A network pharmacology approach to explore the pharmacological mechanism of *Epimedium brevicornum* in sexual dysfunction

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Abstract: Network pharmacology is a research method for designing multi-target drug molecules based on the theory of systems biology and network analysis of biological systems by selecting specific signal nodes to show the multi-component and multi-target attributes of traditional Chinese medicine (TCM). In the present study, we predicted the mechanism of *Epimedium brevicornum* (EB) in the treatment of sexual dysfunction (SD) using network pharmacology. Moreover, chemical constituents of EB were queried using the Traditional Chinese Medicine Systems Pharmacology (TCMSP) server, and the targets related to SD were obtained using Genecards and Online Mendelian Inheritance in Man (OMIM). An intersection of genes was discovered. Subsequently, protein-protein interaction (PPI) networks were constructed with Cytoscape 3.7.1 software and the STRING database. Additionally, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were performed using WebGestalt. A total of 21 active drug ingredients were identified in the TCMSP database. Besides, 1003 disease targets from the GeneCards and OMIM databases were screened. Furthermore, 67 common targets for drugs and diseases were obtained using the Venny online tools. The results of this study preliminarily verified the basic pharmacological activity of EB in the treatment of SD, laying a foundation for elucidating its mechanism of action.

Keywords: *Epimedium brevicornum*; Network pharmacology; Sexual dysfunctions; Component-target

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1. Introduction

Traditional Chinese medicine (TCM) has been widely used in China for thousands of years. TCM has multiple targets and multi-channels and is multi-functional in the treatment of diseases at the molecular level, which also has its own benefits and characteristics, such as safety, efficiency, time-saving, and inexpensiveness[1–3]. However, the underlying molecular mechanisms of TCM remain unclear. Therefore, it is important to explore a new method and strategy to systematically and comprehensively clarify the mechanism of TCM. *Epimedium brevicornum* (EB) is a representative TCM that displays antiosteoporosis and kidney-toning activities and has been widely used for the treatment of bone diseases and gonadal dysfunction[4–10]. Currently, this herb is commonly used alone or in conjunction with other herbs in traditional herbal drug prescriptions to prevent and treat osteoporosis, depression, sexual dysfunction (SD), and cardiovascular diseases[11–15]. SD refers to a problem occurring during any phase of the sexual response cycle that prevents an individual from experiencing satisfaction from sexual activity[16]. SD includes orgasm disorders, arousal disorders, and interest or desire disorders[17–19]. The two most common male SDs are premature ejaculation and erectile dysfunction[20,21]. SD has severe adverse effects

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on a patient's quality of life, such as marital discord, anxiety, and poor self-image[22,23]. Therefore, effective clinical treatments for SD are necessary. However, the specific active components and molecular mechanisms of EB in SD remain unclear.

Network pharmacology is a systems biology-based method that expounds the role of TCM in the human biological network from an overall perspective by integrating pharmacology, omics, system biology, and computational biology[24–27]. In recent years, network pharmacology, as a new drug analysis method, has played an important role in exploring the mechanism of drug action. Through the construction of the biological system network, the selection of special signal nodes, and the analysis of the biological functions between the active components, protein targets, and signal pathways, the mechanism of action of TCM in the treatment of diseases can be analyzed with multiple components, levels, and targets at the molecular level.

In the present study, a comprehensive network pharmacology approach was established to investigate the potential pharmacological mechanism of EB on SD by network pharmacology analysis. First, the active compounds of EB were downloaded from the Traditional Chinese Medicine Systems Pharmacology (TCMSP) server[28,29]. Next, a drug-target network was constructed to provide a systematic overview of the potential target genes and the mechanism of action for EB. Therefore, this study offered an effective tool for investigating the mechanism of action of EB in treating SD. Finally, the targets related to SD were predicted by GeneCards and Online Mendelian Inheritance in Man (OMIM). Furthermore, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were performed using WebGestalt.

2. Material and methods

2.1. Active compounds and corresponding target collection

Major chemical compounds were obtained from TCMSP (https://tcmspw.com/index.php). The screening criteria were as follows: oral bioavailability (OB) ≥ 30% and drug-like (DL) properties ≥ 0.18. Also the targets related to the active compounds were screened using the TCMSP database, and then the obtained target gene names were changed to Gene ID from UniProt (http://www.uniprot.org/) database. After removing duplicate genes, overlapping target genes and active compounds were collected as candidate targets.

2.2. Collection of candidate target genes

The target genes associated with "SD" disease were collected from OMIM (https://www.omim.org)[30] and GeneCards (https://www.genecards.org)[31]. After removing duplicate genes, the overlapping target genes related to SD and active compounds were collected as candidate targets.

2.3. Network construction

Two networks were constructed: (1) a network of active compounds and targets of EB (compound-compound targets); and (2) in order to clarify the relationship between disease and drug targets, the two targets were intersected by Venny, and a network of compounds, common targets, and disease (compound-common target-disease) was constructed. Finally, Cytoscape 3.7.1 software was employed to visualize and analyze the networks. In the networks, the nodes represented target compounds, and edges indicated interactions.

2.4. Protein-protein interaction (PPI) network construction

The candidate targets were inputted into STRING (https://string-db.org) to obtain relevant information about protein interactions, and a PPI network was established using Cytoscape, followed by topological analysis.

2.5. GO and KEGG pathway analyses

To investigate the functional annotation and involved pathways of genes, GO and KEGG enrichment analyses were performed using WebGestalt (http://www.webgestalt.org/option.php).

3. Results

3.1. Active compounds and candidate targets of EB

In the present work, a total of 130 compounds were identified from the TCMSP database. After the screening was performed for OB ≥ 30% and DL ≥ 0.18, 23 active compounds of EB were obtained (Table 1). A total of 384 EB-related genes were identified from the TCMSP database (Table 2). These targets were also transformed into gene names and gene IDs *via* the UniProt database.

3.2. Disease target prediction

A total of 1052 SD-related genes were obtained *via* GeneCards and OMIM. After merging SD-related targets and active compound targets, 1003 overlapping targets were recognized and considered candidate targets.

3.3. Compound-compound target network analysis

A compound-compound target network was constructed to elucidate intuitive interactions in EB using Cytoscape 3.7.1 software. Figure 1 shows that the network of compound-target consists of 203 nodes (21 active ingredient nodes and 182 ingredient target nodes) and 382 edges. Most compound nodes were related to multiple target nodes, such as quercetin and luteolin. The network suggested that many compound targets could be adjusted by multiple ingredients (e.g., PTGS2, NCOA2, and AR in Table 3), which might be vital compound targets in EB against multiple diseases, revealing that the procedure of EB in the treatment disease had the characteristics of multiple components and multiple targets. According to the topology of degree score, the top four active ingredients were quercetin, luteolin, kaempferol, and *C*-homoerythrinan, 1,6-didehydro-3,15,16-trimethoxy-(3.beta.), with scores of 131, 49, 48, and 30, respectively (Table 3). These findings indicated that a single compound had multiple targets, and these targets were potentially related to the mechanism of action of EB. Consequently, we could have an approximate observation of the relevance between active compounds and targets *via* compound-compound target network analysis.

3.4. Drug-compound-target-disease network analysis

By searching the keyword “sexual dysfunction”, 1003 disease targets were obtained from OMIM and GeneCards databases. Figure 2 shows that 67 common targets for drugs and diseases were obtained using the Venny online tools. Figure 3 and Table 4 indicate that the network of drug-compound-target-disease consisted of 85 nodes and 219 edges. The network depicts the potential relationships between the compounds and the targets, thereby revealing the potential pharmacological mechanisms of EB for the treatment of SD. The nodes with the highest degree of connections to other compounds or targets represented hubs within the entire network and hence were potential drugs or targets. For example, the compound with the highest degree of connection was quercetin (degree = 46). Kaempferol, luteolin, and

C-homoerythrinan, 1,6-didehydro-3,15,16-trimethoxy-(3.beta.) also had higher degrees of connections of 18, 17, and 16, respectively. These findings indicated that a single compound affected multiple targets, and these targets were potentially related to the mechanism of action of EB.

Table 1. Active compounds of EB.

MOL ID	Molecule	OB (≥ 30%)	DL (≥ 0.18)
MOL001510	24-Epicampesterol	37.58	0.71
MOL001645	Linoleyl acetate	42.10	0.20
MOL001771	Poriferast-5-en-3beta-ol	36.91	0.75
MOL001792	DFV	32.76	0.18
MOL003044	Chryseriol	35.85	0.27
MOL003542	8-Isopentenyl-kaempferol	38.04	0.39
MOL000359	Sitosterol	36.91	0.75
MOL000422	Kaempferol	41.88	0.24
MOL004367	Olivil	62.23	0.41
MOL004373	Anhydroicaritin	45.41	0.44
MOL004380	*C*-Homoerythrinan, 1,6-didehydro-3,15,16-trimethoxy-, (3.beta.)-	39.14	0.49
MOL004382	Yinyanghuo A	56.96	0.77
MOL004384	Yinyanghuo C	45.67	0.5
MOL004386	Yinyanghuo E	51.63	0.55
MOL004388	6-Hydroxy-11,12-dimethoxy-2,2-dimethyl-1,8-dioxo-2,3,4,8-tetrahydro-1*H*-isochromeno[3,4-h]isoquinolin-2-ium	60.64	0.66
MOL004391	8-(3-Methylbut-2-enyl)-2-phenyl-chromone	48.54	0.25
MOL004394	Anhydroicaritin-3-*O*-α-L-rhamnoside	41.58	0.61
MOL004396	1,2-Bis(4-hydroxy-3-methoxyphenyl)propan-1,3-diol	52.31	0.22
MOL004425	Icariin	41.58	0.61
MOL004427	Icariside A7	31.91	0.86
MOL000006	Luteolin	36.16	0.25
MOL000622	Magnograndiolide	63.71	0.19
MOL000098	Quercetin	46.43	0.28

Table 2. Targets of EB.

MOL ID	Molecule	Target
MOL001510	24-Epicampesterol	Progesterone receptor
MOL001645	Linoleyl acetate	Prostaglandin G/H synthase 1
MOL001771	Poriferast-5-en-3beta-ol	Nuclear receptor coactivator 2
MOL001792	DFV	Prostaglandin G/H synthase 1
MOL003044	Chryseriol	Nuclear receptor coactivator 1
MOL003542	8-Isopentenyl-kaempferol	Estrogen receptor
MOL000359	Sitosterol	Nuclear receptor coactivator 2
MOL000422	Kaempferol	Antileukoproteinase
MOL004367	Olivil	Prostaglandin G/H synthase 2
MOL004373	Anhydroicaritin	Nuclear receptor coactivator 1
MOL004380	*C*-Homoerythrinan, 1,6-didehydro-3,15,16-trimethoxy-, (3.beta.)	Prostaglandin G/H synthase 1
MOL004382	Yinyanghuo A	Nuclear receptor coactivator 2
MOL004384	Yinyanghuo C	Androgen receptor
MOL004386	Yinyanghuo E	Nuclear receptor coactivator 2
MOL004388	6-Hydroxy-11,12-dimethoxy-2,2-dimethyl-1,8-dioxo-2,3,4,8-tetrahydro-1*H*-isochromeno[3,4-h]isoquinolin-2-ium	Prostaglandin G/H synthase 1
MOL004391	8-(3-Methylbut-2-enyl)-2-phenyl-chromone	Cyclin-A2
MOL004396	1,2-Bis(4-hydroxy-3-methoxyphenyl)propan-1,3-diol	Estrogen receptor
MOL004427	Icariside A7	Nuclear receptor coactivator 2
MOL000006	Luteolin	Prostaglandin G/H synthase 1
MOL000098	Quercetin	Glutathione S-transferase Mu 2

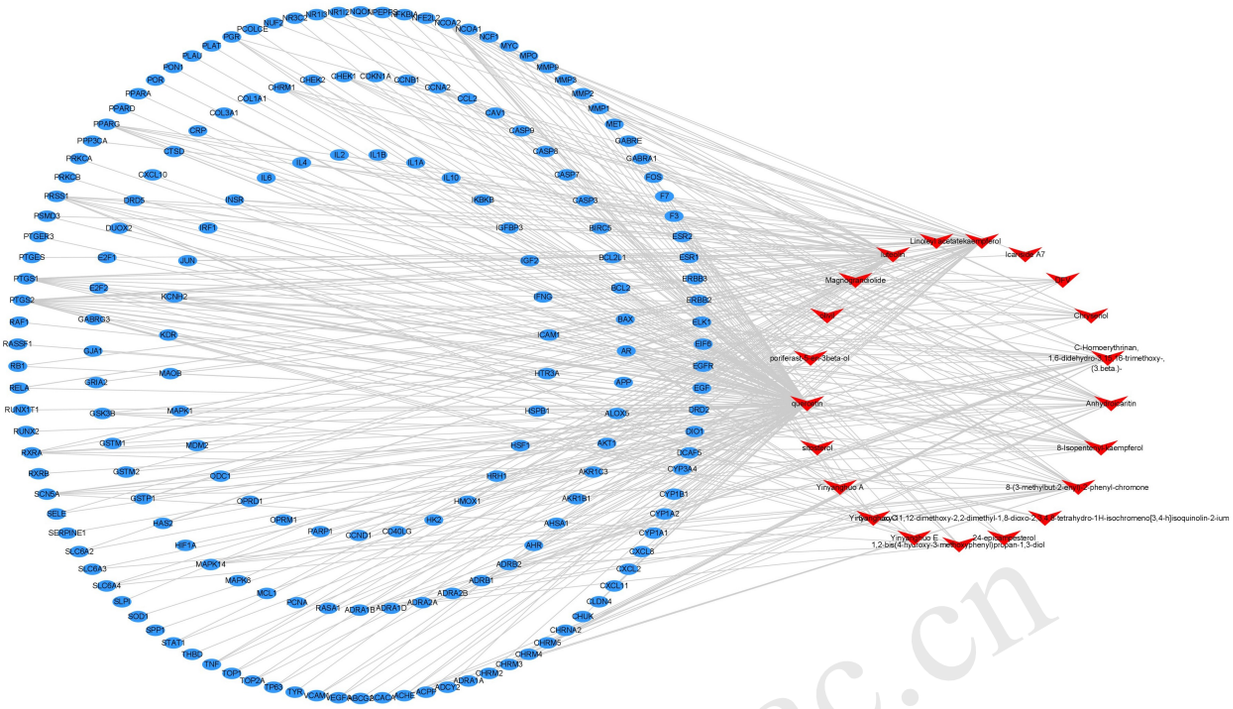

Figure 1. Compound-target network. Red arrows represent active ingredients in *Epimedium*. Red circles represent targets of Epimedium. Edges represent the interaction between ingredients and targets.

Table 3. Topology characteristics of hub nodes from the compound network.

Shared name	Degree	Type
Quercetin	131	Molecule
Luteolin	49	Molecule
Kaempferol	48	Molecule
C-Homoerythrinan, 1,6-didehydro-3,15,16-trimethoxy-, (3.beta.)-	30	Molecule
Anhydroicaritin	25	Molecule
8-(3-Methylbut-2-enyl)-2-phenyl-chromone	22	Molecule
8-Isopentenyl-kaempferol	17	Molecule
PTGS2	17	Gene
NCOA2	16	Gene
AR	11	Gene
Chryseriol	11	Molecule
PTGS1	11	Gene
PRSS1	9	Gene

Table 4. Topology characteristics of the drug-compound-target-disease network.

Shared name	Degree	Type
Quercetin	46	Molecule
Kaempferol	18	Molecule
Luteolin	17	Molecule
C-Homoerythrinan, 1,6-didehydro-3,15,16-trimethoxy-, (3.beta.)-	16	Molecule
8-(3-Methylbut-2-enyl)-2-phenyl-chromone	12	Molecule
Anhydroicaritin	9	Molecule

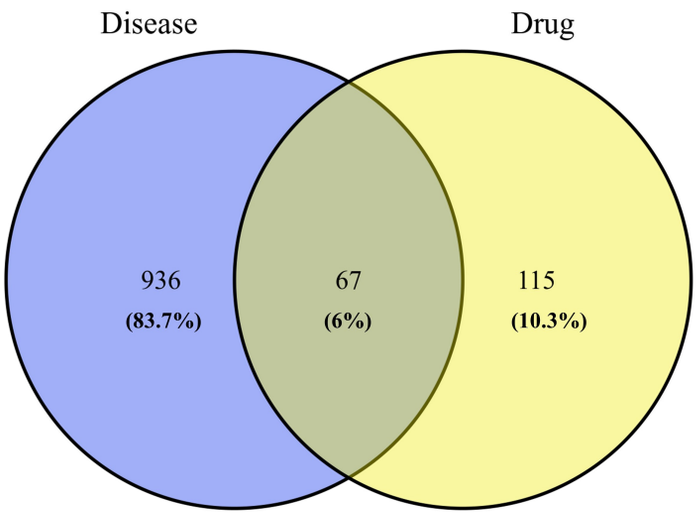

Figure 2. Venn diagram of disease and drug targets.

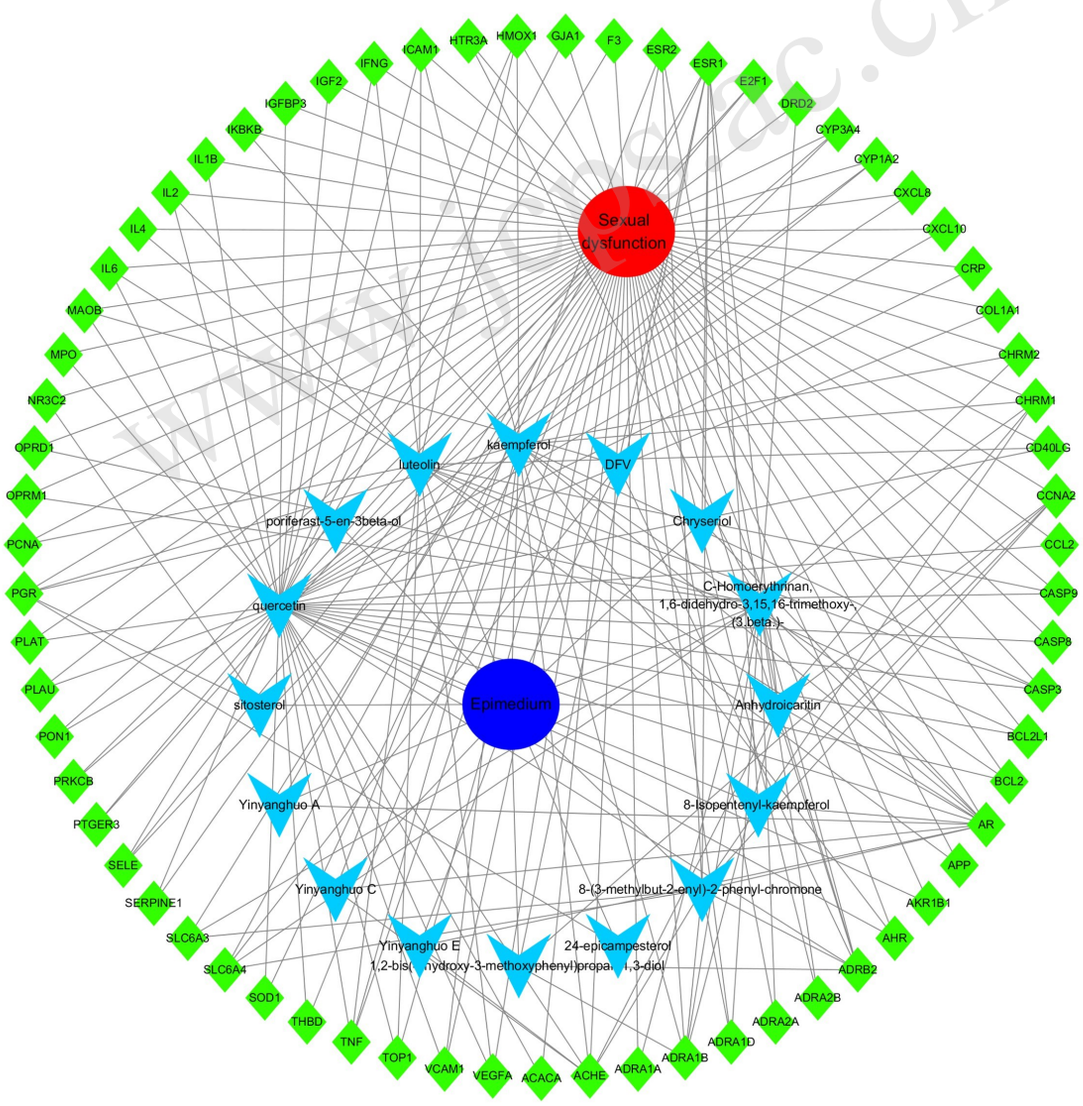

Figure 3. Drug-compound-target-disease network map.

3.5. PPI network analysis

The PPI network contained candidate targets of EB remedying SD and their interacting proteins. The network contained 66 nodes and 604 edges. The nodes represented targets, edges represented associations between targets, and the average node degree value was 18. Generally, the greater the degree, the closer the effect. Figure 4a shows that the depth of color represented the value of the degree. We screened the top 10 degrees of genes, and their close functional relationship is presented in Figure 4b. Figure 4c shows the values of the 30 targets; the targets interleukin-6 (IL-6), VEGFA, tumor necrosis factor (TNF), CASP3, CXCL8, IL1B, APP, CCL2, ESR1, and SERPINE1 were located at the core of the network, playing key regulatory roles in the protein interaction network. It could be hypothesized that these were the key targets of EB in the treatment of SD.

3.6. GO and KEGG pathway enrichment analyses

To further investigate the 67 identified target genes, GO and KEGG enrichment analyses were performed using WebGestalt. Figure 5 shows the results of GO analysis, which mainly included biological processes, cellular components, and molecular functions. The main terms of biological processes were response to stimulus (GO:0050896 67/67), biological regulation (GO:0065007 67/67), multicellular organismal process (GO:0032501 63/67), metabolic process (GO:0008152 62/67), and cell communication (GO:0007154 57/67) (Figure 5a, Table 5). The top five terms of cellular components were membrane (GO:0016020 45/67), endomembrane system (GO:0012505 32/67), extracellular space (GO:0005615 32/67), nucleus (GO:0005634 30/67), and membrane-enclosed lumen (GO: 0031974 28/67) (Figure 5b, Table 6), whereas the major terms of molecular

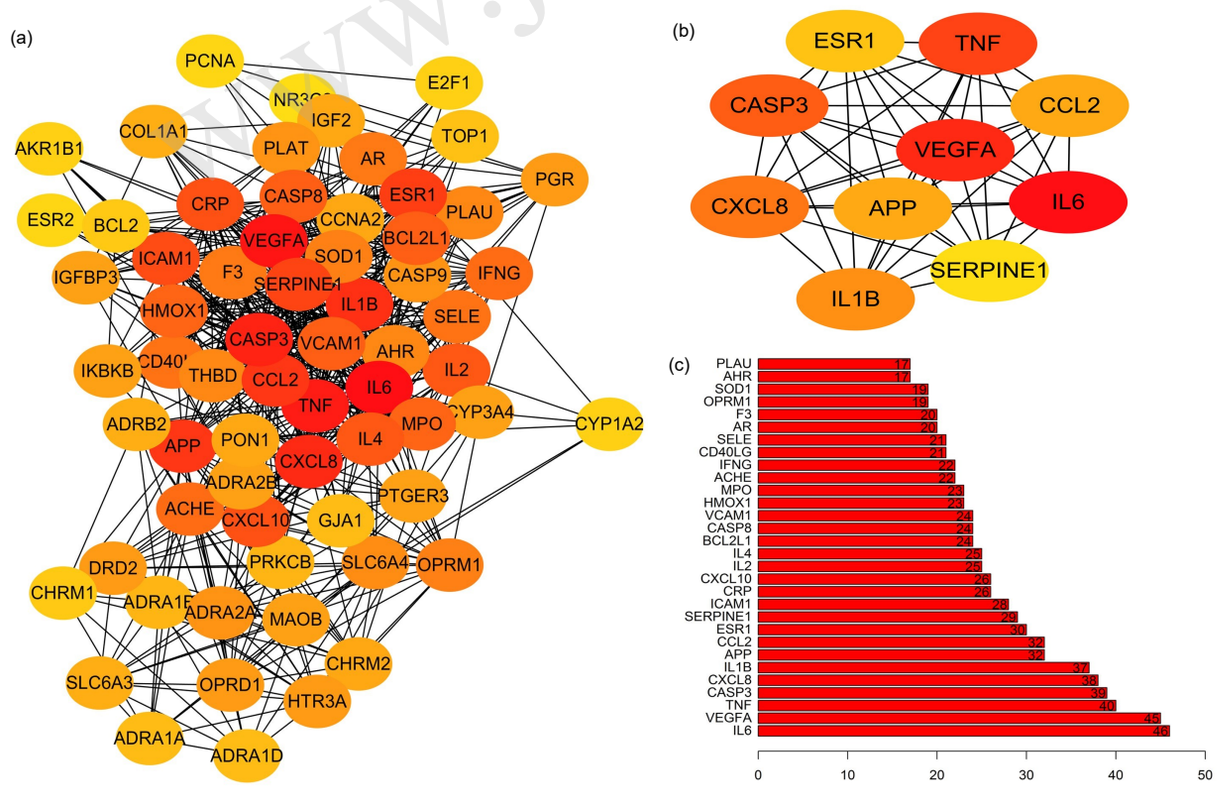

Figure 4. (a) PPI network; (b) the top 10 degrees of genes; (c) the values of the target degree.

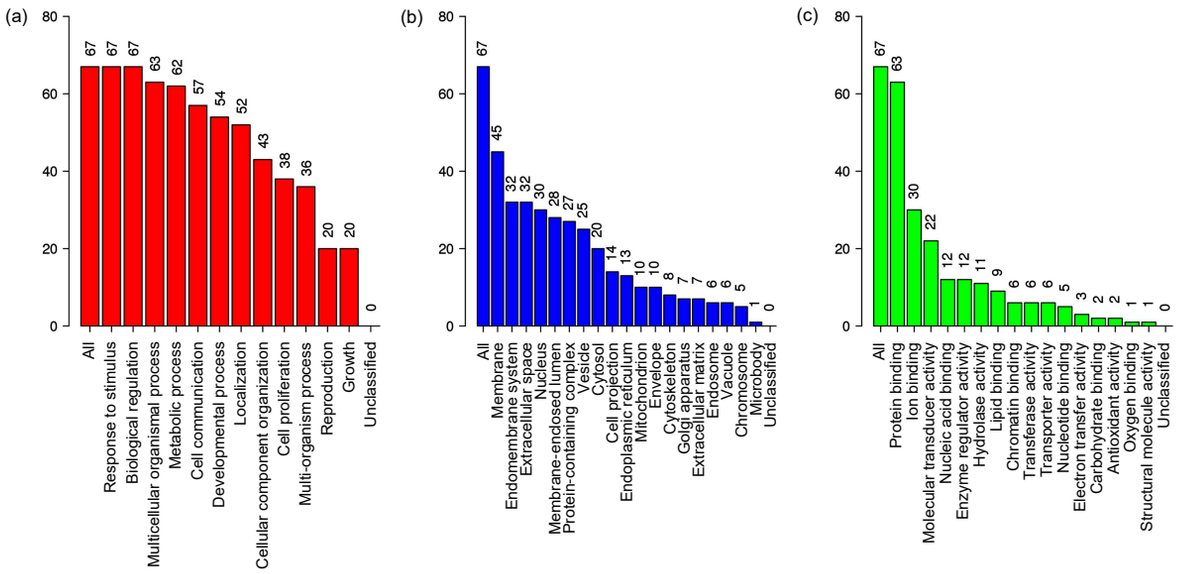

Figure 5. GO map of putative target genes. (a) Biological process categories; (b) cellular component categories; (c) molecular function categories.

Table 5. Biological process categories.

GO ID	GO term	Number of target genes
GO:0050896	Response to stimulus	67
GO:0065007	Biological regulation	67
GO:0032501	Multicellular organismal process	63
GO:0008152	Metabolic process	62
GO:0007154	Cell communication	57
GO:0032502	Developmental process	54
GO:0051179	Localization	52
GO:0016043	Cellular component organization	43
GO:0008283	Cell proliferation	38
GO:0051704	Multi-organism process	36
GO:0000003	Reproduction	20
GO:0040007	Growth	20

Table 6. Cellular component categories.

GO ID	GO term	Number of target genes
GO:0016020	Membrane	45
GO:0012505	Endomembrane system	32
GO:0005615	Extracellular space	32
GO:0005634	Nucleus	30
GO:0031974	Membrane-enclosed lumen	28
GO:0032991	Protein-containing complex	27
GO:0031982	Vesicle	25
GO:0005829	Cytosol	20
GO:0042995	Cell projection	14
GO:0005783	Endoplasmic reticulum	13
GO:0005739	Mitochondrion	10
GO:0031975	Envelope	10
GO:0005856	Cytoskeleton	8
GO:0005794	Golgi apparatus	7
GO:0031012	Extracellular matrix	7
GO:0005768	Endosome	6
GO:0005773	Vacuole	6
GO:0005694	Chromosome	5
GO:0042579	Microbody	1

functions included protein binding (GO:0005515 63/67), ion binding (GO:0043167 30/67), molecular transducer activity (GO:0060089 22/67), nucleic acid binding (GO:0003676 12/67), and enzyme regulator activity (GO:0030234 12/67) (Figure 5c, Table 7). To further identify the potential pathways involved in the inhibitory effects of EB against SD, a KEGG pathway enrichment analysis of the 67 genes was performed. Figure 6 shows that a total of 20 enriched pathways of EB against SD were identified ($P < 0.05$).

Table 7. Molecular function categories.

GO ID	GO term	Number of target genes
GO:0005515	Protein binding	63
GO:0043167	Ion binding	30
GO:0060089	Molecular transducer activity	22
GO:0003676	Nucleic acid binding	12
GO:0030234	Enzyme regulator activity	12
GO:0016787	Hydrolase activity	11
GO:0008289	Lipid binding	9
GO:0003682	Chromatin binding	6
GO:0016740	Transferase activity	6
GO:0005215	Transporter activity	6
GO:0000166	Nucleotide binding	5
GO:0009055	Electron transfer activity	3
GO:0030246	Carbohydrate binding	2
GO:0016209	Antioxidant activity	2
GO:0019825	Oxygen binding	1
GO:0005198	Structural molecule activity	1

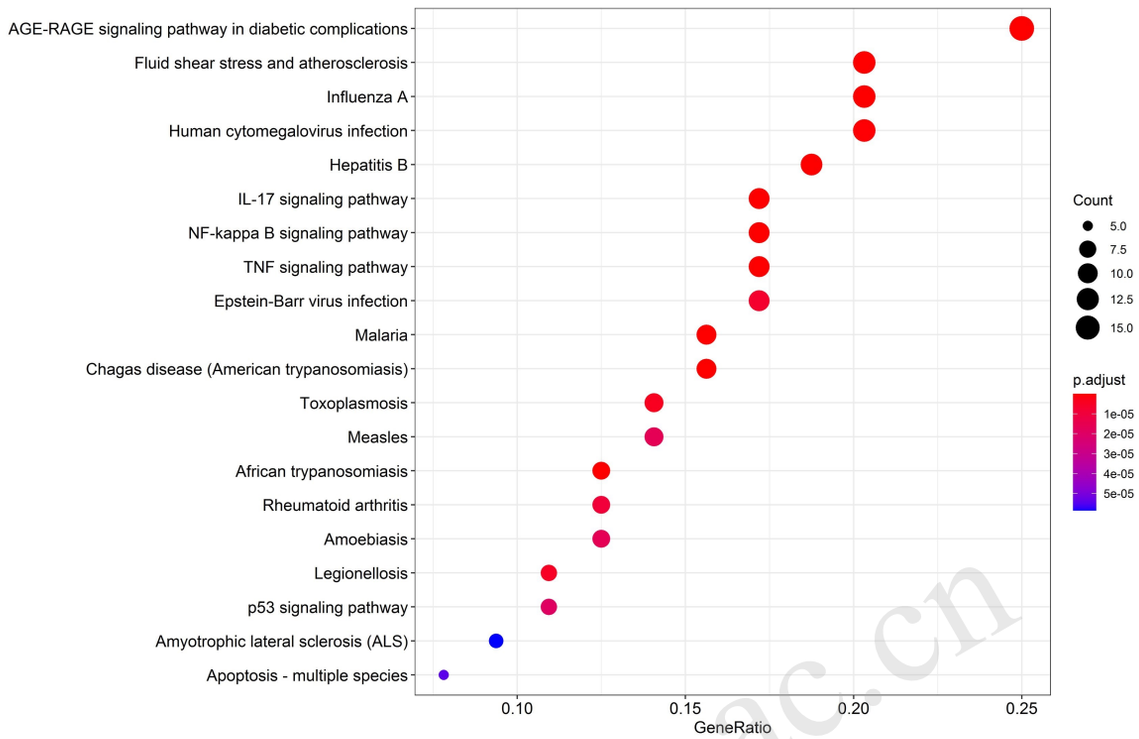

Figure 6. KEGG pathway analysis of putative target genes.

4. Discussion

EB, a Chinese herb, is a multiple-target therapeutic medicament that has been effectively used for the treatment of complex diseases, such as osteoporosis, depression, and SD. It has multiple targets and possesses multiple pharmacological activities and is thus used for treating SD with proven efficacy. Hence, EB can be a promising resource that may be utilized as a lead compound or an active ingredient for future drug discovery.

In recent years, along with the generalization of systems biology, network pharmacology has become an important method to analyze the mechanism of complex components of traditional Chinese herbs, thereby providing a new strategy for the study of TCM. In the present study, the TCMSP database was used to predict the chemical composition and targets of EB. A total of 23 compounds of EB were found, of which 2 compounds had no corresponding target. Further target prediction indicated that 67 targets were related to 16 active ingredients.

To further explore the relationship of the compound, common target, and pathway, we constructed compound-target, drug-compound-target-disease, and PPI networks. These networks revealed that EB had multiple targets, components, and pathways against SD. PPI network analysis was used to identify key proteins associated with EB therapy for SD. Three topological features of each node in the network were calculated to find the major nodes. The top 10 key targets, namely, IL-6, VEGFA, TNF, CASP3, CXCL8, IL1B, APP, CCL2, ESR1, and SERPINE1, played key regulatory roles in the protein interaction network. It could be hypothesized that these might be the key targets of EB in the treatment of SD.

TNF, a multi-functional pro-inflammatory cytokine mainly secreted by macrophages, is involved in the regulation of various biological processes, including

cell proliferation, differentiation, apoptosis, lipid metabolism, and coagulation, and it is closely related to diseases, such as autoimmune diseases, insulin resistance, and cancer. In patients with erectile dysfunction, TNF-α levels in serum are increased and are inversely related to sexual performance[32]. Caspase 3 (CASP3), a type of cysteinyl aspartate-specific protease, functions as an important effector in the apoptotic process, whereas CASP3 plays a primary role in the incidence and development of apoptosis[33]. Estrogen receptor alpha (ESR1) encodes the estrogen receptor, which is important for hormone binding, DNA binding, and transcription activation. A previous study has shown that the damage incurred by the developing penis in hyperestrogenism involves estrogen receptors (i.e., ESR1), and estrogen can also mediate erectile dysfunction that is induced by metabolic syndrome[34]. Furthermore, the loss of the VEGFA subtype in the testes of male mice causes an imbalance in spermatogenic stem cell homeostasis, resulting in decreased sperm counts and low fertility rates[35]. Therefore, EB or the combination of the above components for some key targets plays a vital role in the regulation of SD.

Approximately 47 GO terms and 100 KEGG pathways were identified using enrichment analysis. Network pharmacology analysis showed that potential biomarkers and therapeutic targets of EB mainly included IL-6, VEGFA, TNF, and CASP3, which were significantly associated with the regulation of apoptosis, the male hormone-related signaling pathways, and biological processes. In addition, these proteins were significantly enriched in apoptosis, IL-17, TNF, and NF-kappa B signaling pathways. SD is a potential complication of treatment for some diseases. In diabetic rats, insulin treatment may be used to recover erectile function by restoring the expression of a sex hormone receptor[36]. Central dopamine plays a fundamental role in the control of sexual function[37]. IL-6 is a cytokine with multiple biological functions, and a previous study has shown that the levels of inflammatory cytokines, such as IL-6, in the semen of infertile patients are significantly increased[38]. Meanwhile, TNF-α can activate MAPK and PI3K/Akt signaling pathways, which may also be the regulatory mechanism of TNF-α on SD. Although the enrichment analysis results showed the potential multi-target, multi-functional, and multi-pathway effects of EB in the treatment of SD, the role of these pathways in drug therapy needs to be further verified through experiments.

In summary, this study showed that EB was an active ingredient or a promising compound for the development of a safe and effective multi-targeted SD medicament. This study provided an effective way to predict and discover new medicines treating SD and their application in future clinical investigations. Although the present research revealed the mechanism of EB in the treatment of SD from the perspective of network pharmacology, it still has some limitations, including the lack of experimental verification, which will be performed in our future research.

5. Conclusions

Collectively, the current study, for the first time, identified the bioactive components of EB, as well as the multiple targets and pathways of EB against SD. A network pharmacology of EB for SD treatment was constructed, including 21 effective compounds and 67 target genes. This analysis showed that EB acted on multiple targets and played a therapeutic role in SD *via* multiple pathways. The results might be helpful in developing a specific drug target for SD pharmacotherapy.

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利用网络药理学探究淫羊藿治疗性功能障碍的药理作用机制

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摘要: 本研究利用网络药理学技术和方法分析淫羊藿治疗性功能障碍的活性组分, 并对潜在的靶点与机制进行整合与分析。利用中药系统药理数据库和分析平台(TCMSP)获取淫羊藿的化学成分及活性成分的作用靶点; 运用OMIM数据库获取与性功能障碍相关的靶点; Cytoscape 3.7.1构建药物-活性成分-靶点基因-疾病网络图; STRING数据库构建靶点蛋白互作网络; WebGestalt数据库对核心靶点基因进行基因本体(GO)及京都基因与基因组百科全书(KEGG)相关通路富集分析。该研究从淫羊藿中筛选得到21种有效成分, 从103个疾病目标中筛选得到67个与之相对应的作用靶点。研究结果初步验证了淫羊藿治疗性功能障碍多成分、多靶点、多途径的作用特点, 为淫羊藿治疗性功能障碍的进一步研究提供参考。

关键词: 淫羊藿; 网络药理学; 性功能障碍; 成分-靶点