Prescription in promoting wound healing in patients with anal fistula. *Evidence-based Complementary and Alternative Medicine: eCAM, 2019*, 3865121.

Tam, T. (2018). Abnormal uterine bleeding. *Ambulatory Gynecology*, 59–70.

Tang, Q., Yang, S., Tong, J., Li, X. u., Wu, J., Wang, S., Zhang, J., Chen, Y. i., & Xu, X. (2018). Hemostasis and uterine contraction promoting effect of the extract from drugs in the Zi-Yin-Tiao-Jing granule, a traditional Chinese compound preparation. *Journal of Ethnopharmacology, 211*, 278–284. https://doi.org/10.1016/j.jep.2017.09.035.

Wei, S., Zhou, X., Niu, M., Zhang, H., Liu, X., Wang, R., Li, P., Li, H., Cai, H., & Zhao, Y. (2019). Network pharmacology exploration reveals the bioactive compounds and molecular mechanisms of Li-Ru-Kang against hyperplasia of mammary gland. *Molecular Genetics and Genomics, 294*(5), 1159–1171. https://doi.org/10.1007/s00438-019-01569-5.

Wu, J., Lin, M., & Liu, X. (2018). Research on the functional mechanism of "Baicalin-Gardenia" based on network pharmacology. *Evaluation and Analysis of Drug-Use in Hospitals of China, 18*(1). 1–6+11.

Wu, Z. (2006). *Ji Yin Gangmu*. Beijing: People's Medical Publishing House.

Yang, Y. (2009). Effect of COX inhibitors on myometrial movement *in vitro*. *Chongqing Medicine, 38*(16). 2011–2012, 2015.

Yao, Y., He, Z., Liu, X., He, Y., Lei, Y., Zhang, S., et al. (2020). Potential material basis of Kangbingdu Keli for the treatment of coronavirus pneumonia 2019 (COVID-19) based on network pharmacology and molecular docking technology. *Chinese Traditional and Herbal Drugs, 51*(6), 1–11.

Yu, J., Chen, H., Zhang, P., & Wu, D. (2017). Expressions and significance of mi R-183 and Akt1 in endometrioid carcinoma. *Progress of Anatomical Sciences, 23*(1), 50–53.

Zhou, G., Cai, Y., Li, S., Jian, Q., Feng, S., Qing, M., et al. (2019). Establishment and comparison of HTLCS and HPLC for detecting the luteolin, apigenin and acacetin in Chuju chrysanthemum. *Food & Machinery, 35*(4), 54–59.

Zong, Y., Ding, M., Jia, K., Ma, S., & Ju, W. (2020). Exploring the active compounds of Da-Yuan-Yin in treatment of novel coronavirus (2019-nCoV) pneumonia based on network pharmacology and molecular docking method. *Chinese Traditional and Herbal Drugs, 51*(3), 1–9.