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Mechanism of Bushen Tongluo granule on osteoarthritis based on network pharmacology and molecular docking technology

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Author contributions

Dan Wang designed the study, analyzed the data and wrote the manuscript. Yue-Lan Zhu and Jiang-Xi Xu revised the manuscript. Zheng-Dong Shen and Yun Du analyzed the data. All authors read and approved the final manuscript.

Competing interests

The authors declare no conflicts of interest.

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Data Availability

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

Abbreviations

OA, osteoarthritis; TCM, traditional Chinese medicine; BSTLG, Bushen Tongluo granule; PPI, protein-protein interaction; MF, molecular function; BP, biological process; GO, Gene Ontology; KEGG,Kyoto Encyclopedia of Genes and Genomes; CC, cellular component; VEGF, vascular endothelial growth factor; MAPK, mitogen-activated protein kinase; TNF, the tumor necrosis factor; TLR, toll-like receptor; Th17, T helper cell 17; HIF-1, hypoxia-inducible factor-1; AKT, protein kinase B; IL, interleukin; VEGFA, vascular endothelial growth factor A; PTGS2, prostaglandin-endoperoxide synthase 2; COX-2, cyclooxygenase-2; iNOS, inducible nitric oxide synthase; MMPs, matrix metalloproteinases; NF-κB, nuclear factor kappa-B.

Peer review information

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Abstract

Objective: In this study, we used network pharmacology and molecular docking technology to analyze the mechanism of Bushen Tongluo granule in the treatment of osteoarthritis. **Methods:** The main active components and corresponding targets of Bushen Tongluo granule were screened from the traditional Chinese medicine systems pharmacology database. The targets related to osteoarthritis were collected from the Online Mendelian Inheritance in Man, Therapeutic Target database, GeneCards, Pharmacogenomics Knowledgebases and Drugbank databases. Cytoscape 3.9.0 software was used to construct the action network diagram of "Bushen Tongluo Granule-Active Component-Target". Builded a protein-protein interaction network from the STRING database. The Bioconductor platform and R language 4.0.3 were used for Gene Ontology function and Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis. Then, selected the pathway most associated with osteoarthritis for specific analysis. Finally, the core genes were screened and verified by molecular docking using AutoDockTools software. **Results:** 71 principal components of Bushen Tongluo granule and 183 potential therapeu-tic targets for osteoarthritis were obtained. Twenty-eight key targets of Bushen Tongluo granule in the treatment of osteoarthritis are enriched in 158 pathways. Among them, the tumor necrosis factor signaling pathway, interleukin-17 signaling pathway, Toll-like receptor signaling pathway, T helper cell 17 cell differentiation and hypoxia-inducible factor-1 signaling pathway involved in key targets are closely related to osteoarthritis. The relevant vital targets were involved in the regulation of DNA transcription factor activity, the response to chemical stress, the response to reactive oxygen species, the response to oxidative stress, the proliferation of muscle cells, the proliferation of epithelial cells and the biological processes such as responses to lipopolysaccharides, responses to molecules of bacterial origin. Molecular docking showed that protein kinase B 1-β-sitosterol, the tumor necrosis factor-naringenin, interleukin-6-luteolin, mitogen-activated protein kinase-quercetin, vascular endothelial growth factor A-quercetin and prostaglandin-endoperoxide synthase 2-luteolin have strong docking activities. **Conclusions:** Bushen Tongluo granule can reduce the inflammatory response and inhibit articular cartilage angiogenesis in the treatment of osteoarthritis, which may be achieved by regulating the inflammatory signaling pathway and hypoxia-inducible factor-1 signaling pathway.

Keywords: Bushen Tongluo granule; osteoarthritis; network pharmacology; molecular docking

Highlights

Osteoarthritis has a certain impact on the body and mind of patients, moreover, the high disability rate has also caused a great burden to the society. At present, the pathogenesis of osteoarthritis has not been fully elucidated and the therapeutic effect is not ideal. In this study, the key targets and active components of Bushen Tongluo Granule were obtained. The molecular docking results showed that protein kinase B 1-β-sitosterol, the tumor necrosis factor-naringenin, interleukin-6-luteolin, vascular endothelial growth factor A-quercetin, mitogen-activated protein kinase 3-quercetin and prostaglandin-endoperoxide synthase 2-lut-eolin have strong docking activities.

Background

Osteoarthritis (OA) is a common disease with a high incidence. The prevalence of knee OA in the United States has doubled since the mid-20th century and the number may increase to 67 million by 2030 [1, 2]. In China, the prevalence of OA is about 14.6% and increases almost linearly after age 40 [3]. At present, about 250 million people worldwide suffer from OA [4]. Study shows OA patients are 1.27 times more likely to have suicidal ideation than normal people [5]. It can be seen that OA seriously affects the physical and mental health of patients; moreover, the high disability rate has also caused a great burden to the society. At present, the pathogenesis of OA has not been fully elucidated. Modern treatments, including drug therapy, non-drug therapy and surgical treatment have been widely used. However, the therapeutic effect is not ideal and there are disadvantages such as uncertain efficacy, many adverse reactions and high cost [6].

Traditional Chinese medicine (TCM) classifies OA in the categories of “Bi Syndrome” and “Bone Paralysis”. “Bone Bi” first appeared in the “Yellow Emperor’s Canon of Internal Classic (unknown author, 221 B.C.E.–220 C.E.)”, which is the source of the theory of Bi syndrome in Chinese medicine. *Su Wen • Bi Lun* contains: “Wind, cold and dampness are mixed together to form Bi”, which was the first to put forward the etiology and pathogenesis of Bi syndrome. In China, TCM therapies are an important method for preventing and treating OA [7]. TCM has the advantages of simplicity, convenience, cheapness and effectiveness and has received extensive attention in the field of clinical treatment and new drug development [8]. Bushen Tongluo granule (BSTLG) is an empirical prescription for OA treatment created by Professor Zhu Yuelan. Professor Zhu believes that “kidney deficiency and collateral obstruction” is the main pathogenesis of OA. Deficiency of the kidney leads to bone marrow loss and bone loss. Insufficiency of kidney essence and deficiency of kidney qi are the causes of OA. Obstruction of collaterals, abnormal functioning of qi, blood and body fluids and failure of normal function due to clumps of diseased collaterals are an important factor in the occurrence of OA. Professor Zhu believes that “tonifying the kidney and dredging collaterals” is an effective method for the treatment of OA. According to the therapeutic principle of tonifying the kidney and dredging collaterals, Professor Zhu founded BSTLG based on years of experience. BSTLG is composed of seven TCM of *Sangjisheng*, *Chuanxiong*, *Baishao*, *Chuanniuxi*, *Jixueteng*, *Gusuibu* and *Chuanshanlong*. Among them, *Gusuibu* and *Chuanniuxi* are the monarch medicines, both of which have the functions of invigorating the kidney and activating blood, reducing swelling and relieving pain. *Sangjisheng*, *Baishao* and *Chuanxiong* are the minister medicines, which assist the monarch medicine in enhancing the functions of nourishing the liver and kidney, promoting blood circulation and dredging collaterals. Accompanied by Jixueteng and *Chuanshanlong*, they help all medicines to reach the disease center. The whole formula is warm but not dry, nourishing but not greasy, static and dynamic, combined with tonifying and nourishing and plays the role of nourishing liver and kidney, promoting blood circulation and dredging collaterals. Our previous study showed that BSTLG could protect damaged chondrocytes by regulating the Wnt/β-catenin signaling pathway and transforming growth factor-β/bonemorphogenetic proteins signaling pathway. It also has multiple functions such as anti-inflammatory, scavenging oxygen free radicals, reducing the damage of articular cartilage matrix, promoting cartilage repair and regulating subchondral bone remodeling. This prescription is used for the treatment of OA, which can significantly improve the symptoms of pain, swelling and functional limitation in patients with OA, reduce inflammatory factors such as interleukin (IL)-1 and the tumor necrosis factor (TNF)-α and improve the quality of life of patients. The previous research group has completed the pharmacodynamics experimental and toxicology studies to prove that BSTLG has no obvious adverse reactions.

As a TCM compound, BSTLG has complex components and studying the mechanism of action is challenging. Network pharmacology can predict and analyze the mechanism of action of Chinese herbal compounds, which is fit for studying the mechanism of TCM compounds [9]. It has become a trend to choose network pharmacology technology to study the pharmacodynamic material basis and molecular mechanism of TCM [10]. Molecular docking technology has been widely used in the field of new drug research and development [11]. We speculate that BSTLG treats OA by acting on multiple signaling pathways and targets. This study aimed to analyze the possible mechanism of action of BSTLG in the treatment of OA using network pharmacology and molecular docking technology.

Methods

Materials

The materials and tools used in this study are detailed in Table 1.

BSTLG’s effective active components screening and identification

BSTLG is composed of *Baishao*, *Chuanniuxi*, *Chuanxiong*, *Chuanshanlong*, *Gusuibu*, *Jixueteng* and *Sangjisheng*, a total of seven TCM. These herbs were sequentially entered into the traditional Chinese medicine systems pharmacology for retrieval. The active compounds in the formula were screened according to oral bioavailability ≥ 30% and drug similarity ≥ 0.18 [12, 13].

BSTLG’s action targets prediction and screening

The potential protein targets of the main active components were collected through the traditional Chinese medicine systems pharmacology database and analysis platform and English names of the screened target proteins were entered into the Uniprot database. We entered the screened main active ingredients into the PubChem database and downloaded the corresponding 2D structure map, imported the 2D structure into the Swiss Target Prediction database for target prediction and evaluated the reliability of the prediction results with the confidence threshold (Probability). It was designated as homo sapiens, screened with $P > 0$ and collected targets corresponding to each active ingredient.

Screening of OA disease targets

Using “OA” as the keyword, we screened Therapeutic Target database, DrugBank, GeneCards, Pharmacogenomics Knowledgebase, Online Mendelian Inheritance in Man, for targets related to OA [14–18]. After deduplication, OA-related targets were obtained and a venn diagram was drawn.

Potential targets of BSTLG in the treatment of OA

Use the venny 2.1 program to match the active ingredient targets of BSTLG with the related targets of OA, and draw the venn diagram to obtain the intersection targets, that is, the therapeutic targets. Cytoscape 3.9.0 software was used to construct the action network diagram of “drugsactive components-potential therapeutic targets of disease”.

Table 1 The materials and tools used in this study

No.	Name	Website
1	Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform	http://tcmspw.com/tcmsp.php
2	Swiss Target Prediction	http//www.swisstargetprediction.ch/
3	Online Mendelian Inheritance in Man	http://www.omim.org/
4	Therapeutic Target Database	http://db.idrblab.net/ttd/
5	GeneCards	http://www.Genecards.org/
6	Pharmacogenetics and Pharmacogenomics Knowledgebase	https://www.pharmgkb.org/
7	Drugbank	https://www.drugbank.ca/
8	Venny 2.1	https://bioinfogp.cnb.csic.es/tools/venny/index.html
9	Cytoscape software version 3.9.0	https://cytoscape.org/
10	STRING Protein-Protein Interactions Database	https://string-db.org/
11	R Language 4.0.3	https://www.r-project.org/
12	Bioconductor	https://www.bioconductor.org/
13	The Research Collaboratory for Structural Bioinformatics Protein Data Bank	http://www.rcsb.org/
14	PyMOL 2.4.0	https://pymol.org/2/
15	Chem 3D	https://www.chemdraw.com.cn/apply.html
16	AutoDockTools-1.5.7	https://autodock.scripps.edu/
17	Uniprot	https://www.uniprot.org/
18	PubChem	https://pubchem.ncbi.nlm.nih.gov/

Construction of a protein-protein interaction (PPI) network for key targets

STRING is used to the retrieval of interacting genes and proteins [19]. Enter the common targets of OA and BSTLG into the STRING database, set the species to "Homo snpiens", set the minimum required interaction score to moderate reliability of 0.400, keep other parameters at their default values and hide the discrete targets, the PPI network is constructed. Then import the obtained "string_interactions.tsv" file into the Cytoscape 3.9.0 software and use the CytoNCA plug-in to perform topology analysis on the network. With the values of betweenness, closeness, degree, eigenvector and local average connectivity-based method and network greater than the median value.

Gene Ontology (GO) function and Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway Enrichment Analysis

Using the Bioconductor platform and R language 4.0.3, GO function enrichment and KEGG pathway enrichment analysis was performed on the key targets of BSTLG in the treatment of OA. The species was set to human, filtered at $P < 0.05$ and the significance of protein-related functions was expressed by the P value. GO enrichment was carried out according to molecular function (MF), biological process (BP) and cellular component (CC).

Molecular docking

Application of molecular docking technology to further verify the mechanism of BSTLG in the treatment of OA. Downloaded 2D structural information of key components from the PubChem database. Used ChemBio 3D software to convert the 2D structure into the 3D structure, optimized the energy of MM2 and completed the preparation of small molecule ligands. Downloaded 3D structures of core target proteins from Protein Data Bank. Next, protein receptors were prepared after removing water molecules and ligands with PyMOL 2.4.0. The protein receptor files were read using Autodocktools software and converted to PDBQT format after hydrotreating ion modification. Converted small molecule ligand files to pdbqt format, then converted to 2D structures to map active pockets. Use AutoDock vina software to do molecular docking and save the lowest binding energy data as the result of molecular docking.

Results

Main components of BSTLG and treatment targets of OA

According to oral bioavailability ≥ 30%, drug similarity ≥ 0.18, 71 compounds were screened from BSTLG, including 13 species of *Baishao*, 4 species of *Chuanniuxi*, 7 species of *Chuanxiong*, 3 species of *Chuanshanlong*, 18 species of *Gusuibu*, 24 species of *Jixueteng*, 2 species of *Sangjisheng*. A total of 47 effective active compounds were obtained after excluding duplicate values and non-targets (Table 2). 703 target genes were obtained by Swiss Target Prediction.

33 OA disease targets were obtained from Online Mendelian Inheritance in Man database, 24 marks were obtained from Therapeutic Target database, 827 targets were obtained from GeneCards database, 9 targets were obtained from Pharmacogenomics Knowledgebase database and 361 targets were obtained from Drugbank database. After deduplication, a total of 966 OA disease targets were obtained (Figure 1). By matching the potential targets of BSTLG with OA targets, 183 cross-genes were obtained, which were potential targets of BSTLG for the treatment of OA (Figure 2).

Construction of regulatory network and PPI network

Using Cytoscape 3.9.0 software to build a network diagram of "drugsactive components-disease targets" (Figure 3). Among them, the top five components of degree value were luteolin (degree = 46), beta-sitosterol (degree = 46), kaempferol (degree = 45), quercetin (degree = 44) and naringenin (degree = 38) (Table 3). These components of BSTLG may be key active components in the treatment of OA. Enter the BSTLG key components into the STRING database to obtain the PPI network. Excluding isolated nodes, the network includes 182 nodes, 2,240 edges, an average node degree of 24.5 and an average local clustering coefficient of 0.575. After calculating the median value of each parameter, 28 targets were obtained; selected the top six displayed (Figure 4, Table 4).

Table 2 BSTLG effective active component information

ID	Mol ID	Component	OB (%)	DL
BS1	MOL000211	Betulinic acid	55.38	0.78
BS2	MOL001918	[[1S, 3S, 6R, 8R, 10S]-8-hydroxy-3-methyl-5-oxo-2,9-dioxatricyclo [4.3.1.03,8] decan-10-yl] methyl benzoate	87.59	0.37
BS3	MOL001930	Benzoylpaeoniflorin	31.27	0.75
BS4	MOL001924	Paeoniflorin	53.87	0.79
BS5	MOL001919	Palbinone	43.56	0.53
CNX1	MOL012286	Betavulgarin	68.75	0.39
CNX2	MOL012298	Rubrosterone	32.69	0.47
CSL1	MOL000133	7-Epi-taxol	45.18	0.24
CSL2	MOL000139	Smitilbin	37.60	0.74
CSL3	MOL000138	Diosgenin palmitate	33.58	0.27
CX1	MOL001494	Wallichilide	42.00	0.19
CX2	MOL002151	2-Methyl-6-((2e,6e)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl)cyclohexa-2,5-diene-1,4-dion e	47.66	0.24
CX3	MOL002140	Ethyl linoleate	65.95	0.27
CX4	MOL002135	Myricanone	40.60	0.51
CX5	MOL000433	CID6037	68.96	0.71
GSB1	MOL004328	Naringenin	59.29	0.21
GSB2	MOL002914	Eriodyctiol (Flavanone)	41.35	0.24
GSB3	MOL005190	Eriodictyol	71.79	0.24
GSB4	MOL001040	(2R)-5,7-dihydroxy-2-(4-hydroxyphenyl)-2,3-dihydro-4h-chromen-4-one	42.36	0.21
GSB5	MOL001978	Aureusidin	53.42	0.24
GSB6	MOL009061	22-Stigmasten-3-one	39.25	0.76
GSB7	MOL009091	Xanthogalenol	41.08	0.32
GSB8	MOL009075	Cycloartenone	40.57	0.79
GSB9	MOL009063	Cyclolaudenol acetate	41.66	0.79
GSB10	MOL000569	Digallic acid	61.85	0.26
GSB11	MOL009076	Cyclolaudenol acetate	39.05	0.79
JXT1	MOL000296	Hederagenin	36.91	0.75
JXT2	MOL000491	CID 25717254	37.50	0.66
JXT3	MOL000493	Campesterol	37.58	0.71
JXT4	MOL000497	Licochalcone a	40.79	0.29
JXT5	MOL000500	(+)-Vestitol	74.66	0.21
JXT6	MOL000502	Cajanin	68.80	0.27
JXT7	MOL000503	Medicagol	57.49	0.60
JXT8	MOL000506	(+)-Sparteine	61.89	0.21
JXT9	MOL000507	Pseudobaptigenin	70.12	0.31
JXT10	MOL000033	(24S)-24-Propylcholesta-5-ene-3beta-ol	36.23	0.78
JXT11	MOL000392	Formononetin	69.67	0.21
JXT12	MOL000417	Calycosin	83.38	0.24
JXT13	MOL000468	8-O-methylretusin	70.32	0.27
JXT14	MOL000469	3-Hydroxystigmast-5-en-7-one	40.93	0.78
JXT15	MOL000483	N-cis-feruloyltyramine	118.35	0.26
M1	MOL000359	3-Epi-beta-sitosterol	36.91	0.75
M2	MOL000358	Beta-sitosterol	36.91	0.75
M3	MOL000006	Luteolin	36.16	0.25
M4	MOL000422	Kaempferol	41.88	0.24
M5	MOL000098	Quercetin	46.43	0.28
M6	MOL000449	Stigmasterol	43.83	0.76

M, represents a common ingredient; M1: *Sangjisheng*, *Chuanxiong*, *Baishao*; M2: *Jixueteng*, *Gusuibu*, *Chuanniuxi*, *Baishao*; M3: *Jixueteng*, *Gusuibu*; M4: *Gusuibu*, *Baishao*; M5: *Sangjisheng*, *Chuanniuxi*; M6: *Jixueteng*, *Gusuibu*. BS, *Baishao*; CNX, *Chuanniuxi*; CSL, *Chuanshanlong*; CX, *Chuanxiong*; GSB, *Gusuibu*; JXT, *Jixueteng*. BSTLG, Bushen Tongluo granule; ID, identity; Mol ID, molecular identity; CID,compound identity; OB, oral bioavailability; DL, drug similarity.

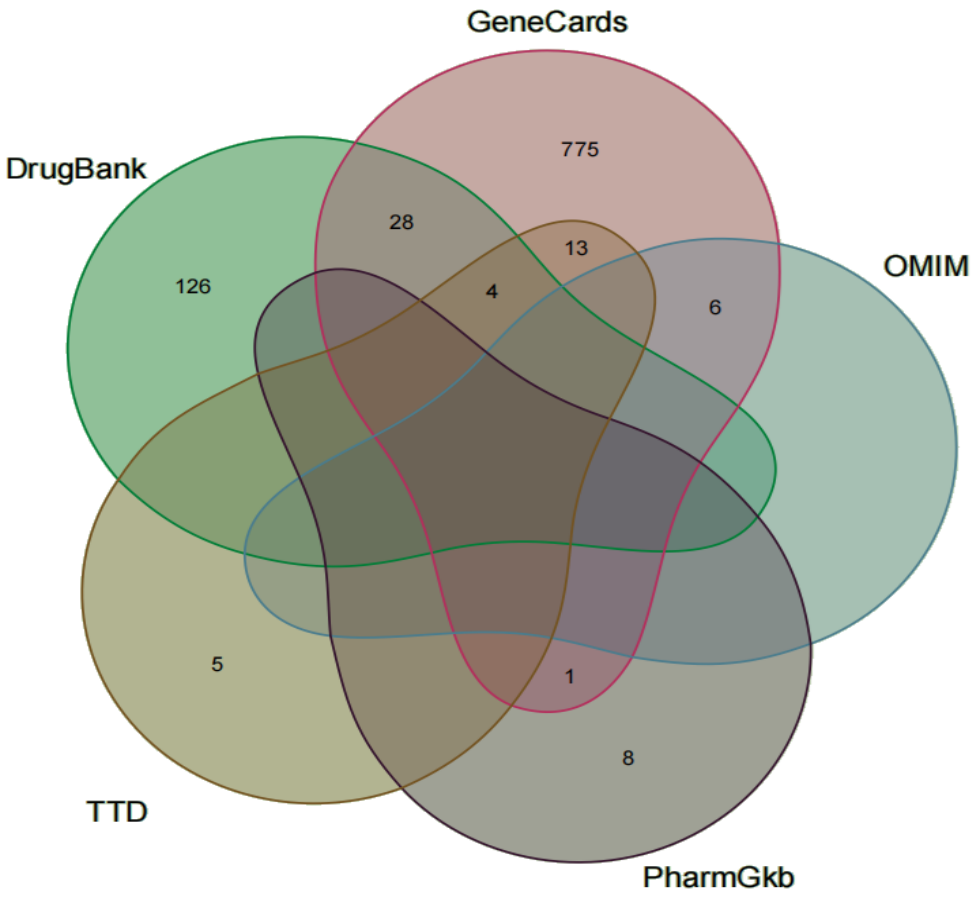


Figure 1 Venn diagram of osteoarthritis disease targets. OMIM, Online Mendelian Inheritance in Man; TTD, Therapeutic Target Database; PharmGkb, Pharmacogenomics Knowledgebase.

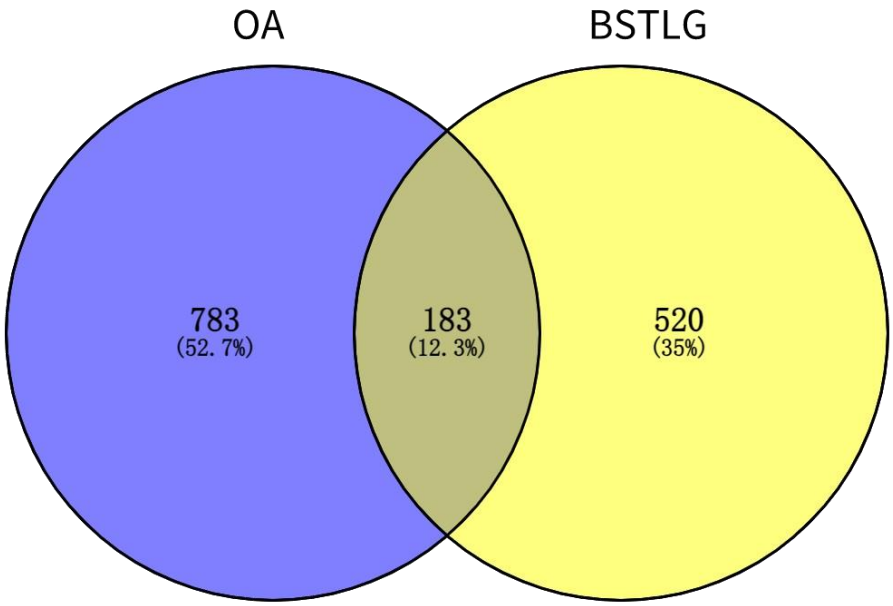


Figure 2 Venn diagram of BSTLG and OA targets. OA, osteoarthritis; BSTLG, Bushen Tongluo granule.

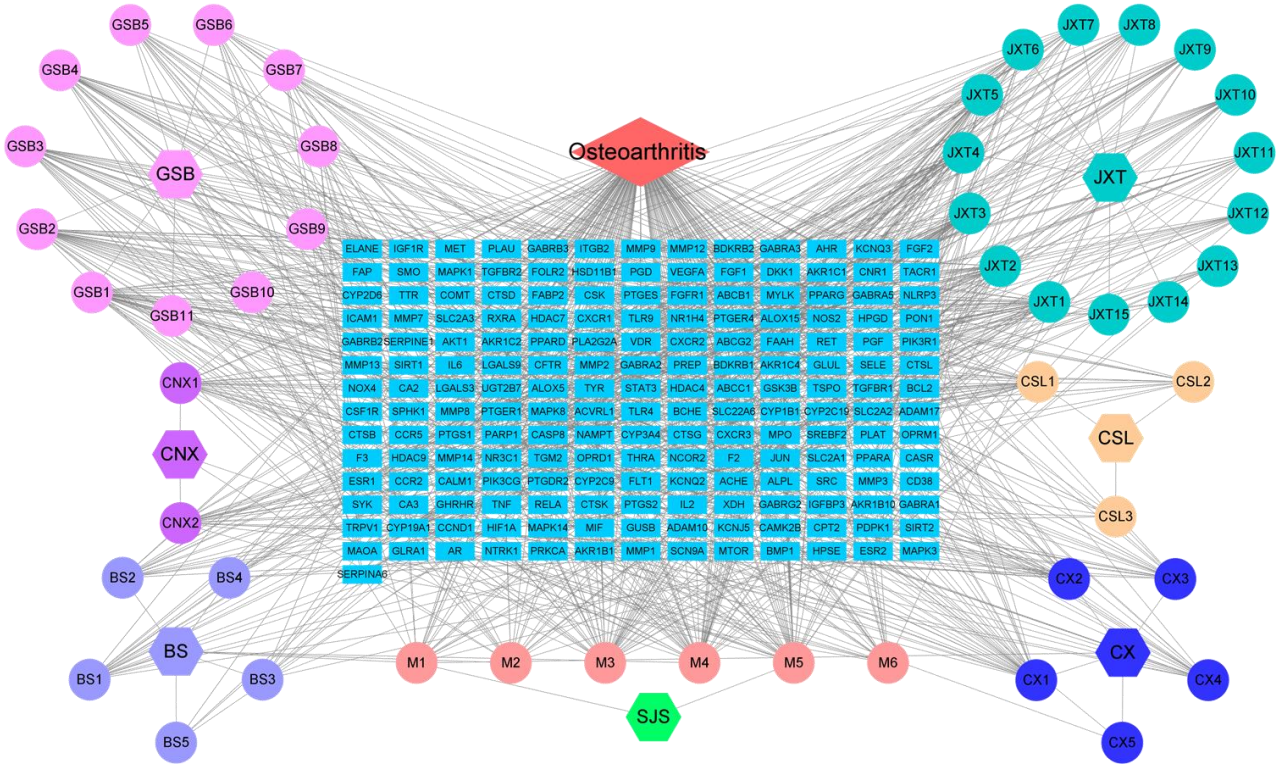
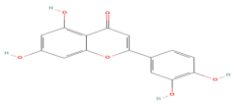
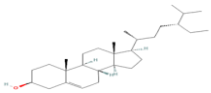
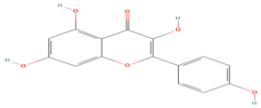
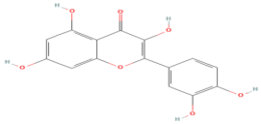
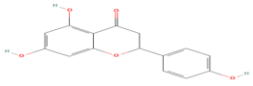


Figure 3 The "BSTLG-Active Component-Target" network. The circular node represents the chemical composition of the drug, the rectangular node represents the gene target and M1-6 represent common components. BSTLG, Bushen Tongluo granule.

Table 3 Key active components

Compound	Degree	Molecular Formula	Structure
Luteolin	46	C15H10O6	
beta-Sitosterol	46	C29H50O	
Kaempferol	45	C15H10O6	
Quercetin	44	C15H10O7	
Naringenin	38	C15H12O5	

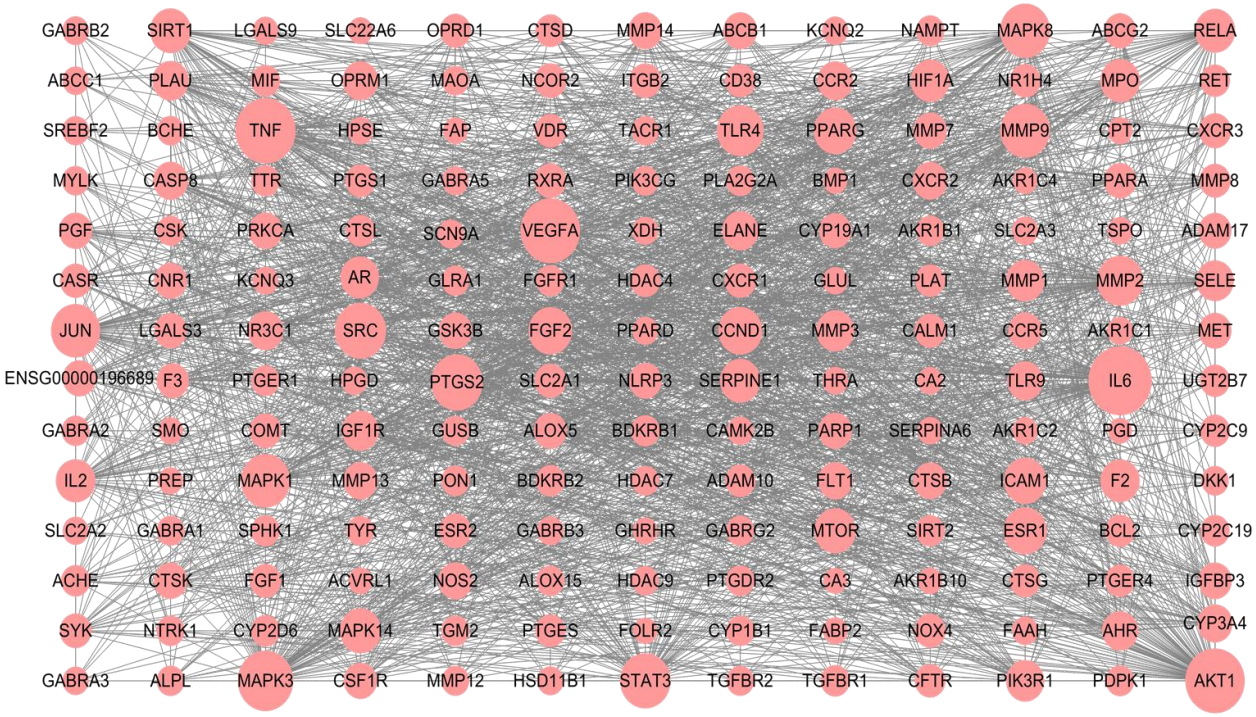


Figure 4 PPI network. The larger the node, the higher the degree. PPI, protein-protein interaction.

Table 4 Core targets (sort by degree)

Target	Betweenness	Closeness	Degree	Eigenvector	LAC	Network
AKT1	176.9814679	0.984126984	61	0.20166935	29.37704918	60.57260217
TNF	174.8618154	0.984126984	61	0.201793283	29.40983607	60.58541891
IL-6	166.2129323	0.96875	60	0.199459612	29.2	58.90008775
VEGFA	152.8554551	0.953846154	59	0.198498651	29.42372881	57.67038097
MAPK3	110.1704908	0.873239437	53	0.184229553	28.33962264	48.63399849
PTGS2	91.7565836	0.861111111	52	0.184275657	28.96153846	47.97650023

LAC, local average connectivity-based method; AKT, protein kinase B; TNF, the tumor necrosis factor; IL, interleukin; VEGFA, vascular endothelial growth factor A; MAPK, mitogen-activated protein kinase; PTGS, prostaglandin-endoperoxide synthase.

GO function and KEGG pathway enrichment analysis

Analysis by GO function, 116 MF, 2032 BP and 50 CC were obtained and the entries with the top 10 enrichment degrees were visualized (Figure 5). The results of bioprocess analysis showed that active components of BSTLG in the human body mainly include response to chemical stress, response to reactive oxygen species, response to oxidative stress, muscle cell proliferation, epithelial cell proliferation, BPs and responses to molecules of bacterial origin. CCs mainly include western cave, membrane microstructure, membrane region, plasma membrane, glutamic acid synaptic, transcriptional regulatory factor complex, pseud, nuclear film and swallowing cup. The MFs mainly include mitogen-activated protein kinase (MAPK) activity, phosphatase binding, protein-threonine/tyrosine kinase activity, growth combination of factor receptor, and nuclear hormone receptor.

KEGG pathway enrichment analysis showed that 28 key targets were enriched in 158 pathways and selected the top 30 items for visualization (Figure 6). Among them, TNF signaling pathway, recombinant human IL-17 signaling pathway, toll-like receptor (TLR) signaling pathway, T helper cell 17 (Th17) cell differentiation and hypoxia-inducible factor-1 (HIF-1) signaling pathway involved in key targets are closely related to OA. Normal articular cartilage is avascular and angiogenesis is an important pathological feature of OA.

Vascular endothelial growth factor (VEGF) is closely related to angiogenesis, which can induce the formation of cartilage neovascularization leading to the occurrence of OA. VEGF can also directly participate in the destruction of cartilage and bone by increasing the permeability of blood vessels and promoting the secretion of various cartilage matrix-degrading enzymes. HIF-1α is one of the hypoxia-inducible factors that regulate VEGF expression; HIF-1α is activated in hypoxic microenviron-ment, leading to upregulation of its downstream target gene VEGF. HIF-1 signaling pathway is related to cartilage angiogenesis and inflammation and is choosed for visualization (Figure 7). The nodes in the red box are the key targets of BSTLG to regulate HIF-1 signaling pathway.

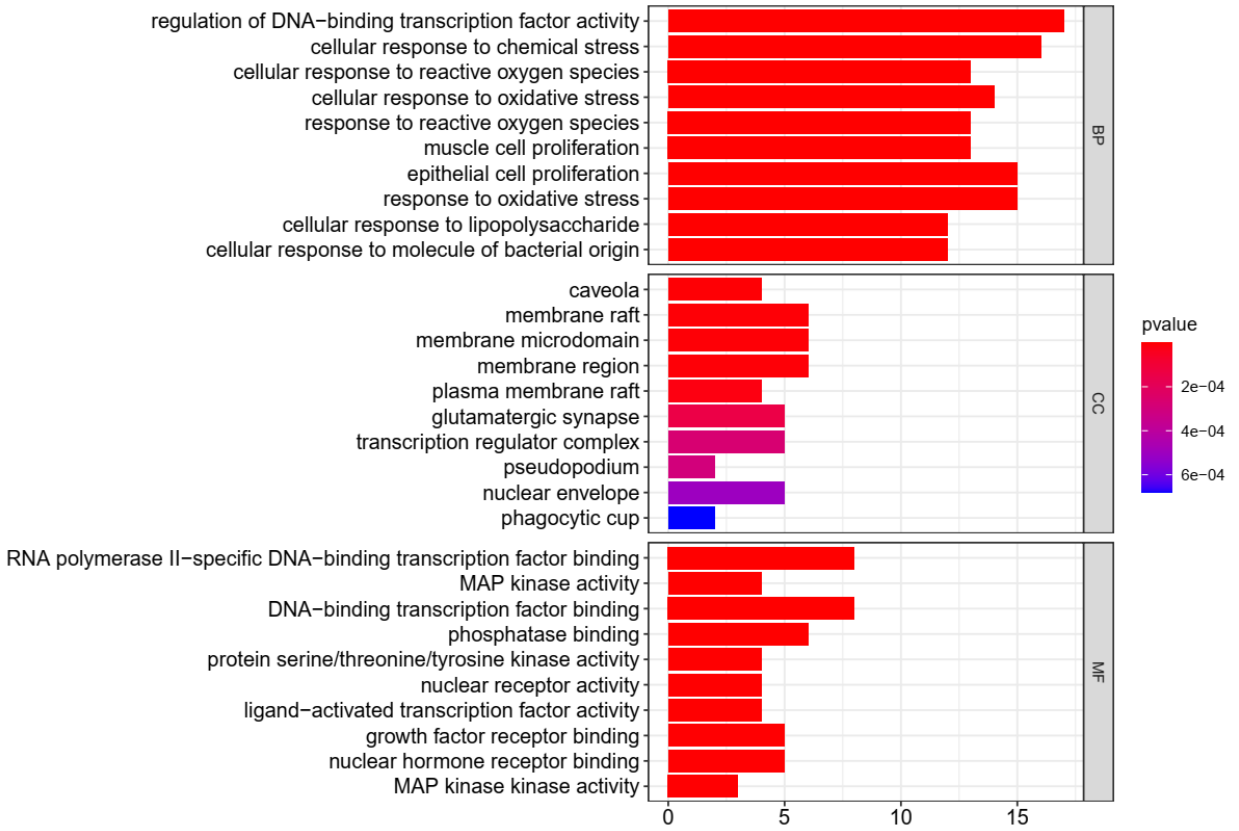


Figure 5 Go function analysis. GO, Gene Ontology.

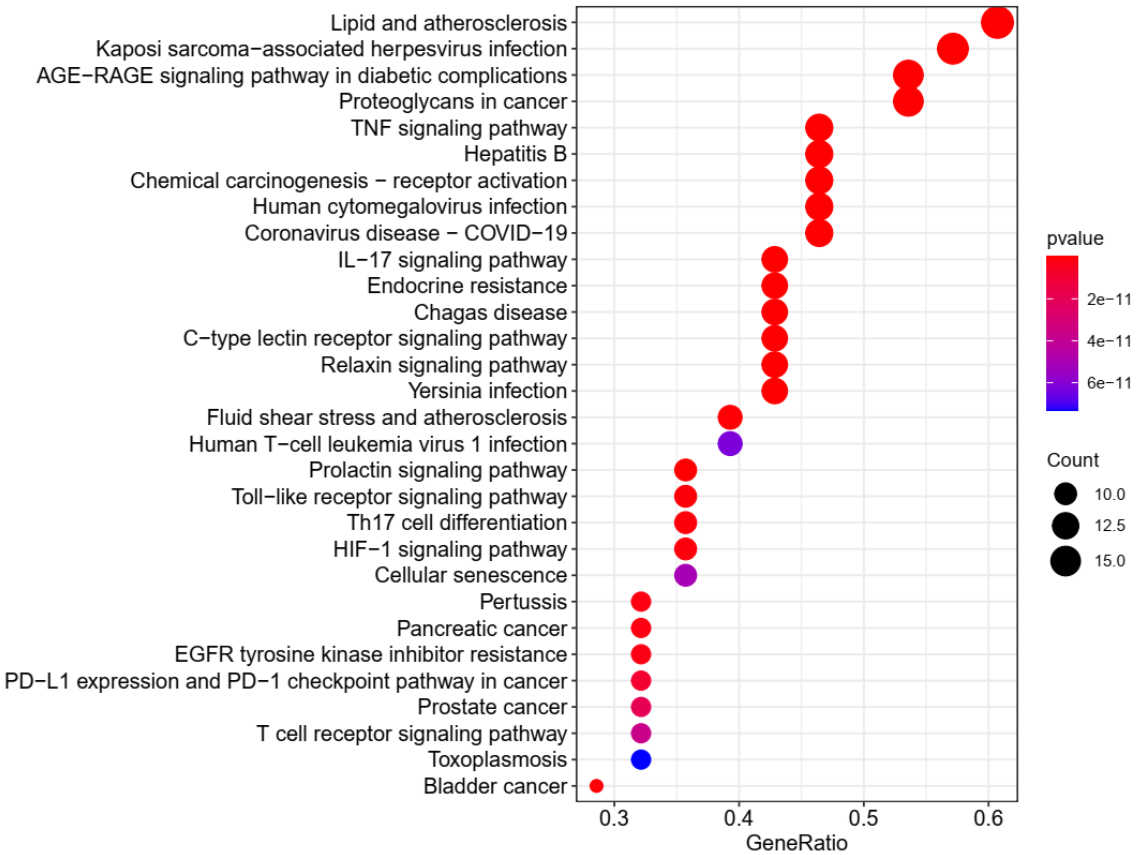


Figure 6 KEGG pathway analysis. KEGG, Kyoto Encyclopedia of Genes and Genomes.

Molecular docking verification

Select the top 5 components luteolin, β-sitosterol, kaempferol, quercetin and naringenin and the top 6 core targets serine/threoine-protein kinase B 1 (AKT1), TNF, IL-6, vascular endothelial growth factor A (VEGFA), MAPK3, prostaglandin-endoperoxide synthase 2 (PTGS2) for molecular analysis. The binding energy between drug component ligands and target receptors is an important indicator to evaluate the binding capacity [20]. When the binding energy is less than –5.0 kcal/mol, the docking affinity is stronger and when the binding energy is less than –7.0 kcal/mol, the docking activity is extremely strong [21].

According to the docking results, all of the binding energy is less than –5.0 kcal/mol and AKT1-β-sitosterol, TNF-naringenin, IL-6-luteolin, VEGFA-quercetin, MAPK3-quercetin and PTGS2-luteolin had the lower binding energies. In addition, quercetin and luteolin acted as ligands with the highest number of receptors. It is speculated from the above molecular docking process that BSTLG is mainly involved in the treatment of OA (Table 5, Figure 8).

Discussion

The composition of TCM is complex and difficult to study. TCM research first need to separate and extract the chemical components of herbs, then carry out molecular identification and finally evaluate the efficacy and activity through animal or cell experiments. These studies have a large workload, a long time, low work efficiency and a certain degree of blindness. Network pharmacology is a new discipline based on systems biology and bioinformatics, through parameter analysis of multi-level biological networks and selection of key nodes to design drug molecules. Using network pharmacology technology to predict

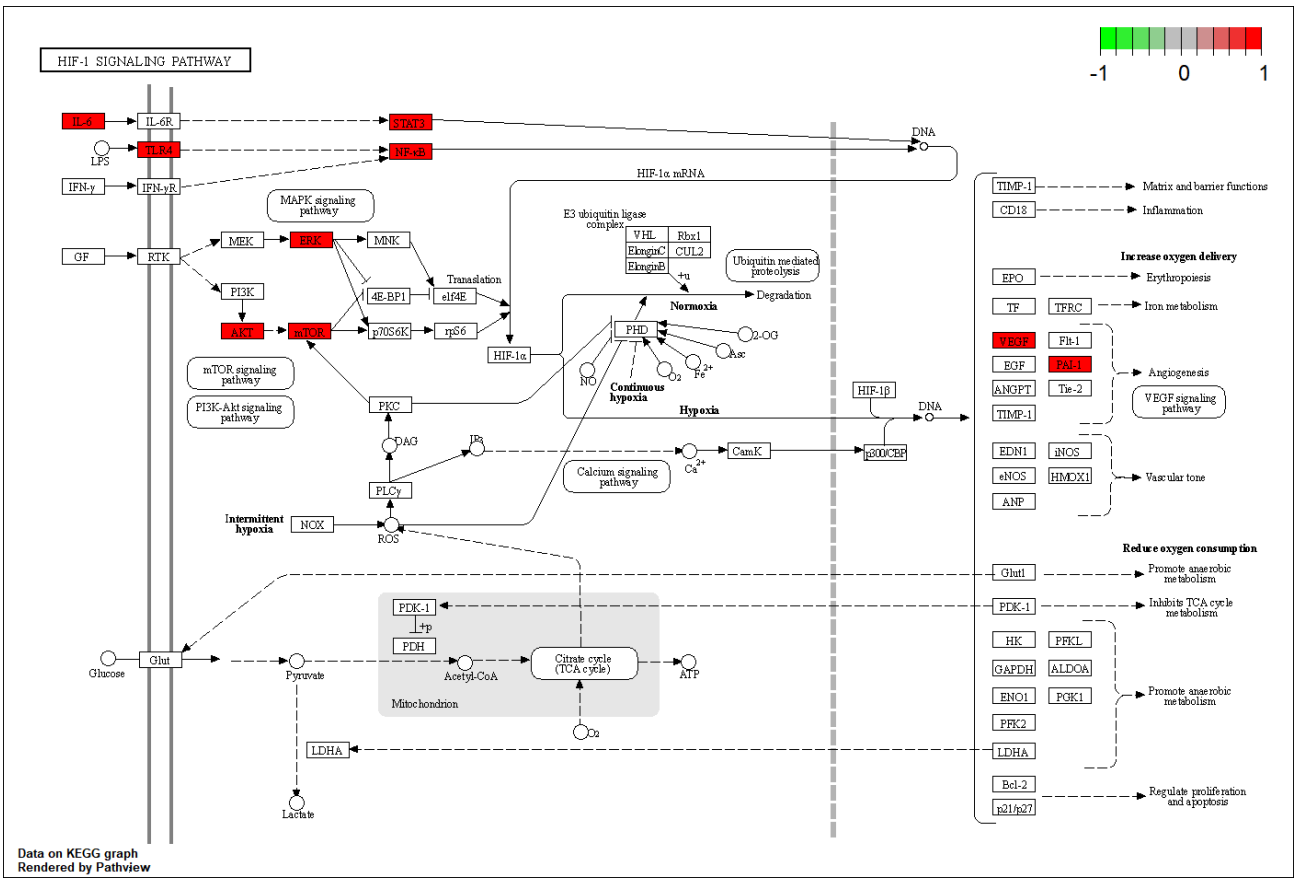


Figure 7 HIF-1 signaling pathway. HIF-1, hypoxia-inducible factor-1.

Table 5 Molecular docking (unit: kcal/mol)

Molecule name	AKT1	TNF	IL-6	VEGFA	MAPK3	PTGS2
Luteolin	–10.1	–8.6	–7.4	–7.3	–8.8	–10
Kaempferol	–9.9	–8.6	–7.0	–7.7	–8.8	–9.3
Quercetin	–10.4	–6.7	–7.1	–8.0	–9.0	–9.8
Naringenin	–7.5	–8.8	–6.9	–7.6	–8.6	–9.2
Beta-Sitosterol	–11.2	–6.6	–6.7	–6.4	–7.3	–8.1

AKT, protein kinase B; TNF, the tumor necrosis factor; IL, interleukin; VEGFA, vascular endothelial growth factor A; MAPK, mitogen-activated protein kinase; PTGS, prostaglandin-endoperoxide synthase.

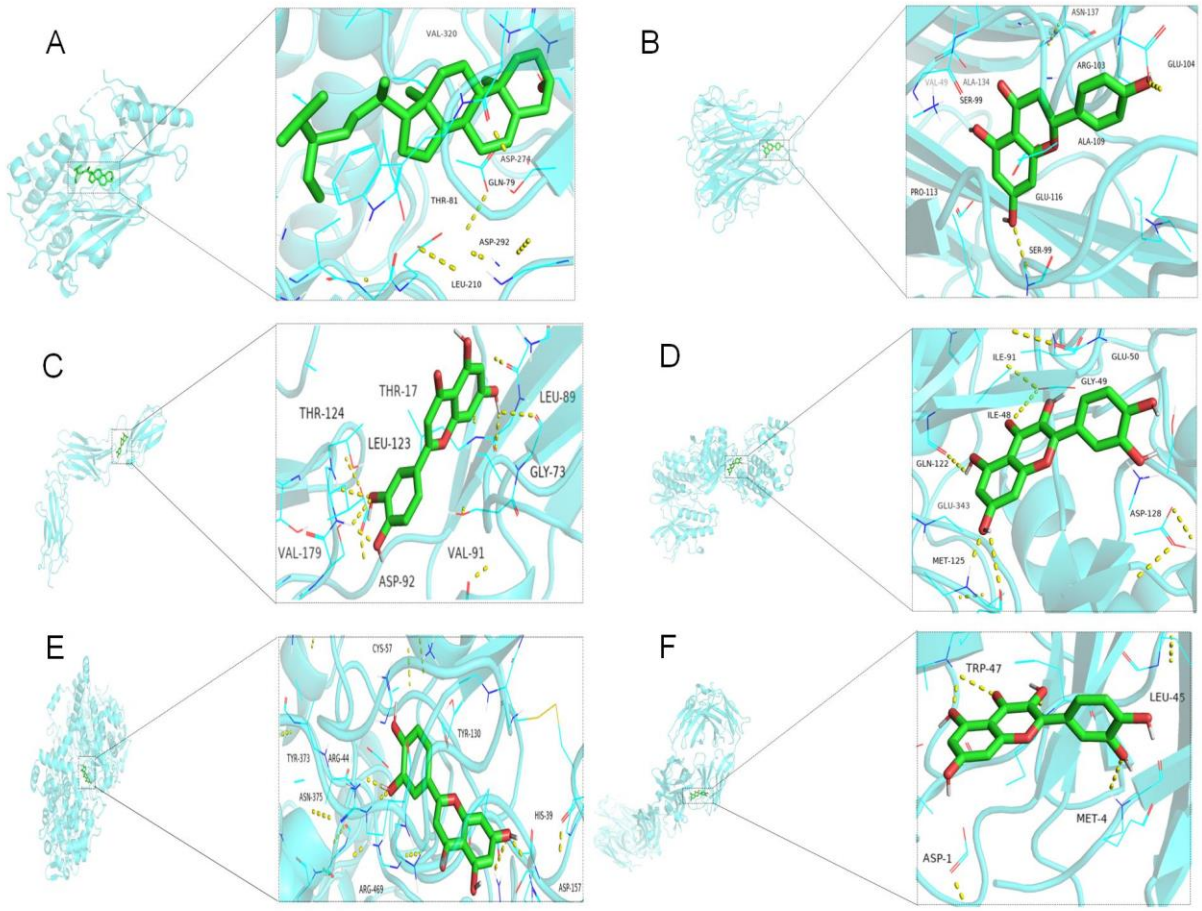


Figure 8 3D Molecular docking model of the 5 key active ingredients with the 6 hub-targets. A, AKT1-β-sitosterol; B, TNF-naringenin; C, IL-6-luteolin; D, MAPK3-quercetin; E, PTGS2-luteolin; F, VEGFA-quercetin. AKT, protein kinase B; TNF, the tumor necrosis factor; IL, interleukin; VEGFA, vascular endothelial growth factor A; MAPK, mitogen-activated protein kinase; PTGS, prostaglandin-endoperoxide synthase.

the active ingredients, exert clinical efficacy, screen out the core targets and important pathways and construct animal or cell models to verify their efficacy, which can save costs and improve scientific research efficiency [22]. Therefore, based on network pharmacology, this study explored the possible mechanism of action of BSTLG in the treatment of OA and further verified the predicted results using molecular docking technology. This study fully verified the molecular mechanism of BSTLG in the treatment of OA and provided a reference for clinical practice. Network topology analysis showed that the potential active components of BSTLG in the treatment of OA mainly included luteolin, β-sitosterol, kaempferol, quercetin and naringenin. Luteolin is a natural flavonoid found in a variety of plants. Has a variety of pharmacological activities, such as anti-inflammatory, acid-lowering, anti-tumor, antibacterial, antiviral, etc. Fei J et al. discovered that luteolin could significantly reduce the production of nitric oxide, TNF-α and the expression of cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS) and matrix metalloproteinases (MMPs) in rat chondrocytes induced by IL-1β and prevent the destruction of cartilage [23]. Sun Y et al. demonstrated that β-sitosterol could reduce expression of IL-6, iNOS, TNF-α and COX-2 in lipopolysaccharide-exposed mouse microglia cells; and alleviate inflammatory response via inhibiting the activation of extracellular regulated protein kinases/p38 and nuclear factor kappa-B (NF-κB) pathways [24]. Zhang F et al. discovered that β-sitosterol could significantly inhibit the level of VEGF in serum and tissue of arthritic rats [25]. Kaempferol belongs to flavonoids, which have anti-cancer, anti-cancer, anti-viral, anti-bacterial, anti-oxidative, anti-inflammatory and other effects, and has attracted more and more attention. Estakhri F et al. discovered that Kaempferol can down-regulate the expression of iNOS and COX-2, decrease the secretion of TNF-α, IL-1β and IL-6 and increase the expression of superoxide dismutase in articular cartilage [26]. Permatasari DA et al. discovered that quercetin could prevent proteoglycan destruction by inhibits IL-1β, TNF-α and MMP-9 expressions on OA model rats [27]. Hassan et al. found that quercetin can inhibit the expression of HIF-1α and enhance the efficacy of chemotherapy drugs [28]. Wang CC et al. discovered that naringenine can relieve the production of MMPs in OA chondrocytes by modulating the NF-κB signaling pathway and alleviate symptoms of joint pain in knee OA model rats [29]. A systematic review and meta-analysis showed that plant flavonoids such as luteolin, quercetin and naringenin could modulate HIF-1 and VEGF signaling pathways, have antiangiogenic activity and flavonoids are promising for the development of antiangiogenic drugs candidates [30]. It can be seen that the above active ingredients can reduce the overall inflammatory response and inhibit angiogenesis related factors.

Through building PPI network, obtained 6 key targets of BSTLG for the treatment of OA, namely AKT1, TNF, IL-6, VEGFA, MAPK3 and PTGS2. AKT1 is closely related to angiogenesis and studies have found that continuous endothelial activation of AKT1 leads to the formation of structurally abnormal blood vessels [31]. Ulici V et al. found that AKT1 leads to angiogenesis and ossification at the end of endochondr-al bone formation [32]. TNF and IL-6 are common inflammatory factors, important mediators involved in the process of OA and key factors causing inflammation. TNF-α and IL-6 can induce the production of matrix metalloproteinases and inhibit the synthesis of proteoglycans and type II collagen, which play a key role in the pathological development of OA [33]. VEGFA belongs to the VEGF

family. There are no blood vessels in normal articular cartilage, so VEGFA is hardly expressed in normal cartilage. VEGFA plays a key role in the regulation of articular cartilage catabolism and angiogenesis. Vadalà et al. found that inhibiting VEGF signaling can slow down OA progression and reduce angiogenesis [34]. MAPK3 belongs to the MAPK signaling pathway, which can mediate the proliferation and hypertrophy of chondrocytes, leading to cartilage calcification and osteophyte formation. PTGS2 (COX-2) is an inducible cyclooxygenase that is generally found in very low levels in normal tissues, but can be induced in inflammation and many pathological processes. COX-2 promotes the conversion of arachidonic acid into prostagla-ndin E2, increased secretion of prostagla-ndin E2 by chondrocytes and synovium, promoting the synthesis of various matrix metalloproteinases by chondrocytes, accelerating the degradation of proteoglyc can and type II collagen in the cartilage matrix, and inhibiting their synthesis. COX-2 selective inhibitor celecoxib can slow down cartilage degeneration in OA and reduce cartilage damage in OA [35]. Therefore, the aforementioned targets may play an important role in the development of inflammation and cartilage angiogenesis in OA.

GO/KEGG enrichment analysis showed that TNF signaling pathway, IL-17 signaling pathway, Toll-like receptor signaling pathway, Th17 cell differentiation and HIF-1 signaling pathway are closely related to OA. TNF-α is an important immunore-gulatory and pro-inflammatory factors, which can participate in the activation of IL1 and the inflammatory response mediated by IL6 and can also participate in the NF-κB inflammatory signaling pathway, which plays a key role in OA [36]. IL-17 is involved in a large number of inflammatory responses in the body and plays an important role in inflammatory diseases. IL-17 gene polymorphism was significantly associated with OA susceptibility. Faust et al.found that intra-articular injection of IL-17 neutralizing antibody reduced the expression of markers of joint degeneration and aging and attenuated articular cartilage damage [37]. TLR signaling pathway is related to the development of OA. TLR receptor-mediated innate immune response of synovial cells is a key link in promoting OA synovitis. Th17 can secrete cytokines such as IL-17, IL-21 and IL-6 and participate in multiple links of the inflammatory response and the imbalance of Th17/Treg cell ratio is closely related to bone joints. Cartilage angiogenesis is a very important pathological feature of OA and a key pathogenic factor of OA. HIF-1 signaling pathway is closely associated with angiogenesis. Under hypoxia, HIF-1α is up-regulated, leading to the activation of its downstream target gene VEGF, which in turn induces processes such as angiogenesis. In OA, inflammation and mechanical stress lead to tissue hypoxia, HIF-1α is activated, HIF-1α can promote the release of inflammatory factors such as TNF-α, IL-6 and aggravate the inflammatory response. Yudoh K et al. found that in OA patients, stronger HIF-1α mRNA expression in chondrocytes was observed in areas of joint degeneration than in normal areas, suggesting that the expression of HIF-1α mRNA is closely related to the progression of articular cartilage degeneration [38].

Finally, this study applied molecular docking technology to verify. The results showed that β-sitosterol could form a docking model with AKT1; luteolin can form a docking model with IL-6 and PTGS2; quercetin can dock with VEGFA and MAPK3; and naringenin can dock with TNF. The above docking results indicate that naringenin and luteolin may reduce joint inflammation by inhibiting the expression of inflammatory factors such as TNF, IL-6 and PTGS2; quercetin and β-sitosterol may reduce cartilage angiogenesis through the angiogenesis-related factors such as VEGFA, AKT1 and MAPK3. According to the calculations with Vina, β-sitosterol can form the most stable molecular docking with AKT1 compared to other components. Abnormal activation of AKT1 can lead to angiogenesis. AKT1 combined with β-sitosterol may inhibit cartilage angiogenesis.

“Luo disease” refers to a series of pathological changes in which the collaterals are affected by evil, which causes the collaterals to run qi and blood and block the function of nourishing the limbs. The pathological basis of “Luo disease” is collateral damage and the main feature is collateral stasis. The blockage of wind-cold-dampness pathogens leads to obstruction of the meridians and collaterals; the function and shape of the collaterals are damaged, resulting in pathological collaterals, which become “sick collaterals”. Anatomically, the new blood vessels and diseased collaterals are also very structurally resemblance. From the perspective of TCM, BSTLG has the effect of promoting blood circulation, removing blood stasis and dredging collaterals and from the perspective of modern medicine, BSTLG may inhibit cartilage angiogenesis by regulating angiogenesis-related factors and pathways.

In this study, the main mechanism of BSTLG in the treatment of OA was predicted by network pharmacology; the main components and targets of the prescription were obtained; and the predicted results were verified by molecular docking technology. This study provides a certain reference for clinical practice, and also provides ideas for experimental research. However, this study also has shortcomings. Although this study has carried out molecular docking verification on the prediction results, it has not carried out experimental verification, so there are still shortcomings.

Next, we will verify the predicted mechanism of BSTLG in the treatment of OA through in vivo experiments or in vitro experiments.

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