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The Mechanism of Action of Huai-Hua Powder in the Treatment of Anal Fissures: A Study Based on Network Pharmacology and Molecular Docking Techniques

Yang Liu[1], Yaping Cui[2], Yumiao Zhang[1], Guangwei Shi[3], Yalei Pan[4,*]

[1]Department of Traditional Chinese Medicine, Shaanxi University of Chinese Medicine, Xianyang, Shaanxi, 712046, China.
[2]Affiliated Hospital of Shaanxi University of Traditional Chinese Medicine, Xianyang, Shaanxi, 712000, China.
[3]Jiuquan Hospital of Traditional Chinese Medicine, Jiuquan, Gansu, 735000, China.
[4]State Key Laboratory of Research & Development of Characteristic Qin Medicine Resources (Cultivation), Co-construction Collaborative Innovation Center for Chinese Medicine Resources Industrialization by Shaanxi & Education Ministry, Shaanxi University of Chinese Medicine, Xianyang, Shaanxi, 712083, China.

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***Corresponding author:** Yalei Pan, State Key Laboratory of Research & Development of Characteristic Qin Medicine Resources (Cultivation), Co-construction Collaborative Innovation Center for Chinese Medicine Resources Industrialization by Shaanxi & Education Ministry, Shaanxi University of Chinese Medicine, Xianyang, Shaanxi, 712083, China.
Email: panyalei588@163.com

Abstract

Objective: To analyze the mechanism of action of Huai Hua Powder in the treatment of anal fissure (AF) based on network pharmacology and molecular docking techniques. **Methods:** The effective chemical components and action targets of Huaihua Powder were searched by Traditional Chinese Medicine Database and Analysis Platform and Bioinformatics Analysis Tool for Molecular Mechanism of Traditional Chinese Medicine platform as well as the relevant literature on China Knowledge Network during 2009-2019. Targets of action were obtained from Pharmmapper and compound-target networks were constructed using Cytoscape 3.7.2 software. GeneCards and OMIM databases were used to search for AF-related genes, and the String online website was used to construct the protein-protein interaction network. Gene ontology function enrichment analysis (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis by DAVID database. AutoDock Vina was used for major molecular docking of compounds to key proteins was performed by Pymol and AutoDock Vinasoftware. **Results:** A total of 63 active ingredients, 25 intersecting targets between Huai Hua Powder and AF were obtained, KEGG pathway enrichment analysis yielded 37 entries, mainly including PI3K-AKT, VEGF, MAPK and other signaling pathways. The molecular docking results showed that 10 major compounds, including lonicerin, hinokiflavone and narirutin, showed good binding to nine key targets, including mitogen-activated protein kinase 1, epidermal factor growth receptor and endothelial-type nitric oxide synthase. **Conclusion:** Through network pharmacology and molecular docking found that Huaihua Powder can act on VEGF receptor, NOS3 and other targets through PI3K-AKT, VEGF, MAPK and other signaling pathways to improve local blood circulation, counteract inflammatory response, analgesia, and thus play a therapeutic effect role in AF.

Keywords

Huai-Hua Powder, anal fissure, network pharmacology, molecular docking, action mechanism

As a common anorectal disease with an incidence of about 1.02% in China [1], anal fissures, which are called fissured haemorrhoids in Chinese medicine, are caused by the internal presence of fire and heat that injures yin or by a deficiency of yin and fluid, and are treated by clearing heat and dampness, reducing swelling and pain, and activating blood circulation to remove blood stasis [2]. Huai-Hua Powder was created by Xu Shuwei in the Southern Song Dynasty and included in the "Catalogue of Ancient Classical Recipes (First Batch)". Composed of four herbs, Sophora Japonica L, Platycladi Cacumen, Schizonepetae spica and ructus aurantii, Huai-Hua Powder is effective in clearing the intestines and stopping bleeding, draining wind and lowering Qi, mainly for the treatment of zang-viscera toxin, intestinal wind, blood in the stool from haemorrhoids with bright red or purple colour and other signs of retention of damp-heat in the interior [3]. Previous studies have found that Huai-Hua Powder has the ability to improve blood circulation in the intestines, reduce blood viscosity, anti-coagulation, invigorate the blood, anti-inflammatory, reduce mucosal edema and relieve pain [4]. In this study, network pharmacology and molecular docking methods were used to investigate the pharmacological basis and mechanism of action of Huai-Hua Powder in the treatment of anal fissures.

1. Materials and methods

1.1 Collection of active ingredients and predicted targets of Huai-Hua Powder

The active ingredients of Huai-Hua Powder were searched in the Systematic Pharmacology Analysis Platform for Traditional Chinese Medicine (https://tcmspw.com/tcmsp.php/, TCMSP) [5], using "Sophora Japonica L", "Platycladi Cacumen" "Schizonepetae spica" and "ructus aurantii" as search terms, and oral bioavailability OB≥30% and drug-like DL≥0.18 were used as screening criteria [6]. At the same time, the bioinformatics analysis tool of Chinese medicine molecular mechanism (http://bionet.ncpsb.org/batman-tcm/, BATMAN-TCM) were also used with the threshold values of "drug-target" similarity model Score cutoff≥20 and Adjust P-value≤0.05 [7] as the screening criteria to search the active ingredients of Huai-Hua Powder. Other active ingredients were supplemented through research literature available in China Knowledge Network (CNKI) within the period of 1 January 2009 to 31 December 2019 [8-13]. The active compound structure was derived using the PubChem (https://pubchem.ncbi.nlm.nih.gov/) database and uploaded that to Pharmmapper (http://www.lilab-ecust.cn/pharmmapper/) [14] to obtain the corresponding predicted target, setting Norm Fit ≥0.9. The predicted targets were converted to official names using Uniprot (https://www.uniprot.org/) [15]. Cytoscape 3.7.2 was used to construct the "drug-component-target" network diagram.

1.2 Acquisition and analysis of the targets of Huai-Hua Powder for the treatment of anal fissures

The keywords "Anal Fissure" were used to search for AF-related targets in GeneCards (https://www.genecards.org/) and OMIM (https://www.omim.org/). The predicted targets were compared with the AF disease targets to find the intersection, which is the target of the active ingredient of Huai-Hua Powder for the treatment of AF. The targets were uploaded to String (https://string-db.org/), and the species "Homo sapiens" was selected to construct the target interaction network.

1.3 Target pathway analysis and visualization

Gene ontolog (GO) biological process enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) metabolic pathway enrichment analysis were performed on key targets at DAVID (https://david.ncifcrf.gov/) [16], with P value <0.05 as the screening condition, in Microbiology Letter (http://www.bioinformatics.com.cn/, Bioinformatics) to form bubble maps.

1.4 Component-target molecular docking

Download crystal structures of key target proteins in RCSB (https://www.rcsb.org/) and core compound structure files in ZINC (http://zinc.docking.org/), process target proteins and core compounds with Pymol, AutoDockTools, apply AutoDock Vina for molecular docking and Pymol to visualise the results.

2. Results

2.1 Screening of active compounds and construction of "drug-component-target" network diagram

The compounds rutin, quercetin and naringin were added according to the literature. Combined with the PubChem results, 12 active compounds were retained from Sophora japonica, 24 from Platycladi Cacumen, 23 from ructus aurantii, and 12 from Schizonepetae spica, with a total of 63 after deletion of duplicates. The screened compounds corresponded to the predicted targets, 70 for Sophora japonica, 75 for Platycladi Cacumen, 88 for ructus aurantii, and 74 for Schizonepetae spica, and a total of 100 targets were obtained after deleting duplicate targets, resulting in a "drug-component-target network diagram" (Figure 1). The diagram contains 166 nodes and 1,310 edges. The circles at each node represent the drug, the octagon represents the active compound, the red in the diamond represents the common predicted target and the blue represents the other targets. Information on the compounds with the top 10 degree values in Figure 1 is shown in Table 1.

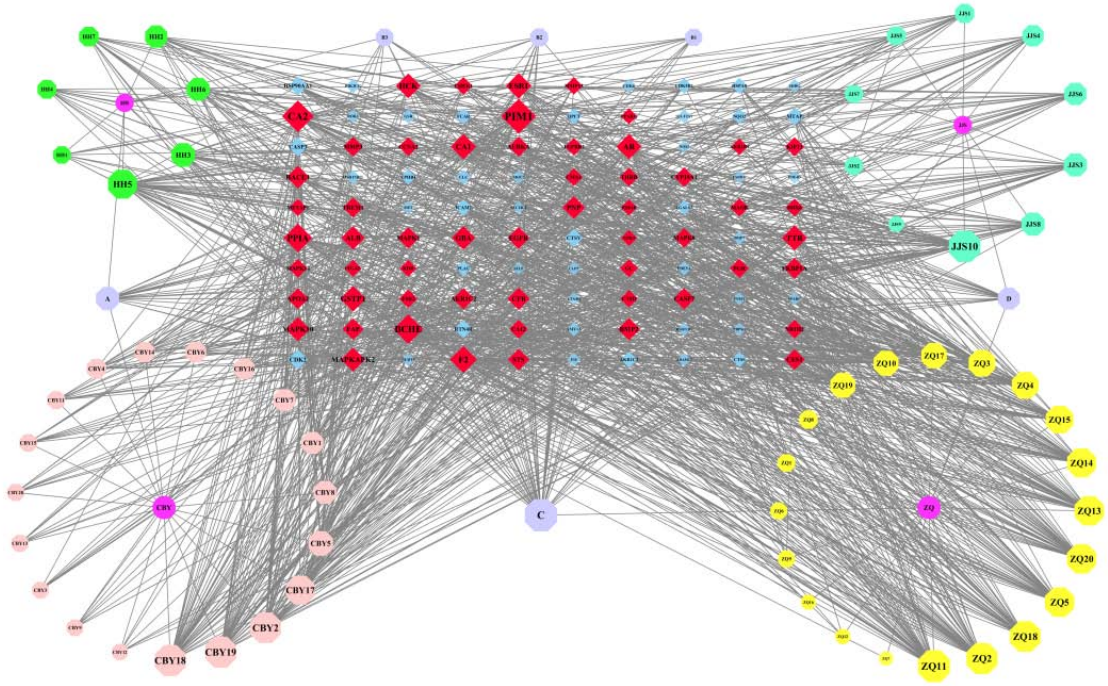


Figure 1. "Drug-component-target" network diagram.

Table 1. Table of basic information on the main active compounds

Identifier	Component	Chemical formula	PubChemCID	OB	DL
CBY2	DNOP	$C_{24}H_{38}O_4$	8346	40.59	0.4
CBY18	Quercitrin	$C_{21}H_{20}O_{11}$	5280459	4.04	0.74
CBY19	Hinokiflavone	$C_{30}H_{18}O_{10}$	5281627	2.51	0.61
ZQ2	Nobiletin	$C_{21}H_{22}O_8$	72344	61.67	0.52
ZQ5	Narirutin	$C_{27}H_{32}O_{14}$	442431	8.15	0.75
ZQ11	Didymin	$C_{28}H_{34}O_{14}$	16760075	38.55	0.24
ZQ18	Lonicerin	$C_{27}H_{30}O_{15}$	10461109	-	-
ZQ20	Marmin	$C_{19}H_{24}O_5$	6450230	38.23	0.31
JJS10	Hesperetin 7-O-glucoside	$C_{22}H_{24}O_{11}$	147394	7.69	0.82
C	Rutin	$C_{27}H_{30}O_{16}$	5280805	3.2	0.68

Notes: 1. CBY-Platycladi Cacumen, ZQ-ructus aurantii, and JJS-Schizonepetae spica; 2. C is a common ingredient of Sophora japonica and Platycladi Cacumen.

2.2 Targets and target interrelationships of Huai-Hua Powder for the treatment of anal fissure

AF-related disease targets were obtained from GeneCards and OMIM respectively, and there were 2,225 targets after deleting duplicates. After intersecting with 100 drug targets, a total of 25 targets of the active ingredient of Huai-Hua Powder for the treatment of AF were obtained. The target interactions are shown in Figure 2, with a total of 23 circular nodes and 106 edge lines. The top 9 core targets are: ALB, CASP3, EGFR, MAPK1, ESR1, MAPK8, KDR, AR, and NOS3.

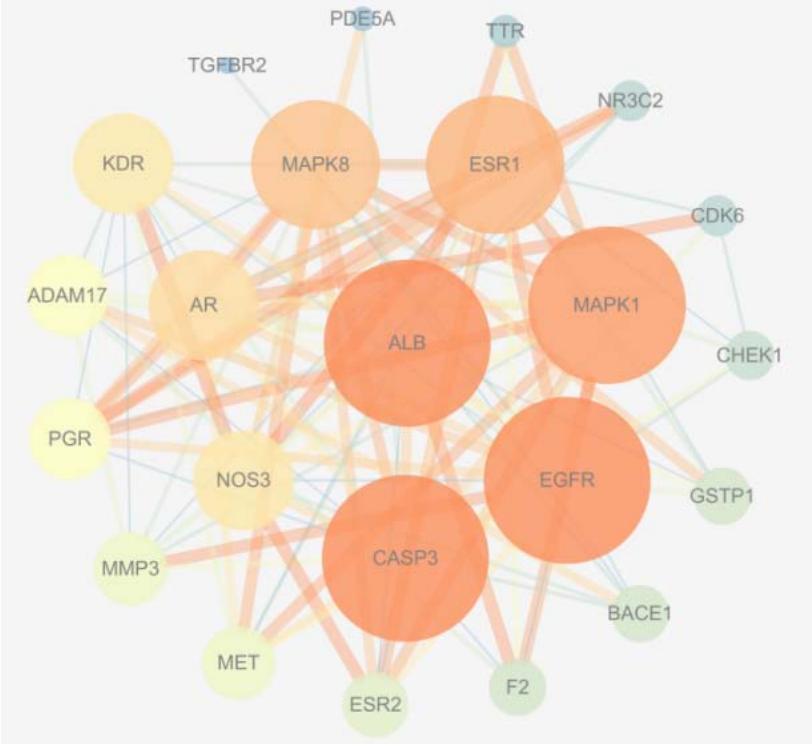


Figure 2. Target Interaction Network Diagram.

2.3 GO and KEGG pathway enrichment results and analysis

GO enrichment analysis showed that Huai-Hua Powder could modulate 92 pathways, of which 55 were biological processes, 13 were cellular components and 24 were molecular functions, the main pathways are shown in Figure 3. 37 entries were enriched for KEGG pathways in Huai-Hua Powder treated AF (Figure 4), the main pathways were PI3K-Akt, Estrogen, Ras, MAPK, Prolactin, TNF, FoxO, Rap1 and VEGF.

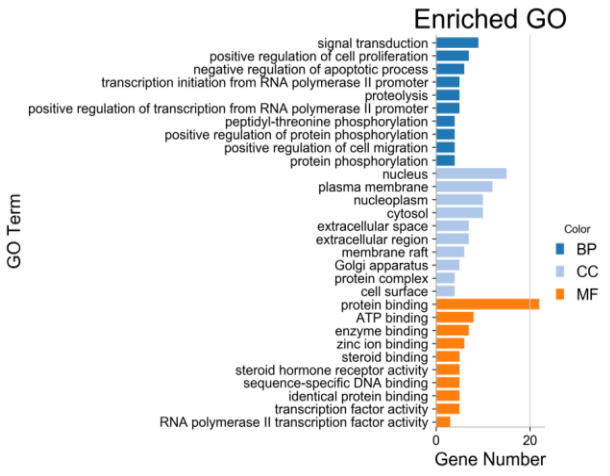


Figure 3. Core target GO enrichment analysis.

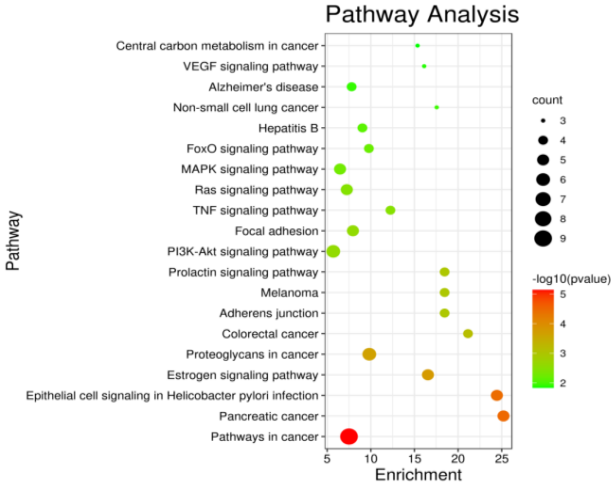


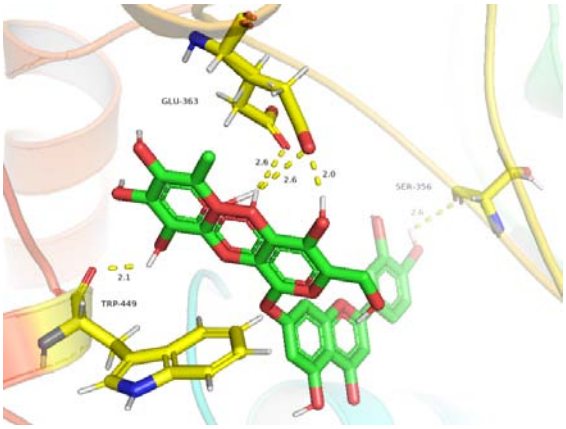
Figure 4. Core target KEGG pathway enrichment analysis.

2.4 Molecular docking

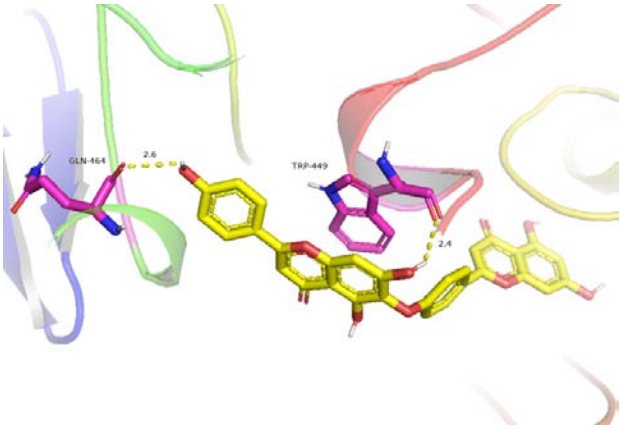
The 10 compounds in Table 1 and the 9 core targets in Figure 2 were selected for molecular docking and the binding energies of the compounds and targets and molecules after docking are shown in Table 2. The lower the free energy of binding for molecular docking, the higher the biological activity between receptor and ligand. The three results with the best binding energy values were taken for visualisation (Figure 5). The docking results show that the active compounds are biologically active, have high binding properties and can form stable structures with the proteins.

Table 2. Active ingredient binding capacity to target proteins

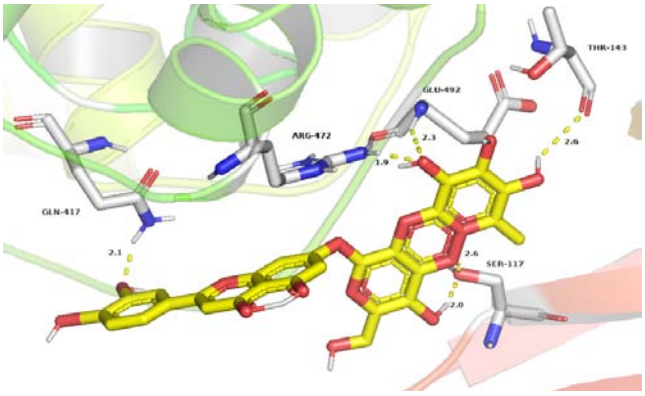
Active ingredients	Combining abilities(kcal/mol)									Average
	EGFR	ALB	CASP3	MAPK1	ESR1	MAPK8	AR	KDR	NOS3	
Didymin	-5.6	-7.5	-4.6	-3.6	-3.5	-7.7	-4.6	-6.4	-9.1	-5.84
Dnop	-3.6	-5.4	-3.7	-4.7	-3.8	-4.5	-2.8	-4.5	-6.8	-4.42
Hesperetin-7-O-glucoside	-5.4	-8.0	-5.7	-8.9	-1.1	-7.3	-4.8	-7.2	-10.2	-6.51
Hinokiflavone	-6.4	-10.9	-7.0	-10.8	-4.5	-7.8	-4.5	-8.9	-12.6	-8.16
Lonicerin	-7.9	-11.2	-9.5	-11.1	-8.4	-10.0	-7.7	-9.3	-13.5	-9.84
Nobiletin	-5.2	-7.0	-5.5	-7.1	-3.8	-7.1	-4.3	-6.2	-8.1	-6.03
Quercitrin	-5.9	-8.7	-6.8	-8.6	-4.0	-7.0	-4.2	-7.2	-10.2	-6.96
Rutin	-5.9	-8.2	-5.8	-9.2	-5.5	-7.7	-4.9	-7.4	-10.5	-7.23
Narirutin	-7.9	-8.6	-7.6	-9.6	-6.2	-8.2	-5.4	-8.3	-9.8	-7.96
Marmin	-7.1	-6.8	-5.1	-8.2	-5.9	-4.8	-4.9	-5.9	-8.1	-6.31



5a. NOS3 and Lonicerin.



5b. NOS3 and Hinokiflavone.



5c. ALB and Lonicerin.

Figure 5. Molecular docking pattern of key compounds to key target proteins.

3. Discussion

The main constituents of Huai-Hua Powder four drugs are flavonoid and coumarins, etc. Studies have shown that flavonoid have a wide antibacterial spectrum and are not easily resistant to drugs [17-18]. Coumarins have pharmacological effects such as anti-inflammatory and analgesic, antibacterial and antiviral [19]. The targets of action of the four drugs in Huai-Hua Powder are clustered and can synergize their effects. The results of GO and KEGG pathway enrichment analysis showed that Huai-Hua Powder can act on multiple biological pathways and regulate multiple signalling pathways to exert therapeutic effects on AF. It was found that Huai-Hua Powder and its components can regulate NO and vascular endothelial growth factor (VEGF) expression through the PI3K-AKT signalling pathway [20], and it can further activate the NOS3 target of the PI3K-AKT signalling pathway through EGFR2 to release NO, and also act on the ERK target to regulate the PGI2 production process and exert anti-inflammatory effects [21]. The active ingredients in t Huai-Hua Powder can also act on two of the MAPK signalling pathways, ERK and JNK, to regulate the inflammatory response and extracellular matrix metabolism [22]. Both the above experimental studies and the present study suggest that Huai-Hua Powder may alleviate the inflammatory response of anal fissures by inhibiting the MAPK signalling pathway. The molecular docking results showed that all 10 major compounds of Huai-Hua Powder showed excellent affinity to 9 key proteins, with stable hydrogen bonds present in each binding site and structurally stable, validating the results of this study.

In summary, the active compounds in Huai-Hua Powder flavonoid and coumarins act on targets such as EGFR, NOS3 and ALB through signalling pathways such as PI3K-Akt, VEGF and MAPK to improve local blood circulation, counteract inflammatory responses and analgesia, and play a therapeutic role in promoting wound healing. This study investigates the material basis and potential effect mechanism of the classical formula Huai-Hua Powder in the treatment of AF based on a network pharmacology approach, providing clues for further in-depth research on the treatment of AF with Sophora japonica.

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