

# Pharmacological mechanism of Xiaoyao San in the treatment of polycystic ovary syndrome based on network pharmacology

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## Research

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# Abstract

**Background :** Xiaoyao San(XYS) has been widely used in the treatment of polycystic ovary syndrome(PCOS), but its mechanism is not clear. The purpose of this study is to elucidate the mechanism of YYS in the treatment of PCOS from the aspects of active components, targets and pathways. The purpose of the study is to explore the molecular mechanism of YYS in the treatment of PCOS.

**Methods :** TCMSP database, UniProt and Perl were used to screen and collect the active components and targets of YYS. The genes related to PCOS were searched in GeneCards database. Collect the related targets of PCOS and YYS, use STRING database and Cytoscape software to process the data visually and analyze topology, and screen the key components and targets in the network. The key targets were enriched by R Project to predict the mechanism of YYS in the treatment of PCOS.

**Results :** 68 active components and 96 drug targets in YYS were screened out. 3648 PCOS related disease targets were collected. 66 targets of YYS for PCOS treatment were obtained after analysis. 21 key targets of NCOA2, PGR, PTGS1, PPARG and AR were constructed after topology analysis. 63 biological functions and 111 biological pathways were obtained after gene ontology(GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes(KEGG) Pathway enrichment analysis.

**Conclusions :** YYS has the characteristics of multi-component, multi-target and multi-path. This study discussed the active components, targets and potential mechanism of YYS in the treatment of PCOS, which provided a new direction for further study of the mechanism of YYS in the treatment of PCOS, and provides more ideas for clinical treatment of PCOS.

## 1. Background

PCOS is a common endocrine disorder with reproductive dysfunction and metabolic abnormality. It is characterized by continuous anovulation, hyperandrogenism and polycystic ovarian changes, often accompanied by IR. The global incidence of PCOS is 5% - 10%. TCM in the treatment of PCOS differentiation diagnosis and treatment [6], liver depression is a non obese type of PCOS patients often show the syndrome type [7]. Guangrong Li et al [8] proposed that emotional failure is an important cause of PCOS, and stagnation of liver Qi is an important pathogenesis of PCOS. Modern medicine shows that the pathogenesis of PCOS is very complex, which is mainly related to the mechanism of human hormone secretion and regulation. In clinic, TCM compound prescription is often used to recuperate patients' menstruation. In addition, the study shows that the complications of PCOS, such as cardiovascular and cerebrovascular diseases, diabetes, endometrial cancer and so on, have brought dual physical and mental harm to women.

TCM is a kind of compound Chinese medicine, which can increase the efficiency and reduce the toxicity. The clinical effect of TCM compound is a comprehensive result of complex biological process in vivo. The research of effective component group and related drug target group is two important problems

faced by the modernization research of TCM compound [10]. In clinic, YYS is widely used in the treatment of PCOS with liver depression, which has a good clinical effect [12–15], but up to now, the material basis and mechanism of YYS is not clear. Network pharmacology is to study the occurrence and development of diseases from the perspective of biological network, and to understand the interaction between drugs and the body [17]. To explore the relationship between YYS and PCOS, a biological network was constructed by means of network pharmacology and information technology. It is helpful to explain the mechanism of YYS in the treatment of PCOS.

Based on the research methods of network pharmacology and Informatics, the potential active components of YYS were analyzed and screened out under the conditions of OB value and DL value. Through the construction of compound component target disease network, the links and interrelations of nodes in the network were analyzed, and the mechanism of YYS in the treatment of PCOS was clarified. The foundation of the research is established.

## **2. Materials And Methods**

### **2.1 The active components of YYS**

YYS is composed of six kinds of medicine, such as Liquorice, Radix Bupleuri, Radix Paeoniae Alba, Angelica, Poria cocos and Rhizoma Atractylodis Macrocephalae. The TCM systems pharmacy database and analysis platform (tcmsp) (<http://tcmsp.com/tcmsp.php>) [19] database was used to find the chemical components of each single drug in YYS, and then ADME analysis was carried out [20]. According to the condition parameters of oral bioavailability (OB)  $\geq 30\%$ , drug like (DL)  $\geq 0.18$ , the active components were screened and collected.

### **2.2 The potential targets of YYS**

In tcmsp database, the corresponding target points of each flavor components of YYS were mined and collected. Using the uniprotkb search function in the protein database (UniProt) (<http://www.uniprot.org/>), enter the free scatter target and set the species as "human", and collect all the target corrected official names (Symbol).

### **2.3 PCOS related targets acquisition**

Enter "polycystic ovary syndrome" in the genecards database (<https://www.genecards.org/>) to search and collect relevant targets of PCOS.

### **2.4 Construction and analysis of "compound-component-target-disease" network**

The intersection of the collected targets was selected to determine the targets of YYS for the treatment of PCOS. After sorting out the existing data and prediction results, import them into the software of Cytoscape 3.7.2 [21] to get the topology network of "compound composition target disease". Each node in

the network represents XYS, polycystic ovary syndrome, target and active components respectively, and the connection between nodes indicates that there is interaction between connected nodes. The potential key active ingredients were obtained after analyzing by Network Analyzer. (Degree represents the importance of nodes in the network)

## **2.5 Construction and analysis of protein protein interaction (PPI) network**

The obtained target proteins were imported into the string [22] database (<https://string-db.org/>), and "Homo sapiens" was selected as the research species. After removing the isolated free nodes, the PPI network was obtained. The key target proteins of XYS in the treatment of PCOS were obtained by analyzing the PPI network.

### **2.6 Gene ontology (GO) functional enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis**

Using R Project [23], Cluster Profiler [24], Colorspace [25], Dose [26], org.Hs.eg.db [27], Pathview [28] and other packages to program, the names of cross target proteins of XYS and PCOS were transformed into official names, and biological function enrichment analysis and biological pathway enrichment analysis were carried out. The threshold which was set P value < 0.05 was used as the enrichment condition.

## **3. Results**

### **3.1 Collection results of active components in XYS**

A total of 928 active components of the six drugs in XYS were collected after screening in tcmsp. Among them, 85 of Radix Paeoniae Alba, 55 from Atractylodes macrocephala, 349 of Radix Bupleuri, 125 from angelica, 250 of Liquorice, and 24 from Poria cocos. Oral bioavailability and chemotaxis are important kinetic parameters for drug screening and evaluation. Set parameters  $OB \geq 30\%$ ,  $DL \geq 0.18$  to screen 928 compounds in XYS and get 76 compounds meeting the conditions. After de duplication, 67 active ingredients with research value in XYS are obtained, as shown in Table 1.

Table.1 Basic information of active components of Xiaoyao San

MOL ID	Molecule Name	OB(%)	DL
MOL001918	paeoniflorgenone	87.59	0.37
MOL004644	Sainfuran	79.91	0.23
MOL001925	paeoniflorin_qt	68.18	0.4
MOL001928	albiflorin_qt	66.64	0.33
MOL001910	11alpha,12alpha-epoxy-3beta-23-dihydroxy-30-norolean-20-en-28,12beta-olide	64.77	0.38
MOL000022	14-acetyl-12-senecieryl-2E,8Z,10E-atractylentriol	63.37	0.3
MOL000020	12-senecieryl-2E,8E,10E-atractylentriol	62.4	0.22
MOL002712	6-Hydroxykaempferol	62.13	0.27
MOL002680	Flavoxanthin	60.41	0.56
MOL000021	14-acetyl-12-senecieryl-2E,8E,10E-atractylentriol	60.31	0.31
MOL013187	Cubebin	57.13	0.64
MOL000211	Mairin	55.38	0.78
MOL000492	(+)-catechin	54.83	0.24
MOL000049	3β-acetoxyatractylone	54.07	0.22
MOL001924	paeoniflorin	53.87	0.79
MOL002717	qt_carthamone	51.03	0.2
MOL000354	isorhamnetin	49.6	0.31
MOL001921	Lactiflorin	49.12	0.8
MOL004609	Areapillin	48.96	0.41
MOL002694	4-[(E)-4-(3,5-dimethoxy-4-oxo-1-cyclohexa-2,5-dienylidene)but-2-enylidene]-2,6-dimethoxycyclohexa-2,5-dien-1-one	48.47	0.36
MOL002710	Pyrethrin II	48.36	0.35
MOL004628	Octalupine	47.82	0.28
MOL004624	Longikaurin A	47.72	0.53
MOL000098	quercetin	46.43	0.28
MOL004653	(+)-Anomalin	46.06	0.66
MOL002757	7,8-dimethyl-1H-pyrimido[5,6-g]quinoxaline-2,4-dione	45.75	0.19
MOL002721	quercetagenin	45.01	0.31

MOL ID	Molecule Name	OB(%)	DL
MOL000300	dehydroeburicoic acid	44.17	0.83
MOL000449	Stigmasterol	43.83	0.76
MOL001919	(3S,5R,8R,9R,10S,14S)-3,17-dihydroxy-4,4,8,10,14-pentamethyl-2,3,5,6,7,9-hexahydro-1H-cyclopenta[a]phenanthrene-15,16-dione	43.56	0.53
MOL000282	ergosta-7,22E-dien-3beta-ol	43.51	0.72
MOL002695	lignan	43.32	0.65
MOL002707	phytofluene	43.18	0.5
MOL004718	$\alpha$ -spinasterol	42.98	0.76
MOL001645	Linoleyl acetate	42.1	0.2
MOL000422	kaempferol	41.88	0.24
MOL000283	Ergosterol peroxide	40.36	0.81
MOL002776	Baicalin	40.12	0.75
MOL002706	Phytoene	39.56	0.5
MOL000028	$\alpha$ -Amyrin	39.51	0.76
MOL000275	trametenolic acid	38.71	0.8
MOL000287	3beta-Hydroxy-24-methylene-8-lanostene-21-oic acid	38.7	0.81
MOL000285	(2R)-2-[(5R,10S,13R,14R,16R,17R)-16-hydroxy-3-keto-4,4,10,13,14-pentamethyl-1,2,5,6,12,15,16,17-octahydrocyclopenta[a]phenanthren-17-yl]-5-isopropyl-hex-5-enoic acid	38.26	0.82
MOL000292	poricoic acid C	38.15	0.75
MOL000279	Cerevisterol	37.96	0.77
MOL000953	CLR	37.87	0.68
MOL002773	beta-carotene	37.18	0.58
MOL000358	beta-sitosterol	36.91	0.75
MOL000359	sitosterol	36.91	0.75
MOL000296	hederagenin	36.91	0.75
MOL001771	poriferast-5-en-3beta-ol	36.91	0.75
MOL000033	(3S,8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-[(2R,5S)-5-propan-2-yl-octan-2-yl]-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol	36.23	0.78

MOL ID	Molecule Name	OB(%)	DL
MOL000006	luteolin	36.16	0.25
MOL000072	8 $\beta$ -ethoxy atractylenolide $\boxtimes$	35.95	0.21
MOL000276	7,9(11)-dehydropachymic acid	35.11	0.81
MOL002698	lupeol-palmitate	33.98	0.32
MOL000289	pachymic acid	33.63	0.81
MOL002714	baicalein	33.52	0.21
MOL002719	6-Hydroxynaringenin	33.23	0.24
MOL004598	3,5,6,7-tetramethoxy-2-(3,4,5-trimethoxyphenyl)chromone	31.97	0.59
MOL004648	Troxerutin	31.6	0.28
MOL001930	benzoyl paeoniflorin	31.27	0.75
MOL000280	(2R)-2-[(3S,5R,10S,13R,14R,16R,17R)-3,16-dihydroxy-4,4,10,13,14-pentamethyl-2,3,5,6,12,15,16,17-octahydro-1H-cyclopenta[a]phenanthren-17-yl]-5-isopropyl-hex-5-enoic acid	31.07	0.82
MOL000273	(2R)-2-[(3S,5R,10S,13R,14R,16R,17R)-3,16-dihydroxy-4,4,10,13,14-pentamethyl-2,3,5,6,12,15,16,17-octahydro-1H-cyclopenta[a]phenanthren-17-yl]-6-methylhept-5-enoic acid	30.93	0.81
MOL000290	Poricoic acid A	30.61	0.76
MOL000291	Poricoic acid B	30.52	0.75
MOL004702	saikosaponin cqt	30.5	0.63
MOL000490	petunidin	30.05	0.31

## 3.2 Collection results of potential targets of YYS

96 potential targets of YYS were obtained after 67 potential targets of active ingredients were found in TCMSP and repeated targets were removed.

## 3.3 Results of PCOS target gene acquisition

A total of 3648 PCOS related disease targets were obtained by searching "polycystic ovary syndrome" in the GeneCards.

## 3.4 Network construction and analysis results

66 intersection targets were obtained by matching 96 drug targets with 3648 disease targets. After visualization, the results are shown in Fig. 1. 66 potential therapeutic targets of YYS for PCOS were determined. The interaction network of "YYS-active ingredient-target-PCOS" was constructed by using the software of cytoscape 3.7.2, as shown in Fig. 2. There are 93 nodes (25 active component nodes, 66 gene

nodes, 1 compound node, 1 disease node) and 373 edges in the network. The larger the degree value of the key active ingredient, the more important it is in the complex network, and the more significant it is in the treatment of diseases. After the network analysis, the key chemical components are quercetin, kaempferol, isorhamnetin, beta-sitosterol, Stigmasterol, sitosterol, 3,5,6,7-tetramethoxy-2-(3,4,5-trimethoxyphenyl)chromone, hederagenin, petunidin, Areapillin, 8 $\beta$ -ethoxy atractylenolide, 3 $\beta$ -acetoxyatractylone,  $\alpha$ -spinasterol, (+)-catechin and so on. The degree statistics results As shown in Table 2. These chemical components are the key active components of YYS in the treatment of PCOS.

Table.2 Key active components of Xiaoyao San in the treatment of PCOS

Mol ID	Mol Name	Degree	Herbal medicine
MOL000098	quercetin	111	Radix Bupleuri, Liquorice
MOL000422	kaempferol	61	Radix Paeoniae Alba, Radix Bupleuri, Liquorice
MOL000354	isorhamnetin	25	Radix Bupleuri
MOL000358	beta-sitosterol	21	Radix Paeoniae Alba, Angelica, Liquorice
MOL000449	Stigmasterol	15	Radix Bupleuri, Angelica, Liquorice
MOL000359	sitosterol	7	Radix Paeoniae Alba
MOL004598	3,5,6,7-tetramethoxy-2-(3,4,5-trimethoxyphenyl)chromone	6	Radix Bupleuri
MOL000296	hederagenin	6	Poria cocos
MOL000490	petunidin	5	Radix Bupleuri
MOL004609	Areapillin	5	Radix Bupleuri
MOL000072	8 $\beta$ -ethoxy atractylenolide	5	Rhizoma Atractylodis Macrocephalae
MOL000049	3 $\beta$ -acetoxyatractylone	5	Rhizoma Atractylodis Macrocephalae
MOL004718	$\alpha$ -spinasterol	4	Radix Bupleuri
MOL000492	(+)-catechin	4	Radix Paeoniae Alba

### 3.5 Construction of PPI network and results of key target screening

In order to further analyze the mechanism of YYS in the treatment of PCOS, 66 intersection targets were analyzed. 66 target genes were imported into the string database to retrieve protein interaction

information. The confidence level was set to 0.4 and isolated target proteins were eliminated. The obtained protein interaction information is visualized and network topology analysis is carried out, and the PPI network related to the effect of XYS on PCOS is constructed as shown in Fig. 3. The nodes represent the targets, while the edges represent the interaction between the targets. After processing the protein interaction information, the results are as shown in Figs. 4 and 24 core targets are selected as shown in Table 3, which are Interleukin-6(IL6), Caspase-3(CASP3), Epidermal growth factor receptor(EGFR), Estrogen receptor(ESR1), Myc proto-oncogene protein(MYC), Vascular endothelial growth factor A(VEGFA), Mitogen-activated protein kinase 8(MAPK8), G1/S-specific cyclin-D1(CCND1), Receptor tyrosine-protein kinase erbB-2(ERBB2), Androgen receptor(AR), Proto-oncogene c-Fos(FOS), Peroxisome proliferator activated receptor gamma(PPARG), Progesterone receptor(PGR), Caveolin-1(CAV1), Nitric oxide synthase, endothelial(NOS3), Caspase-8(CASP8), Hypoxia-inducible factor 1-alpha(HIF1A), Caspase-9(CASP9), Retinoblastoma-associated protein(RB1), Estrogen receptor beta(ESR2), Intercellular adhesion molecule 1(ICAM1), Nuclear factor erythroid 2-related factor 2(NFE2L2), Insulin-like growth factor II(IGF2), NAD(P)H dehydrogenase [quinone] 1(NQO1). It is speculated that these targets may be the key targets of XYS in the treatment of PCOS.

Table.3 Key targets of Xiaoyao San in the treatment of PCOS

Gene	Full Name	Degree
IL6	Interleukin-6	45
CASP3	Caspase-3	44
EGFR	Epidermal growth factor receptor	42
ESR1	Estrogen receptor	42
MYC	Myc proto-oncogene protein	41
VEGFA	Vascular endothelial growth factor A	41
MAPK8	Mitogen-activated protein kinase 8	40
CCND1	G1/S-specific cyclin-D1	37
ERBB2	Receptor tyrosine-protein kinase erbB-2	35
AR	Androgen receptor	33
FOS	Proto-oncogene c-Fos	33
PPARG	Peroxisome proliferator activated receptor gamma	28
PGR	Progesterone receptor	26
CAV1	Caveolin-1	25
NOS3	Nitric oxide synthase, endothelial	25
CASP8	Caspase-8	24
HIF1A	Hypoxia-inducible factor 1-alpha	23
CASP9	Caspase-9	21
RB1	Retinoblastoma-associated protein	20
ESR2	Estrogen receptor beta	19
ICAM1	Intercellular adhesion molecule 1	19
NFE2L2	Nuclear factor erythroid 2-related factor 2	19
IGF2	Insulin-like growth factor II	18
NQO1	NAD(P)H dehydrogenase [quinone] 1	18

### 3.6 Results of GO functional enrichment analysis

Using R Project and program package, 85 enrichment results were obtained by GO functional enrichment analysis of 66 cross target genes ( $p < 0.05$ ,  $q < 0.05$ ). The top 20 enrichment results are shown in Fig. 5, and the specific information is shown in Table 4. The first 20 biological function enrichment analysis

results are all molecular function related items, mainly involving the steroid hormone receptor activity, steroid binding, DNA-binding transcription activator activity, RNA polymerase II-specific nuclear receptor activity, transcription factor activity, direct ligand regulated sequence-specific, DNA binding, nuclear hormone receptor binding, RNA polymerase II transcription factor binding oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, hormone receptor binding, integrin binding, ubiquitin protein ligase binding, oxidoreductase activity, acting on NAD(P)H, heme protein as acceptor, activating transcription factor binding, estrogen receptor binding, ubiquitin-like protein ligase binding, cysteine-type endopeptidase activity involved in apoptotic process, steroid hormone receptor binding, monooxygenase activity, kinase regulator activity and heme binding.

Table.4 Cross gene biological function information of compound and disease

ID	Description	P-value	Count	Function
GO:0003707	steroid hormone receptor activity	5.98E-08	6	MF
GO:0005496	steroid binding	6.18E-08	7	MF
GO:0001228	DNA-binding transcription activator activity, RNA polymerase II-specific	6.08E-07	11	MF
GO:0004879	nuclear receptor activity	8.40E-07	5	MF
GO:0098531	transcription factor activity, direct ligand regulated sequence-specific DNA binding	8.40E-07	5	MF
GO:0035257	nuclear hormone receptor binding	1.53E-06	7	MF
GO:0001085	RNA polymerase II transcription factor binding	1.74E-06	7	MF
GO:0016705	oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen	2.07E-06	7	MF
GO:0051427	hormone receptor binding	5.64E-06	7	MF
GO:0005178	integrin binding	9.67E-06	6	MF
GO:0031625	ubiquitin protein ligase binding	1.19E-05	8	MF
GO:0016653	oxidoreductase activity, acting on NAD(P)H, heme protein as acceptor	1.38E-05	3	MF
GO:0033613	activating transcription factor binding	1.61E-05	5	MF
GO:0030331	estrogen receptor binding	1.78E-05	4	MF
GO:0044389	ubiquitin-like protein ligase binding	1.84E-05	8	MF
GO:0097153	cysteine-type endopeptidase activity involved in apoptotic process	2.18E-05	3	MF
GO:0035258	steroid hormone receptor binding	2.37E-05	5	MF
GO:0004497	monooxygenase activity	3.38E-05	5	MF
GO:0019207	kinase regulator activity	0.00012068	6	MF
GO:0020037	heme binding	0.000147572	5	MF

### 3.7 Results of KEGG pathway enrichment analysis

Using R Project and program package, 111 enrichment results were obtained by KEGG functional enrichment analysis of 66 cross target genes ( $p < 0.05$ ,  $q < 0.05$ ). The top 20 of the enrichment results are shown in Fig. 6, and the specific information is shown in Table 5. The first 20 are AGE-RAGE signaling pathway in diabetic complications, Fluid shear stress and atherosclerosis, Colorectal cancer, Kaposi sarcoma-associated herpesvirus infection, Prostate cancer, Proteoglycans in cancer, Bladder cancer,

Breast cancer, Hepatitis B, Endocrine resistance, Hepatocellular carcinoma, Estrogen signaling pathway, EGFR tyrosine kinase inhibitor resistance, Thyroid hormone signaling pathway, Non-small cell lung cancer, Prolactin signaling pathway, p53 signaling pathway, Platinum drug resistance, Apoptosis, MicroRNAs in cancer and other biological pathways.

Table.5 Cross gene biological pathway information of compound and disease

ID	Description	pvalue	Count
hsa04933	AGE-RAGE signaling pathway in diabetic complications	1.24E-11	12
hsa05418	Fluid shear stress and atherosclerosis	3.97E-11	13
hsa05210	Colorectal cancer	4.74E-11	11
hsa05167	Kaposi sarcoma-associated herpesvirus infection	1.22E-10	14
hsa05215	Prostate cancer	1.80E-10	11
hsa05205	Proteoglycans in cancer	4.19E-10	14
hsa05219	Bladder cancer	7.59E-10	8
hsa05224	Breast cancer	1.19E-09	12
hsa05161	Hepatitis B	3.66E-09	12
hsa01522	Endocrine resistance	3.75E-09	10
hsa05225	Hepatocellular carcinoma	5.54E-09	12
hsa04915	Estrogen signaling pathway	8.14E-09	11
hsa01521	EGFR tyrosine kinase inhibitor resistance	9.01E-09	9
hsa04919	Thyroid hormone signaling pathway	2.50E-08	10
hsa05223	Non-small cell lung cancer	3.91E-08	8
hsa04917	Prolactin signaling pathway	6.27E-08	8
hsa04115	p53 signaling pathway	7.85E-08	8
hsa01524	Platinum drug resistance	8.76E-08	8
hsa04210	Apoptosis	9.01E-08	10
hsa05206	MicroRNAs in cancer	9.19E-08	14

## 4. Discussion

PCOS is one of the most common gynecological endocrine disorders. In western medicine, insulin sensitizers, compound cyproterone acetate, laparoscopic ovarian drilling or follicular puncture are often

used to treat PCOS. However, due to the side effects of hormone drugs, patients are prone to have adverse symptoms. In recent years, TCM has been widely used in the treatment of PCOS, with significant therapeutic effect and high patient satisfaction. TCM can effectively regulate the menstrual cycle of women, improve the related symptoms of patients with PCOS, and restore normal ovulation [33].

As one of the famous prescriptions of TCM, YYS has the remarkable effect of soothing the liver and relieving the depression, nourishing the blood and strengthening the spleen. It is widely used in the treatment of PCOS with liver depression as the main treatment, and has a significant effect [39]. In the treatment of polycystic ovary model rats with modified or Modified YYS, it was found that Modified YYS could up regulate the expression of FSHR and LHR protein in granulosa cells of polycystic ovary rats, and reduce the levels of serum testosterone (T), anti Mullerian hormone (AMH) and egg yolk The expression intensity of AMH protein in the nest. Therefore, AMH can be regulated to reduce the androgen level in rats and improve PCOS [40].

TCM plays an important role in disease prevention and treatment through multi-component and multi-target, which makes the research on the material basis of the efficacy of TCM complex extremely difficult. Network pharmacology can systematically predict and reveal the action and mechanism of different drug molecules. In this study, the mechanism of action of TCM compound was comprehensively analyzed and explained from the molecular network level, and then integrated with disease analysis to achieve the overall analysis of medical theory [45].

In this study, TCM holistic view, principle of syndrome differentiation and treatment, new mode of network target network pharmacology were effectively combined. Using TCMSP, GeneCards, R Project and Cytoscape, the active ingredients and targets were screened and collected, and "YYS-Active ingredients-Target-PCOS" Physical network was constructed. In order to fully elucidate the potential mechanism of YYS in the treatment of PCOS, the key nodes in the network were analyzed and the related biological functions and pathways were studied.

The main chemical components of YYS in the treatment of PCOS are quercetin, kaempferol, isorhamnetin, beta sitosterol, stigmasterol, sitosterol, 3,5,6,7-tetramethoxy-2 - (3,4,5-trimethoxyphenyl) chromone, hederagenin, petunidin, areapillin, 8  $\beta$  - ethoxy atractylolide  $\square$ , 3  $\beta$  - acetoxyatractylone,  $\alpha$  - spinosterol, (+) - catch. Many studies have shown that the active components of YYS have certain pharmacological activities. Quercetin, as a phytoestrogen and antidiabetic, can improve follicular formation in diabetic mice. Quercetin administration in diabetic mice increased the volume of ovaries and growing follicles, the number of growing follicles and corpus luteum, and significantly reduced the number of atretic follicles. Quercetin may be used to treat PCOS and IR by inhibiting TLR / NF -  $\kappa$  B signaling pathway and improving microenvironment inflammation of IR in PCOS [47]. Isorhamnetin can inactivate NF -  $\kappa$  B signaling pathway and inhibit apoptosis and inflammation [49]. Phytosterol (stigmasterol, sitosterol, beta sitosterol) has the effects of regulating lipid metabolism, antioxidation and preventing atherosclerosis [51], and it is possible to treat the disorder of glycolipid metabolism in patients with PCOS. Hederagenin has antitumor, antidepressant, antibacterial, anti-inflammatory, anti diabetes and

other pharmacological effects [52]. Catechin can not only enhance the oxidation and energy consumption of fat, but also play an obvious role in the metabolism and distribution of fat, especially in reducing visceral fat. It can also achieve anti-inflammatory effect by regulating NF -  $\kappa$  B factor to alleviate glycolipid metabolic disorder syndrome [54]. Therefore, the efficacy of YYS in the treatment of PCOS is achieved through the joint action of a variety of active compounds in various TCM.

According to the results of GO analysis, YYS can treat PCOS by regulating the expression of multiple genes. The collected key targets such as IL6, CASP3, EGFR, ESR1, MYC, VEGFA, AR, PGR and so on are all involved in the disease regulation of PCOS, and play an important role in inflammatory response, cell proliferation and apoptosis, human reproductive and sexual development.

The results of KEGG analysis showed that many biological pathways were involved in many targets regulated by YYS. After analyzing the existing research work, it is found that the collected biological pathways are closely related to the potential therapeutic mechanism and pathogenesis of PCOS. The main biological pathways regulated by the collected cross-linked targets (the cross-linked targets are filled with red in the pathway map,  $\log_{2}FC = 1$ ) are: AGE-RAGE signaling pathway in diabetic complications (Fig. 7), Fluid shear stress and atherosclerosis (Fig. 8), Colorectal cancer, Proteoglycans in cancer, Bladder cancer, Breast cancer, Hepatitis B, Endocrine resistance, Hepatocellular carcinoma, Estrogen signaling pathway (Fig. 9), EGFR tyrosine kinase inhibitor resistance, Thyroid hormone signaling pathway, Prolactin signaling pathway, p53 signaling pathway, Platinum drug resistance, Apoptosis, MicroRNAs in cancer, PI3-Akt signaling pathway, NF-kappa B signaling pathway, MAPK signaling pathway, Wnt signaling pathway, etc. Among them, the biological pathways that have been confirmed include: ages receptor (RAGE) is a member of immunoglobulin superfamily, rage on cell membrane can activate several signal pathways after binding with corresponding ligands, and it is expressed in many kinds of cells, which is an indispensable ligand receptor with important functions in human body [56]. Rage can also activate multiple signaling pathways such as PI3K-Akt and NF -  $\kappa$  B through linkers. PI3K-Akt signaling pathway is the main pathway regulating serum insulin, and activation of PI3K-Akt pathway can improve IR [57]. Down regulation of TLR4 / NF -  $\kappa$  B inflammatory signaling pathway and down-regulation of inflammatory cytokines such as IL-6 and TNF -  $\alpha$  can effectively inhibit oxidative stress effect [58]. Inhibition of Wnt pathway can inhibit the expression of calcitonin in the endometrium of polycystic ovary rats, promote the normal secretion of sex hormones, reduce the activity of creatine kinase isoenzyme (CK-MB) and lactate dehydrogenase (LDH), and improve the symptoms of PCOS[63,64]. PCOS(PCOS) is a female endocrine disease involving multiple biological pathways. The regulation of key biological pathways is very important for the treatment of PCOS.

Many studies have shown that patients with PCOS have the following complications: type II diabetes, coronary heart disease, atherosclerosis, endometrial cancer and other diseases [66]. Low shear force is closely related to the occurrence of atherosclerotic plaques and is a local risk factor for atherosclerotic formation. Shear force may become one of the indicators to predict atherosclerosis [68]. The most common metabolic disorder caused by PCOS is abnormal lipid metabolism, which is mainly manifested in the increase of apoB, apoB / ApoA1 ratio [69]. In clinic, apoB / ApoA1 ratio and non-high-density

lipoprotein cholesterol are often used to predict coronary heart disease before blood lipid measurement [70]. Phosphatidylinositol 3-kinase / protein kinase B (PI3K / Akt) signaling pathway is an important pathway of cell proliferation in the body, which plays an important role in IR of pregnant women and endometrial proliferation [71]. The target gene of MDM2 is p53. Activation of PI3K / Akt pathway will lead to the increase of MDM2 expression, the rapid degradation of p53 and the abnormal proliferation of cells [72]. Pi3k-akt-mdm2 pathway plays an active role in endometrial proliferation and the development of endometrial cancer [73]. Therefore, PCOS is closely related to endometrium related diseases. The pathogenesis of PCOS has been proved to be closely related to IR (IR), hyperandrogenemia, hyperinsulinemia and obesity. It is generally believed that IR is the core of PCOS, and the existing work shows that the main cause of PCOS after IR is secondary hyperinsulinemia, through direct stimulation of ovarian androgen secretion increase, synergistic enhancement of luteinizing hormone induced steroidal hormone synthetase, interference of hypothalamus pituitary gonadal axis, enhancement of the amplitude and frequency of gonadotropin pulse release, leading to To induce ovarian dysfunction, to inhibit the synthesis and secretion of SHBG, to induce hyperandrogenemia, to inhibit the synthesis and secretion of IGFBP-1  $\alpha$ , to increase the level of IGF-1, and to stimulate the synthesis of androgen and hyperinsulinemia, we can promote the anti Mullerian hormone (AMH) Generation inhibition and ovulation of [74,75]. Therefore, PCOS is a complex metabolic disorder syndrome which is closely related to many diseases. Its complex pathogenesis makes it difficult to determine the treatment method, the clinical cure rate is low and the recurrence rate is high.

It is found that the potential mechanism of XYS in the treatment of PCOS lies in the target and biological pathway. The potential mechanism of XYS in the treatment of PCOS is as follows: XYS can reduce the expression of IL-6 inflammatory cytokines, inhibit the effect of oxidative stress and alleviate the long-term inflammatory state of cell tissues, so as to reduce the production of ROS, avoid the production of IR or reduce the symptoms of IR. Jiawei XYS can reduce the positive expression of EGFR protein, improve the ovarian function of PCO rats, promote the normal growth and development of follicles, and improve the ovarian polycystic change and ovulation disorder as a whole, suggesting that the treatment of PCOS with XYS may be related to the reduction of androgen level and the regulation of EGFR protein expression in ovarian granulosa cells [76]. XYS may reduce the expression of androgen receptor (AR) and the secretion of insulin in  $\beta$  cell of islet of Langerhans, so as to avoid the disorder of  $\beta$  cell function and the increase of testosterone concentration in the body, so as to reduce the prevalence of IR [77,78]. XYS may activate estrogen signal and steroid hormone biosynthesis by regulating the protein expression of PGR, ESR1 and ESR2 genes, stimulate ovulation and mediate follicle rupture [79], and alleviate the symptoms of PCOS. XYS may regulate the vegf-vegfr2 signal pathway, up regulate the content of VEGF and its related proteins, promote angiogenesis, so as to treat premature ovarian failure [80,81], improve the ability of ovarian hormone secretion, and alleviate the clinical symptoms of PCOS. In addition, XYS has some therapeutic effects on PCOS, type II diabetes, premature ovarian failure and endometrial cancer.

In this study, the mechanism of XYS in the treatment of PCOS was preliminarily elucidated, but it is difficult to determine whether the data is comprehensive because the components and target information of XYS are all from the existing database. In this study, we did not take into account the deeper chemical

reactions of various chemical components of the drug, and ignored the influence of YYS type, dose, and the complexity and difference of human body on drug metabolism and response.

## 5. Conclusions

Based on the network pharmacology method and information tool platform, this study analyzes the multi-component target PCOS network of YYS and the target gene function and metabolic pathway regulated by YYS, and explains the potential mechanism of YYS in the treatment of PCOS. The target predicted in this paper is consistent with the pharmacological action reported in the known literature, which shows the accuracy of target prediction, and reveals the characteristics of YYS's multi-component, multi-target and multi-channel integrated regulation. YYS can restore the hormone secretion level and relieve the disorder of cell function by regulating the secretion process of inflammatory factors, inhibiting the effect of oxidative stress, regulating steroid biosynthesis, promoting epithelial cell production and so on. This study comprehensively analyzed and clarified the mechanism of YYS in the treatment of PCOS in combination with the node connections and enrichment results in the biological network, so as to lay a foundation for further animal experiments or clinical verification of TCM in the later stage, and to provide ideas for the research and development of new clinical drugs of PCOS.

## Availability Of Data And Materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

## Abbreviations

YYS

Xiaoyao San

PCOS

Polycystic ovary syndrome

IR

Insulin Resistance

TCM

traditional Chinese medicine

## Declarations

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### Contributions

C.Y.Z., Y.J.H., W.D.Z., Y.Y.Z., Y.T.L., J.L.L., and X.Y.W. designed the experiment plan. C.Y.Z., Y.J.H., W.D.Z. and Y.Y.Z. collected data on traditional Chinese medicine prescriptions. C.Y.Z., Y.J.H. and Y.T.L collected disease targets and related information. C.Y.Z. and Y.Y.Z visualized the data. C.Y.Z. and W.D.Z analyzed the data. C.Y.Z. and Y.J.H. wrote the manuscript. J.L.L. and X.Y.W revised the manuscript. All of the authors read and approved the final manuscript.

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Correspondence to Xiying Wei or Junling Li.

### Ethics declarations

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

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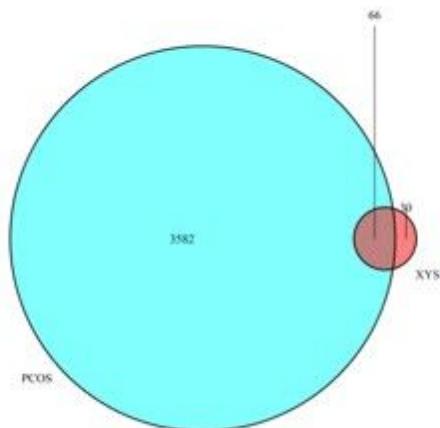
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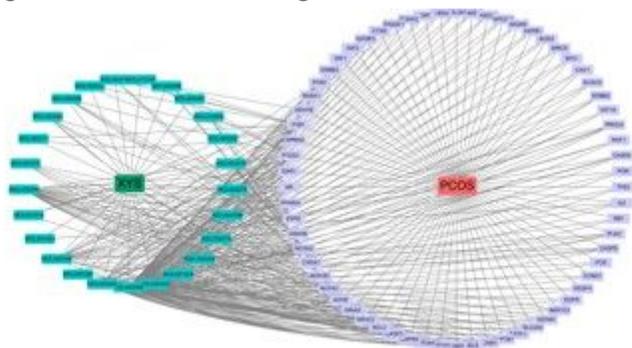
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## Figures



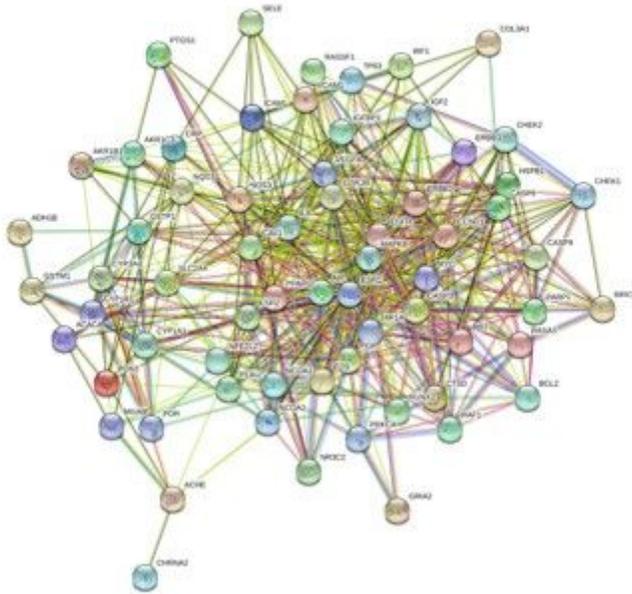
**Figure 1**

Venn diagram of the cross target of XYX and PCOS. There are 66 potential targets in XYX for PCOS.



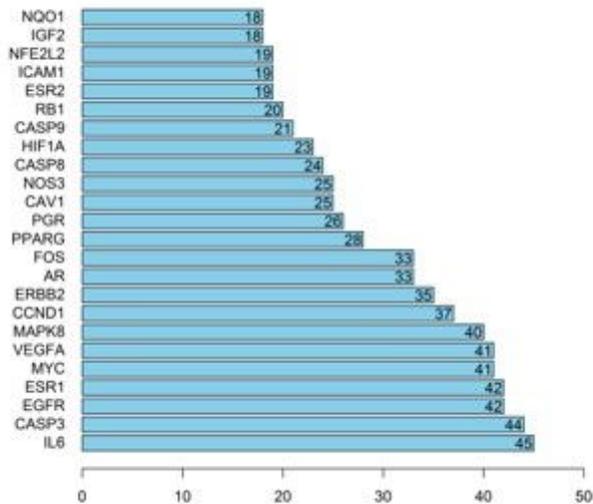
**Figure 2**

“XYS-Active component-Target-PCOS” network There are 93 nodes (25 active component nodes, 66 gene nodes, 1 compound node, 1 disease node) and 373 edges in the network.



**Figure 3**

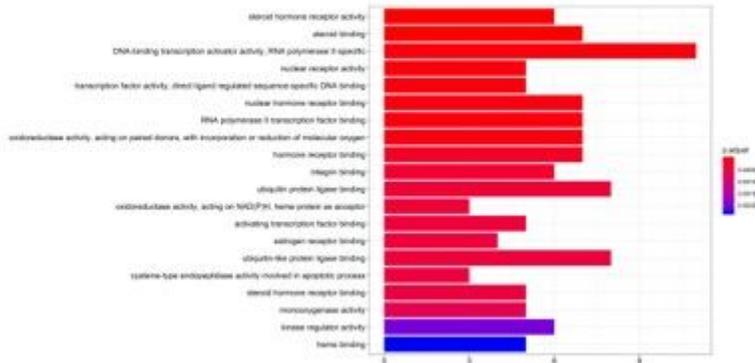
PPI network of XYs in the treatment of PCOS



**Figure 4**

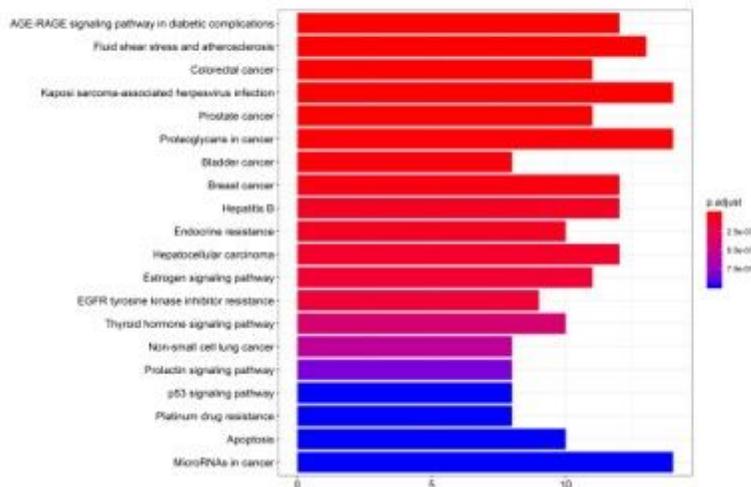
Key genes in protein interaction The targets with high degree in the figure were Interleukin-6(IL6), Caspase-3(CASP3), Epidermal growth factor receptor(EGFR), Estrogen receptor(ESR1), Myc proto-oncogene

protein(MYC), Vascular endothelial growth factor A(VEGFA), Mitogen-activated protein kinase 8(MAPK8), G1/S-specific cyclin-D1(CCND1), Receptor tyrosine-protein kinase erbB-2(ERBB2) and so on.



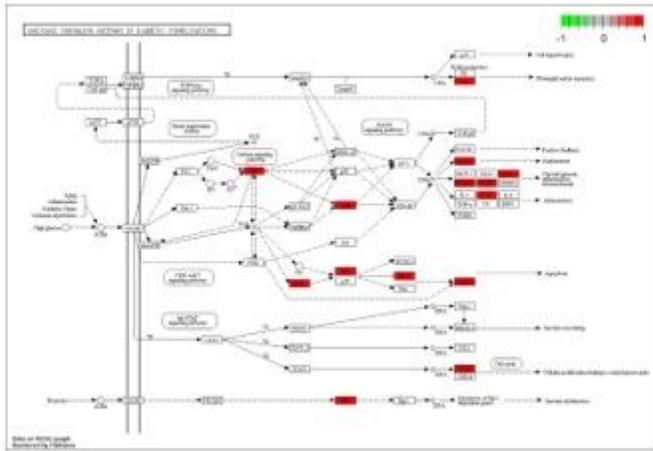
**Figure 5**

Visualization results of GO functional enrichment analysis The first 20 biological function enrichment analysis results are all molecular function related items, mainly involving the steroid hormone receptor activity, steroid binding, DNA-binding transcription activator activity, RNA polymerase II-specific nuclear receptor activity, transcription factor activity, direct ligand regulated sequence-specific, DNA binding, nuclear hormone receptor binding and so on.



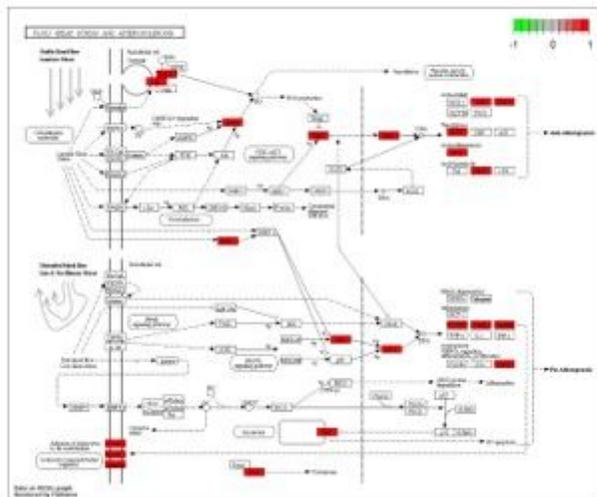
**Figure 6**

Visualization results of KEGG pathway enrichment analysis The first 20 are AGE-RAGE signaling pathway in diabetic complications, Fluid shear stress and atherosclerosis, Colorectal cancer, Kaposi sarcoma-associated herpesvirus infection, Prostate cancer, Proteoglycans in cancer, Bladder cancer, Breast cancer and so on.



**Figure 7**

AGE-RAGE signaling pathway in diabetic complications



**Figure 8**

Fluid shear stress and atherosclerosis

