Network Pharmacology-based Mechanistic Study on Prunellae Spica for the Treatment of Endometrial Cancer

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Abstract: Objective: To explore the potential mechanism of action of Prunellae Spica on endometrial cancer (EC) by network pharmacology and molecular docking. Method: TCSMP database was used to screen the Prunellae Spica ingredients and corresponding targets.Screen target genes for endometrial cancer from GeneCards, OMIM, and TTD databases,and the intersection targets were analyzed for functional enrichment and biological pathways. Perform molecular docking between the top 5 core targets and the active ingredients of Prunellae Spica. Results: Network pharmacological analysis showed that there were 83 key targets of Prunellae Spica in the treatment of EC.The results of the GO analysis showed that 544 biological processes (BP), 52 cell components (CC) and 104 molecular functions (MF) were included. Molecular docking was performed between the top 5 core targets and the active ingredients of Prunellae Spica, and the results showed that the active ingredients of Prunella vulgaris have good binding ability to the key targets. Conclusion: In summary, the mechanism of action of Prunellae Spica in the treatment of EC involves multiple targets and signaling pathways. It is speculated that TP53, AKT1, JUN, BCL2, MYC, TNF, and CASP3 are key targets of Prunellae Spica in the treatment of EC, but further experimental verification is still needed.

***Keywords*:** Prunellae Spica, endometrial cancer, network pharmacology, mechanism

1. Introduction

Endometrial cancer (EC) is among the three major malignant tumors of the female reproductive system, with annually increasing morbidity and mortality rates[1]. Most patients can recover from EC after early diagnosis and surgery but the prognosis for those with the advanced or recurrent disease is extremely poor. The five-year survival rate for patients with stage IV EC is a meager 15%[2]. The treatment options for advanced EC typically include chemoradiotherapy and effective measures and drugs are currently lacking. Chemoradiotherapy can lead to side effects including suppression of the bone marrow, gastrointestinal adverse reactions, sexual dysfunction[3], and hair loss, necessitating the identification and development of safer and more effective treatment methods. TCM shows obvious advantages with fewer adverse actions. It benefits the inhibition of tumor recurrence and metastasis and improves patients' quality of life[4]. Prunellae spica extract exhibits various biological activities including regulation of immune function, microenvironmental regulation of tumor metastasis, and antioxidant, antibacterial, anti-inflammatory, and antiviral properties[5]. Studies have shown that Prunellae spica is crucial in treating colon adenocarcinoma[6]and cancer of the uterus[7]. Although Prunellae spica's application is gradually expanding in clinical treatment and understanding of its properties is deepening through confirmatory experiments, the pharmacological mechanism of action is largely unclear.

Network pharmacology as a concept was first proposed in 2007 by Prof. Hopkins[8], who suggested that the pharmacological effects of medications may not be exerted in a point-wise manner but maybe within network interactions. A biological network of drug-component-target-disease can be constructed using network pharmacology to reveal the mechanism of drug action affecting disease from the perspective of multiple levels and systems. In this study, network pharmacology and molecular docking methods were used to investigate the effective components of Prunellae Spica in inhibiting endometrial cancer and the possible mechanism of action.

2. Materials and methods

2.1 Identifying Active Components of Prunellae Spica

Drug components can be downloaded from the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP, https://old.tcmsp-e.com/tcmsp.php).After typing in the search term, "Prunellae spica" in the TCMSP database, based on the distribution, absorption, excretion, and metabolism characteristic parameters of the

compounds, molecules with OB ≥ 30% and DL ≥ 0.18 were selected. Relevant information on selected components and corresponding targets was downloaded, and drug target names were converted to gene identifiers in the UniProt database (https://www.uniprot.org/). The corresponding chemical structures were downloaded and saved in MOL2 format.

2.2 Acquisition of EC Targets

We will use "endometrial carcinoma" as the search term, and search related genes in GeneCards (https://www.genecards.org/Guide/Sources), filtering criteria: relevance score > 10), TTD and OMIM (https://www.omim.org/) databases to obtain related target genes of endometrial carcinoma.

2.3 Venn Diagram

The intersection target genes of Prunellae spica and EC were obtained by using the online online Venn diagram drawing tool (https://bioinfogp.cnb.csic.es/tools/venny/).

2.4 Constructing the PPI Network

A PPI network was generated for the common targets between Prunellae spica and EC using the STRING database (https://string-db.org/). The network was imported into Cytoscape 3.8.2 for visualization and analysis to obtain core targets.

2.5 GO and KEGG Enrichment Pathway Analyses

Common target genes of Prunellae spica and EC were uploaded onto the DAVID database (https://david.ncifcrf.gov/summary.jsp) to acquire and download GO terms and KEGG pathways. The downloaded data were screened for the species “homo sapiens” with $P < 0.05$ and $FDR < 0.05$. The results were sorted in the ascending order of the P-values. The top 20 functional annotation terms in each GO branch, namely molecular function (MF), cellular component (CC), and biological process (BP), and the top 20 pathways from KEGG pathway enrichment analysis were visualized and analyzed.

2.6 Construct network diagram

Cytoscape software (v3.8.2) was used to establish the active ingredient-intersection target correlation network diagram to further predict the core relationships between targets and disease pathway networks.

2.7 Molecular Docking of Prunellae Spica with its Key Targets

The pathway diagram of drug active ingredient-intersection target network was constructed. Structural data on small molecules were downloaded from TCMSP and saved in Mol2 format after screening the active components. Related data were imported into AutodockTools-1.5.6 to add atomic charges and assign atom types when setting the flexible bond as rotatable. The results generated were saved in pdbqt format.

PDB (Protein Data Bank) (https://www.rcsb.org/) was used to retrieve the crystal structure of the target protein. Protein molecules after removing irrelevant small molecules using Pymol 2.5.0, were imported into AutoDockTools-1.5.6 to add hydrogen atoms, calculate charges, and assign atom types. According to the small molecule-protein interactions, the center, height, width, and length of the Grid Box were determined, and finally, batch molecular docking was performed using AutoDock, and visualized using Pymol 2.5.0. Calculations for molecular docking were performed using a Genetic Algorithm to analyze the interaction pattern of the compounds with the target proteins and with the active site residues. The results of docking suggestive of a strong binding affinity were imported into the Pymol software, to generate three-dimensional diagrams for visualization.

3. RESULTS

3.1 Retrieval of Active Components and Shared Target Genes

Through the TCMSP database, the search term "prunellae spica" was entered, and 11 main active ingredients of prunellae spica were screened out according to the screening conditions (OB≥ 30%, DL≥ 0.18) (Table1). These targets were converted into gene names through UniProt database, and 185 target genes were obtained. endometrial carcinoma is selected as the key word, and 1290 genes related to endometrial carcinoma are found in GeneCards (ilter condition: relevance score□10)), TTD and OMIM. The intersection of endometrial carcinoma is obtained through the online Venn diagram drawing tool. The obtained Prunellae Spica and endometrial cancer target overlapping genes, 83 intersection targets were found (Figure 1).

3.2 Construction of the PPI Network

The PPI network diagram of the common target off Prunellae spica and EC was constructed (Figure2A), and imported into Cytoscape3.8.2 for screening and visualization of the protein network (Figure2B). The larger the value of Degeree, the larger the icon, the greater the role in the network diagram, and the more likely it is the target gene that plays a key role,

TP53, AKT1, JUN, BCL2, MYC, TNF, CASP3 may be the key target (Figure 2B).

Table 1. eleven main active components of Prunellae spica

MOL ID	Melecule Name	MW	OB(%)	DL
MOL000358	beta-sitosterol	414.79	36.91	0.75
MOL000422	kaempferol	286.25	41.88	0.24
MOL004355	Spinasterol	412.77	42.98	0.76
MOL000449	Stigmasterol	412.77	43.83	0.76
MOL004798	delphinidin	303.26	40.63	0.28
MOL000006	luteolin	286.25	36.16	0.25
MOL006767	Vulgaxanthin-I	339.34	56.14	0.26
MOL006772	poriferasterol monoglucoside_qt	412.77	43.83	0.76
MOL006774	stigmast-7-enol	414.79	37.42	0.75
MOL000737	morin	302.25	46.23	0.27
MOL000098	quercetin	302.25	46.43	0.28

OB: Bioavailability DL: Drug-like properties

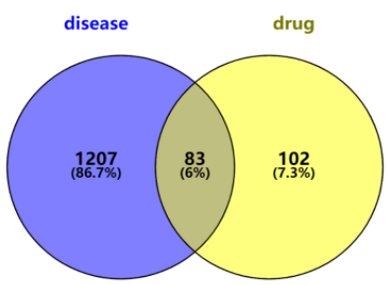

Figure 1. Wayne map of common target genes of Prunella and EC

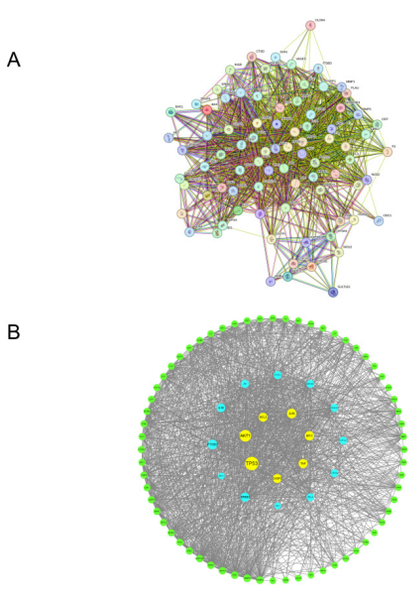

Figure 2. Analysis of 83 common potential targets of prunella and endometrial cancer. PPI network diagram (A) and PPI network diagram after visual analysis (B), where the yellow icon is the target with a higher Degree in the network diagram.

3.3 GO and KEGG Analyses of Prunellae Spica and EC targets

Eighty-three common targets were uploaded to the DAVID database for GO and KEGG pathway enrichment analyses. A total of 702 items were obtained by GO analysis, including 544 biological processes, including positive regulation of apoptosis process, positive regulation of gene expression, and negative regulation of apoptosis process. A total of 52 cell

composition items were obtained, including extracellular space, macromolecular complex, etc. A total of 106 molecular function items were obtained, including enzyme binding sites, protein binding, and cytokine activity. The first 20 GO analysis results are shown in Figure 3A.

A total of 137 pathways were selected after screening in the database. The gene targets of Prunellae spica for endometrial cancer were enriched in cancer pathway, prostate cancer pathway, apoptosis pathway and other pathways. The analysis of the top 20 KEGG channels is shown in Figure 3B.

3.4 Construction of Network Diagrams

By constructing the drug active ingredient-intersection target map (Figure 4), it can be seen that the active ingredients of Prunella can directly or indirectly act on multiple targets in endometrial cancer, among which MOL000422 (Kaempferol;), MOL000006 (Luteolin), MOL000098 (Quercetin), the active ingredients of drugs play an important role in treatment.

3.5 Molecular Docking

To analyze the feasibility of Prunellae spica for EC treatment, we conducted molecular docking of the active components of Prunellae spica together with the key EC targets. Molecular docking was conducted between the top 3 drug active components with degree values selected from the active component-intersection target diagram, namely MOL000006 (luteolin), MOL000098 (quercetin), and MOL000422 (kaempferol), and key targets with top five degree values in the PPI network diagram, including AKT1 (PDB ID: 4GV1), TP53 (PDB ID: 6RL3), BCL2 (PDB ID: 4LXD), JUN (PDB ID: 5J41), TNF (PDB ID: 2E7A), MYC (PDB ID: 6S9Z), and CASP3 (PDB ID: 1QX3). The binding energy was < 0 kcal·mol-1, indicating stable binding of the compound to the protein receptor since the lower binding energy represents the stronger binding affinity of the compound to the protein receptor. The results of molecular docking are shown in Table 2. Therefore, we found that quercetin, luteolin and kaempferol have good binding activity with key targets, indicating that Prunellae spica may have obvious anti-endometrial cancer effects.

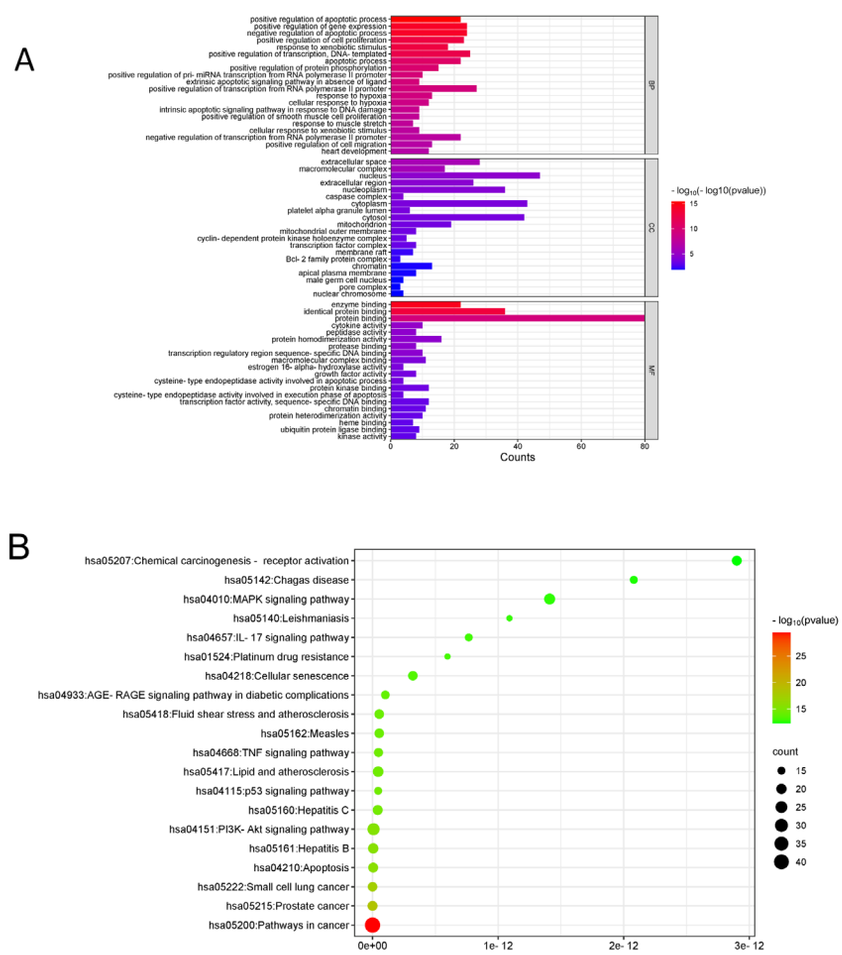

Figure 3. GO analysis (A) and KEGG functional enrichment analysis (B) of the common target of Prunella and EC. The bubble size represents the number of genes on the enrichment pathway, and the color represents the qualue value.

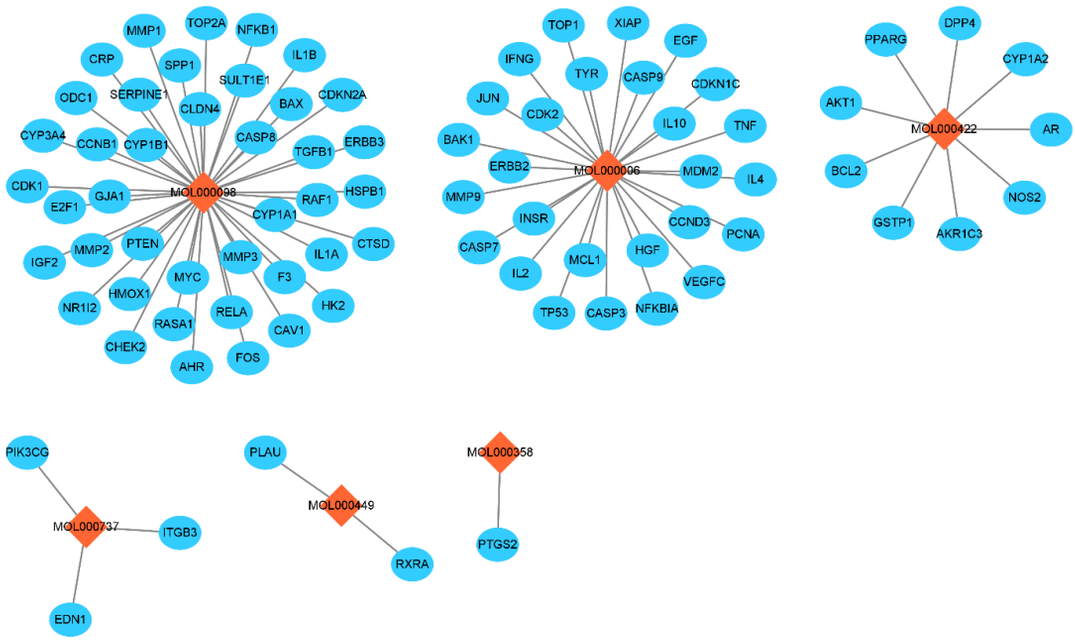

Figure 4. Drug active ingredients of Prunellae spica - intersection target network diagram

Table 2. The results of molecular docking

Ingredient	Target	Binding energy(kcal.mol-1)
MOL000098	TP53 (6RL3)	-6.15
MOL000006	TP53 (6RL3)	-5.11
MOL000422	TP53 (6RL3)	-5.14
MOL000098	AKT1(4GV1)	-5.08
MOL000006	AKT1(4GV1)	-5.52
MOL000422	AKT1(4GV1)	-4.77
MOL000098	JUN (5J41)	-4.76
MOL000006	JUN (5J41)	-5.13
MOL000422	JUN (5J41)	-5.69
MOL000098	BCL2(4LXD)	-4.76
MOL000006	BCL2(4LXD)	-5.02
MOL000422	BCL2(4LXD)	-5.27
MOL000098	TNF(2E7A)	-4.69
MOL000006	TNF(2E7A)	-5.91
MOL000422	TNF(2E7A)	-5.82
MOL000098	MYC(6S9Z)	-7.14
MOL000006	MYC(6S9Z)	-7.56
MOL000422	MYC(6S9Z)	-7.13
MOL000098	CASP3 (1QX3)	-4.53
MOL000006	CASP3 (1QX4)	-5.53
MOL000422	CASP3 (1QX5)	-4.72

4. Discussion

Globally, approximately 76,000 deaths due to EC are reported annually. EC is the second leading cause of cancer-related deaths among women in China. Surgery is the most effective therapeutic option for treating patients with early-stage EC, while those with advanced metastatic EC show a poor prognosis despite comprehensive treatments including aggressive

chemotherapy, surgery, and radiotherapy. TCM shows great potential in preventing and treating tumors and other diseases. The separation and extraction of active components from TCM is crucial for the development of new drugs[9]. Network pharmacology provides multifaceted perspectives on investigating the relationship between diseases and drugs.

In this study, we selected 83 common target genes and used STRING database for PPI analysis to preliminarily conclude that Prunellae spica plays a potential key role in the treatment of endometrial cancer. GO analysis showed that these 83 targets may be involved in the positive and negative regulation of apoptosis and the positive regulation of gene expression. In addition, KEGG analysis showed that the cross-gene between Prunellae spica and endometrial carcinoma has extensive anti-cancer effects, and there is a good enrichment in the cancer pathway. In addition, I constructed the PPI network map for 83 common target genes and analyzed the central hub of the network. Six key genes, namely TP53, AKT1, JUN, BCL2, MYC, TNF and CASP3, were obtained. After the molecular docking verification, the active ingredient of the drug has good binding energy with the key target, and it is speculated that the active ingredient of the drug may play an anticancer role by acting on the key target.

Several recent reports have confirmed that these three active components have many key biological functions, including anti-inflammatory, anti-tumor, and antioxidant activities. Some reports show that quercetin inhibits the migration and proliferation of EC cells, regulates the cell cycle, and induces apoptosis. Quercetin's anti-tumor effect is related to ferroptosis induction in EC cells[10]. Experimental evidence for luteolin efficacy in the treatment of EC is lacking but its role in the treatment of colorectal cancer in combination with the chemotherapy drug, cisplatin, has been documented. It acts by inducing mitochondrial dysfunction and apoptosis [11]. Luteolin can inhibit the migration and proliferation of HCC cells, increase G2/M phase ratio, and induce apoptosis. It plays an anti-HCC role through AKT- or MAPK-JNK signaling-mediated ESR1 action [12]. Luteolin is key in fighting against various cancers such as multiple myeloma and ovarian cancer. Kaempferol, a key active component, exerts no significant toxicity in human monocytes and normal cell lines at non-cytotoxic concentrations. It is a novel therapeutic candidate for EC which acts through HSD17B1-related estrogen metabolism pathways[13]. Lei et al. showed that kaempferol exerted prominent anti-cancer effects by activating mitochondria-mediated apoptotic pathways and inducing cell cycle arrest at the G2/M phase. It inhibits EC through apoptosis induction, cell cycle arrest, and inhibition of the mTOR/PI3K/Akt signaling pathway[14].

In summary, the mechanism of Prunellae spica's treatment action of EC was initially explored using network pharmacology and molecular docking analyses. Luteolin, kaempferol, and quercetin may be the core active components of Prunellae spica for EC treatment. The active components of Prunellae spica may produce therapeutic effects on EC by inhibiting gene targets including AKT1, TP53, BCL2, JUN, TNF, Myc, and CASP3.

5. Conclusion

In this study, we validated the mechanism of Prunellae spica related to EC by network pharmacology, and identified multiple active drug-acting components of Prunellae spica, which were further validated by molecular docking. Therefore, our results suggest a possible underlying mechanism of Prunellae spica for the treatment of endometrial cancer. However, further experimental validation is still needed.

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List of abbreviations

Endometrial cancer = EC
Herapeutic Target Database = TTD
Online Mendelian Inheritance in Man = OMIM
Protein-protein interaction = PPI
Gene Ontology = GO
Kyoto Encyclopedia of Genes and Genomes = KEGG
Traditional Chinese medicine = TCM
Hepatocellular carcinoma = HCC
Molecular weights = MW
Drug-likeness = DL
Oral bioavailability = OB
Half-life = HL
Molecular function = MF
Cellular component = CC
Biological process = BP
Protein Data Bank = PDB

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