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A Network Pharmacology Approach to Estimate the Active Ingredients and Potential Targets of *Cuscutae semen* in the Treatment of Osteoporosis

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

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Background: Osteoporosis is a metabolic osteopathy characterized by abnormal bone mass and microstructure that has become a public health problem worldwide. *Cuscutae semen* (CS) is a traditional Chinese medicine (TCM) that has a positive effect on the prevention and treatment of osteoporosis. However, the mechanism of CS is unclear. Therefore, this study aimed to reveal the possible molecular mechanism involved in the effects of CS on osteoporosis based on a network pharmacology approach.

Material/Methods: The inactive and active ingredients of CS were identified by searching the pharmacology analysis platform of the Chinese medicine system (TCMSP), and the targets of osteoporosis were screened in the relevant databases, such as GeneCards, PubMed, and the Comparative Toxicogenomics Database (CTD). The network of "medicine-ingredients-disease-targets (M-I-D-T)" was established by means of network pharmacology, and the key targets and core pathways were determined by R analysis. Molecular docking methods were used to evaluate the binding activity between the target and the active ingredients of CS.

Results: Eleven active ingredients were identified in CS, and 175 potential targets of the active ingredients were also identified from the TCMSP. Moreover, we revealed 22 539 targets related to osteoporosis in the 3 well-established databases, and we determined the intersection of the disease targets and the potential targets of the active ingredients; 107 common targets were identified and used in further analysis. Additionally, biological processes and signaling pathways involved in target action, such as fluid shear stress, atherosclerosis, cancer pathways, and the TNF signaling pathway, were determined. Finally, we chose the top 5 common targets, *CCND1, EGFR, IL6, MAPK8*, and *VEGFA,* for molecular docking with the 11 active ingredients of CS.

Conclusions: This study suggested that CS has multiple ingredients and multiple targets relevant to the treatment of osteoporosis. We determined that the active ingredient, sesamin, may be the most crucial ingredient of CS for the treatment of osteoporosis. Additionally, the network pharmacology method provided a novel research approach to analyze the function of complex ingredients.

MeSH Keywords: **Medicine, Chinese Traditional • Osteoporosis • Pharmacology**

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Background

Osteoporosis (OP) is a systemic disease characterized by reduced bone strength and damaged bone microstructure, resulting in increased bone fragility and proneness to fracture [1]. According to the World Health Organization (WHO) statistics, 200 million people suffer from OP, and with the aging of the global population, this figure will continue to increase [2]. As a result of this phenomenon, the soaring expense of medical treatment and nursing has become an indisputable fact. It is predicted that in China, expenses due to OP-related fracture treatment will reach 25.43 billion dollars in 2050 [3]. At present, anti-osteoporosis drugs mainly include anti-catabolic agents, anabolic agents and supplements that utilize other mechanisms, such as calcium, vitamin K2 and strontium [4].

Because traditional Chinese medicine (TCM) can play a positive role in the treatment of OP [5], an increased number of researchers have begun to explore Chinese herbal medicines. *Cuscutae semen* (CS), which is derived from the dry and mature seeds of *Cuscuta australis* R. Br. or *Cuscuta chinensis* Lam., is a widely used Chinese medicine with a long history of use [6,7]. Previous studies have shown that CS can nourish the kidneys and liver, prevent miscarriages and protect the eyesight [8]. Moreover, it also plays a certain role in the treatment of impotence and the seminal inhibition of the growth of tumor cells [9,10]. In addition to these biological functions, Yao et al. showed that CS can promote the proliferation of bone marrow mesenchymal stem cells and osteoblasts and inhibit osteoclast activity in rat bone cells. Moreover, Yang et al. demonstrated that CS can induce osteogenic activity in human osteoblast-like MG-63 cells [11]. Although some ingredients of CS have been extracted and verified [12–14], the identities of numerous other components and how they relieve OP by influencing bone metabolism are still largely unknown.

Recently, the concept of network pharmacology has been proposed as a new method to predict the mechanisms of the effects of drug therapy on disease at the whole organismal level [15]. With the aid of molecular biology and related database information, network pharmacology has shifted from the traditional "one drug, one target" strategy to the "drug-target-pathway-disease" strategy to provide a more comprehensive understanding of TCM mechanisms [16]. Therefore, this study adopted a network pharmacology approach to analyze and construct an "ingredient-target-pathway" network of the effects of CS on the treatment of OP. The multitarget and multiple pathway network of CS was also revealed from a holistic point of view, providing a reference for further exploring the mechanism underlying its treatment effects on OP.

Material and Methods

Acquisition of chemical ingredients and screening of active ingredients

"*Cuscutae semen*" was used as the key word to search for all the chemical information about CS in the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) (*http://lsp.nwsuaf.edu.cn/tcmsp.php*) [17] and in the literature review. By referring to multiple standards in the literature, an oral availability (OB) greater than 30% and a pharmacokinetic value (DL) greater than 0.18 were used as the limiting conditions to screen the active ingredients in the database [18]. As a result, 11 chemical ingredients were identified, and PubChem (*http://pubchem.ncbi.nlm.nih.gov/*) was used to retrieve the active ingredients and obtain their 3D structures in mol2 format for further analysis.

Target prediction of the active ingredients

The targets of the active ingredients in CS were queried by using the TCMSP target module, and the target protein name was transformed into a gene name by using Perl (*http://www.perl.org/*) and the UniProt database (*http://www.UniProt.org/*).

Target prediction of disease

To reveal the genes possibly related to disease, "osteoporosis" was used as the keyword, and the GeneCards database (*https://www.genecards.org/*), PubMed website (*https://www.ncbi.nlm.nih.gov*) and CTD database (*https://ctdbase.org/tools/batchQuery.go*) were utilized. All of these online tools are continuously updated with information about human genes and genetic diseases, providing a relatively comprehensive overview of research results. We removed duplicate targets from the search results.

Intersection of active ingredients and disease targets

We first downloaded the R package (*https://www.rproject.org/*) and entered the command code to install the toolkit for drawing a Venn diagram in R. Then, by using the previously prepared files that contained the active ingredients of CS and the disease targets, a specific command code was entered in R, which generated the Venn diagram and a list describing the specific outcomes of the analysis. This "ingredients to disease" list was used in the following steps.

Network construction and analysis

The "ingredients to disease" list was imported into Cytoscape 3.6.1 software (*https://cytoscape.org/*); then, the CS ingredient names and OP names were also introduced into Cytoscape to construct the model of the medicine-ingredients-disease-targets (M-I-D-T)

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Table 1. Active components of *Cuscutae semen* (CS).

Mol ID	Mol name	OB (%)	DL
MOL001558	Sesamin	56.55	0.83
MOL000184	NSC63551	39.25	0.76
MOL000354	Isorhamnetin	49.6	0.31
MOL000358	beta-Sitosterol	36.91	0.75
MOL000422	kaempferol	41.88	0.24
MOL005043	Campest-5-en-3beta-ol	37.58	0.71
MOL005440	Isofucosterol	43.78	0.76
MOL005944	Matrine	63.77	0.25
MOL006649	Sophranol	55.42	0.28
MOL000953	CLR	37.87	0.68
MOL000098	Quercetin	46.43	0.28

network. In the network construction, nodes were used to represent molecules or target proteins, and edges were used to represent the relationships among ingredients, disease and targets.

Construction of the protein interaction network

The "ingredients to disease" list was imported into the Search Tool for the Retrieval of Interacting Genes (STRING) database (*http://string-db.org*), which is a protein–protein interaction (PPI) database that can search for known proteins and predict PPIs [19]. In the operating interface, we limited the species to "Homo sapiens" and set the minimum interaction threshold to 0.7 to determine the relationships between potential targets of CS in the treatment of OP. Next, we utilized the R package to screen the hub proteins. The basic principle was to determine the number of junction nodes between all proteins and the top 30 proteins.

Gene ontology and pathway enrichment analysis

Bioconductor (*http://www.bioconductor.org/*) provides tools for the analysis and interpretation of high-throughput genomic data. It uses the programming software R, which is an open source and open development software [20]. With the help of the R package, we successfully installed this useful analysis tool and then ran the code. The enrichment analysis of Gene Ontology (GO) functions and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways were carried out on the genes in the ingredient-OP target network, and the results were obtained (*P*-adjusted value <0.01). By using the count score, we selected the top 20 for presentation.

Molecular docking

Five targets with a maximum count score in the target interaction network obtained from the PPI analysis were selected. They were mitogen-activated protein kinase 8 (MAPK8), epidermal growth factor receptor (EGFR), cyclin D1 (CCND1), interleukin-6 (IL-6), and vascular endothelial growth factor A (VEGFA). All of these were searched in the PDB database (*http://www.rcsb.org/pdb/home/home.do*), which is the most important database containing atomic-level 3D structural data for biological macromolecules (proteins, nucleic acids and sugars) [21], and the conformations were screened according to the following conditions: 1) protein structure was obtained by an x-ray diffraction method; 2) protein structure had a resolution less than 3 Å; 3) protein was typed; and 4) protein structures reported in previous docking studies were preferred. Then, Autotools was used to remove the excess protein chains and ligands, and hydrogenation was performed to remove the water molecules. AutoGrid was used to calculate the energy lattice, to set the grid box coordinates, and to set the distance of each small grid point to 0.1 nm. Finally, Autodock Vina 4.2 (*http://vina.scripps.edu/*), which is an open source program used for molecular docking that was designed and implemented by Dr. Oleg Trott at the Molecular Graphics Lab at The Scripps Research Institute [22], was used for batch docking of potential active ingredients in CS with the 5 proteins, and the results returned 9 conformations. The predominant conformation was analyzed and plotted with Free Maestro by Schrodinger (*https://www.schrodinger.com/freemaestro*).

Results

Active ingredients of CS

Seventy-six chemical ingredients of CS were identified in the TCMSP database. After setting the filtering criteria mentioned above, 11 active ingredients of CS were determined, which are shown in Table 1.

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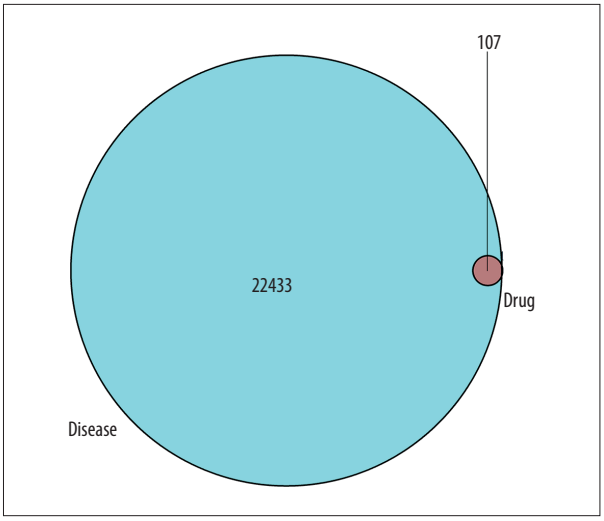

Figure 1. Venn diagram of 107 potential common targets.

Potential targets of the active ingredients

Through searching the TCMSP target module, 175 potential targets of CS active ingredients and their corresponding symbols were collected (Supplementary Table 1).

Potential targets of OP

In this study, 3 internationally recognized databases of disease genes were searched, and 22 539 potential targets were retrieved after removing the duplicate targets. These targets were closely related to the occurrence and development of OP (Supplementary Table 2).

Ingredient and disease targets intersection

After inputting the potential targets of the ingredients and the disease targets into the R platform, the intersection of the 2 types of targets was determined. The Venn diagram showed that 107 potential targets had relationships with active ingredients and OP (Figure 1). The common gene names of the 107 potential targets are shown in Supplementary Table 3.

M-I-T-D network

As shown in Figure 2, a medicine-ingredients-targets-disease network was generated and indicated that these 4 components had close relationships with each other.

PPI network

In the "ingredients and disease intersection" part of the PPI network, OP targets and active ingredient potential targets showed 107 duplicate genes. These genes may become targets for the treatment of OP. To study the interaction of the targets *in vivo* and search for the hub genes, PPI network analysis of the potential target groups was carried out (Figure 3). The linked tables with different colors represent the different meanings of the biological information. Moreover, the top 30 genes that had a close relationship with other genes were represented via a bar plot that clearly described these 30 gene targets in terms of their key positions in the PPI network. These genes were *MAPK8, EGFR, CCND1, IL-6, VEGFA, ESR1, AR, MYC, CASP3, PPARG, RELA, DECR1, NCOA1, ERBB2, FOS, ICAM1, NOS3, CYP1A1, PRKCA, CYP2B6, PGR, CASP8, CAV1, CYP3A4, GSTP1, NCOA2, RB1, VCAM1, AHR*, and *CD44* (Figure 4).

GO and KEGG pathway enrichment analysis

To illustrate the mechanism underlying the effects of CS active ingredients on OP more comprehensively and concretely, we performed GO enrichment analysis of the 107 common targets in the ingredient-disease target network. As a result, the top 20 enriched GO terms were identified, which are shown in a bar plot (*P*-adjusted value <0.01, Figure 5). For example, in biological processes, the targets of CS were enriched in cofactor binding (GO: 0048037), proximal promoter sequence-specific DNA binding (GO: 0000987), DNA-binding transcription activator activity, RNA polymerase II proximal promoter sequence-specific binding (GO: 0001228), RNA polymerase II proximal promoter sequence-specific DNA binding (GO: 0000978), protein heterodimerization activity (GO: 0046982), chromatin binding (GO: 0003682), ubiquitin-like protein ligase binding (GO: 0044389), enzyme activator activity (GO: 0008047), and other processes. Meanwhile, a dot plot indicated the gene ratio of the number of target genes involved in one biological process to the number of all annotated genes. The higher the ratio, the higher the level of enrichment is. The size of the dot reflects the number of target genes in the analysis, and the different colors of the dots indicate the different *P*-adjusted value ranges (Figure 6).

To elucidate the critical pathways among the 107 potential targets in terms of OP therapy, the top 20 pathways were filtered according to a *P*-adjusted value < 0.01 (Figure 7) and included pathways involved in fluid shear stress and atherosclerosis (hsa05418), Kaposi sarcoma-associated herpesvirus infection (hsa05167), proteoglycans in cancer (hsa05205), human cytomegalovirus infection (hsa05163), hepatitis B infection (hsa05161), Epstein-Barr virus infection (hsa05169), prostate cancer (hsa05215), AGE-RAGE signaling in diabetic complications (hsa04933), hepatocellular carcinoma (hsa05225), apoptosis (hsa04210), TNF signaling (hsa04668), measles infection (hsa05162), breast cancer, (hsa05224), colorectal cancer (hsa05210), thyroid hormone signaling (hsa04919), pancreatic cancer (hsa05212), p53 signaling (hsa04115), bladder cancer (hsa05219), prolactin signaling (hsa04917), and platinum drug resistance (hsa01524).

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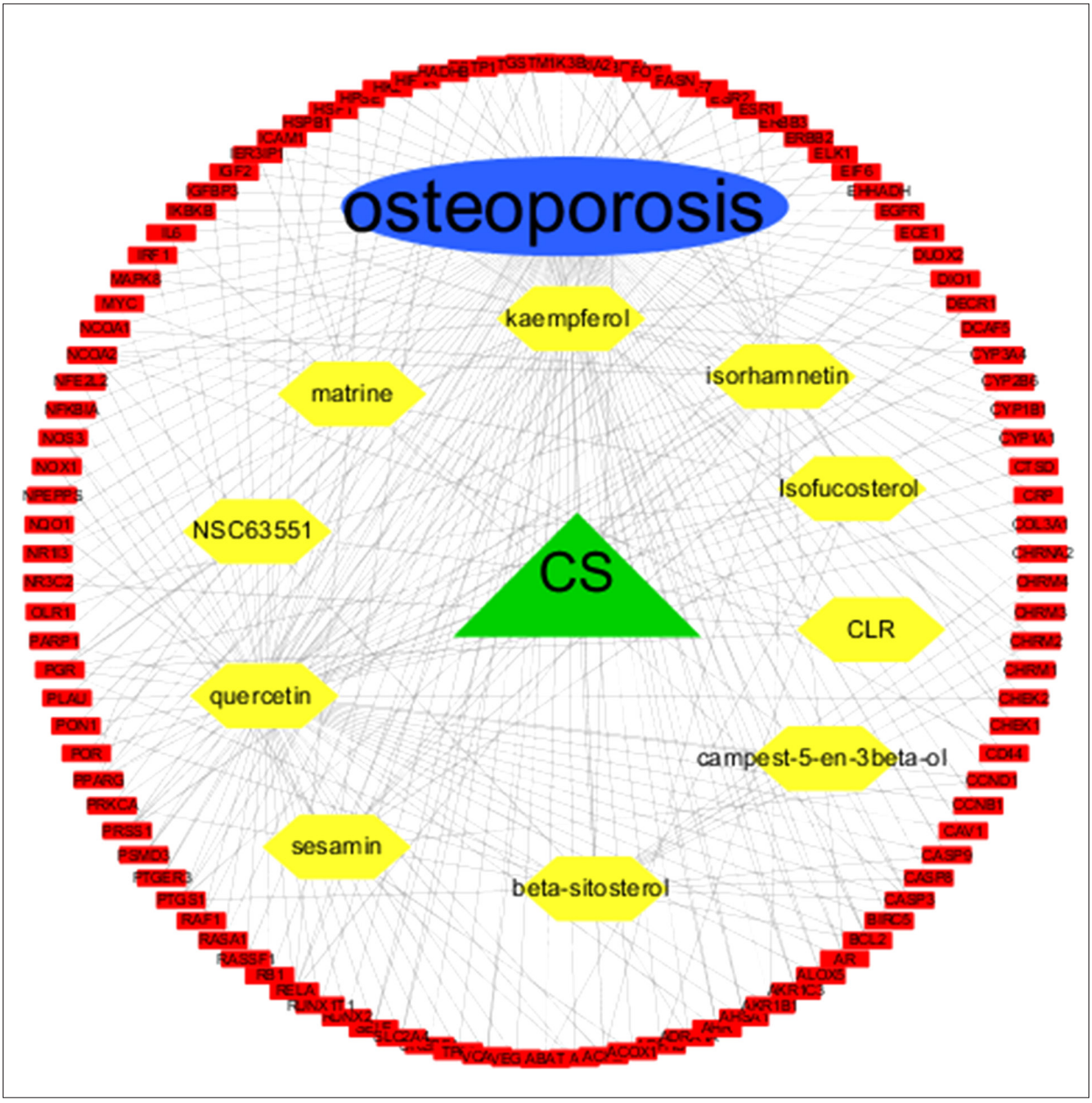

Figure 2. A medicine-ingredients-targets-disease network of 4 parts. CS: *Cuscutae Semen*; yellow: active ingredients of CS; red: 107 potential common targets.

By applying an identical analytical method as that used for the GO analysis, a dot plot showing the relevant pathways was obtained that showed the same data as the GO dot plot (Figure 8).

Active ingredient-main target molecular docking

Because 107 potential targets were obtained, the top 5 targets (*CCND1, EGFR, IL-6, MAPK8*, and *VEGFA*), which had higher scores, were selected for molecular docking with 11 active ingredients of CS. The ranking of the binding free energy of the 11 small molecule compounds was achieved, as shown in Table 2. This table indicates that the active ingredient sesamin bound to all 5 proteins better than the other ingredients.

We observed that all 11 ingredients entered the active pocket of the enzyme. For example, the analysis of sesamin indicated that sesamin bound to the active pocket of CCND1 and interacted with the H-bond of the amino group on LYS180 and weakly interacted via an aromatic hydrogen bond with CYS73 and GLU75 (Figure 9). In Figure 10, sesamin bound to the active pockets of EGFR and interacted with MET769, ALA698, and ARG817 to form hydrogen bonds and with GLN767, ASP813,

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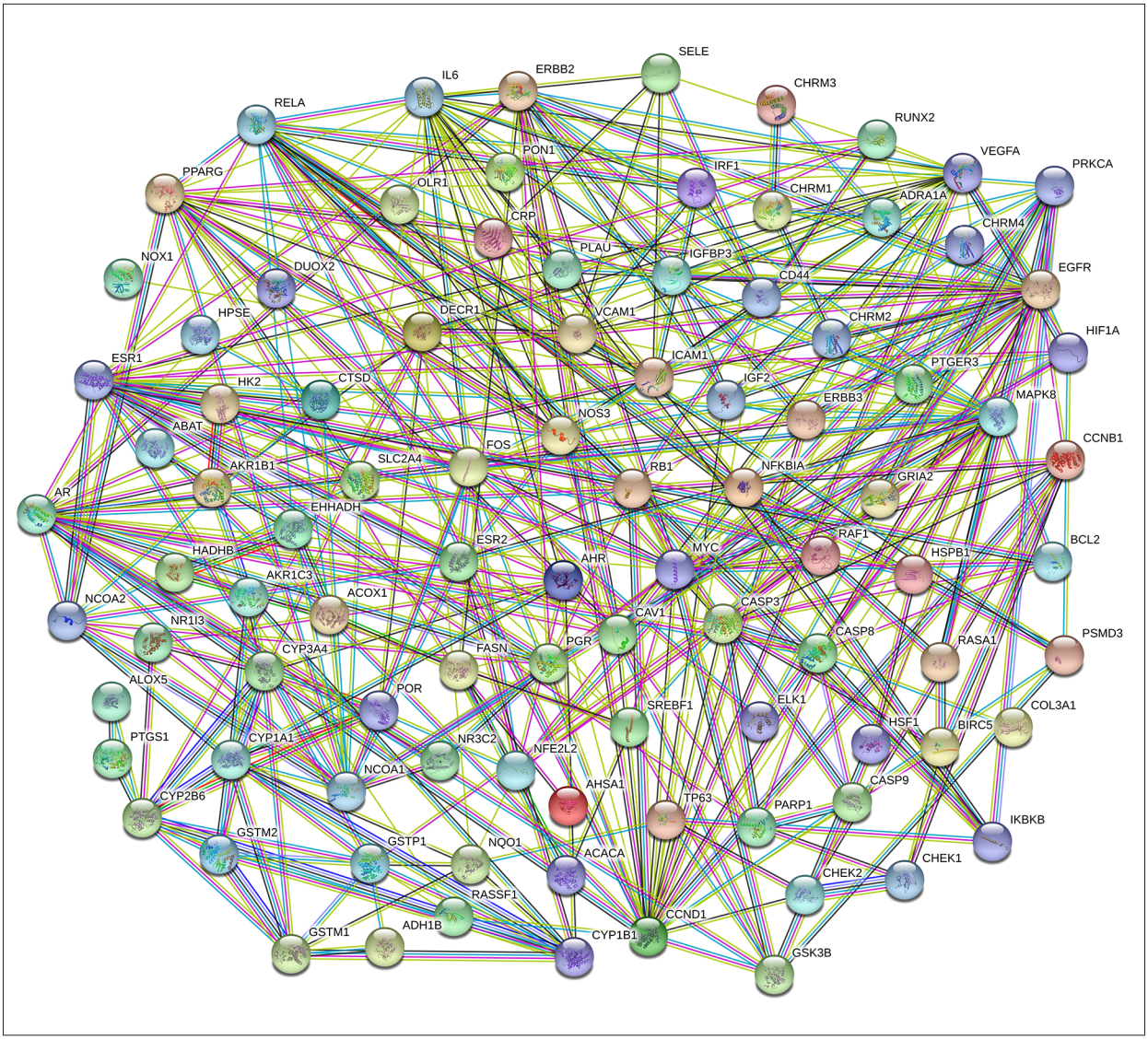

Figure 3. Protein–protein interaction (PPI) network analysis of 107 potential target: cyan line: from curated databases; purple line: experimentally determined; green line: gene neighborhood; red line: gene fusions; blue line: gene co-occurrence; yellow line: text mining; black line: co-expression; baby blue line: protein homology.

and PHE699 to form weakly aromatic hydrogen bonds. Sesamin combined with the active pocket of IL-6 and interacted with and formed weakly aromatic hydrogen bonds with GLU42, THR43, and ASP 160, as shown in Figure 11. Additionally, sesamin was linked to the active pocket of MAPK8 and interacted with MET111 to form hydrogen bonds. It also interacted with ASN156, ASP169, GLU109, and MET109 to form weakly aromatic hydrogen bonds (Figure 12). Finally, sesamin bound to the active pocket of VEGFA and interacted with SER25 and TRP28 to form hydrogen bonds. The aromatic ring of RP28 interacted equally with the Pi-Pi bond and weakly with ASP47 to form aromatic hydrogen bonds (Figure 13).

Discussion

OP is characterized by low bone mass and a higher risk of fracture due to bone fragility. CS is a kind of bioactive ingredient extracted from plants. In recent years, it has been shown to play a vital role in bone metabolism through antioxidative and antiapoptotic effects [23]. Because of the variety of components in CS, the mechanism underlying its influence on OP is still unclear. Therefore, this study used a network pharmacology method to uncover the relevant relationships from multiple angles.

For example, certain studies on the relationship between sesamin, quercetin, and kaempferol and OP have been reported.

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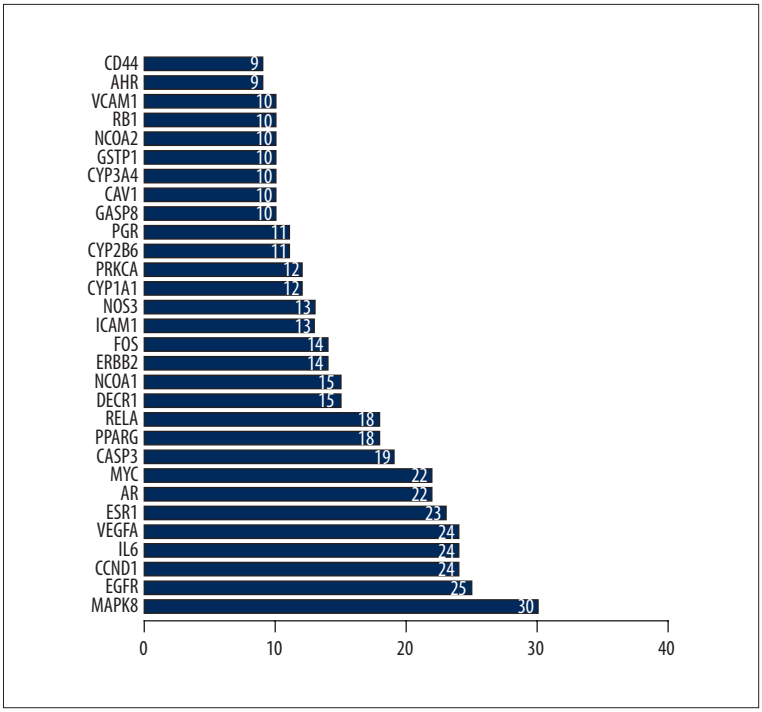

Figure 4. Top 30 targets from protein–protein interaction (PPI) network.

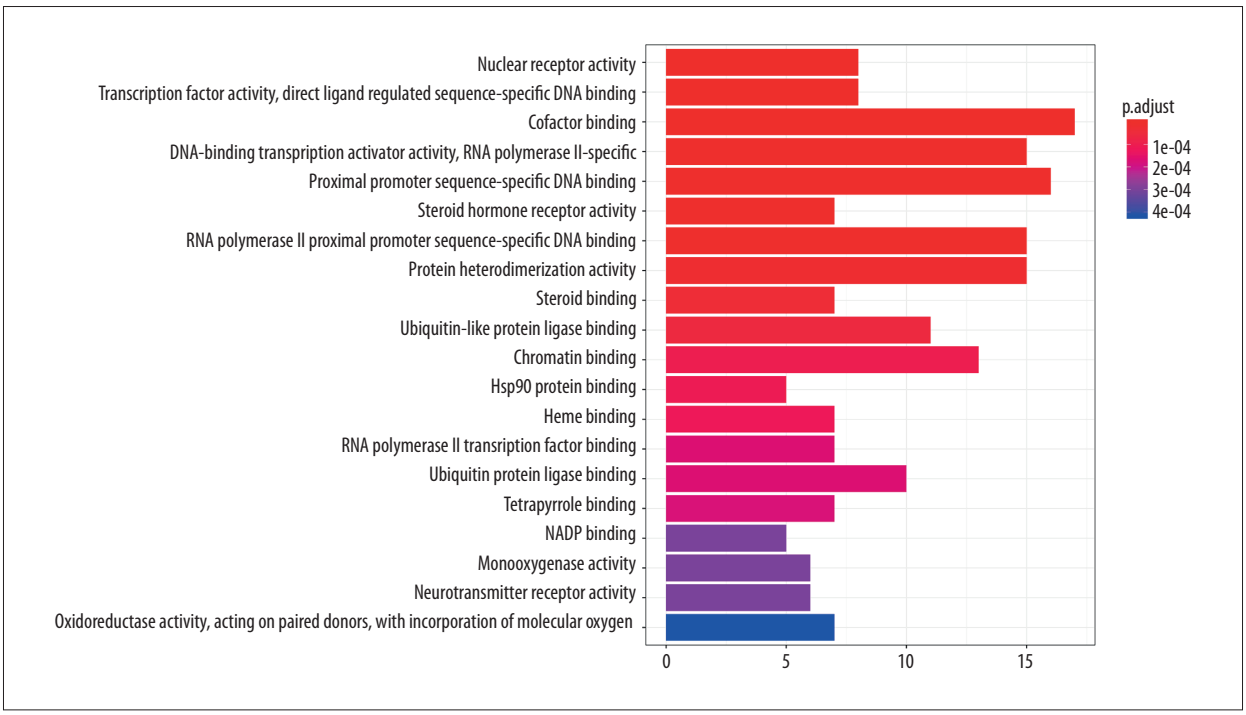

Figure 5. Top 20 enriched Gene Ontology (GO) terms selected from 107 common targets. (*P*-adjust value <0.01).

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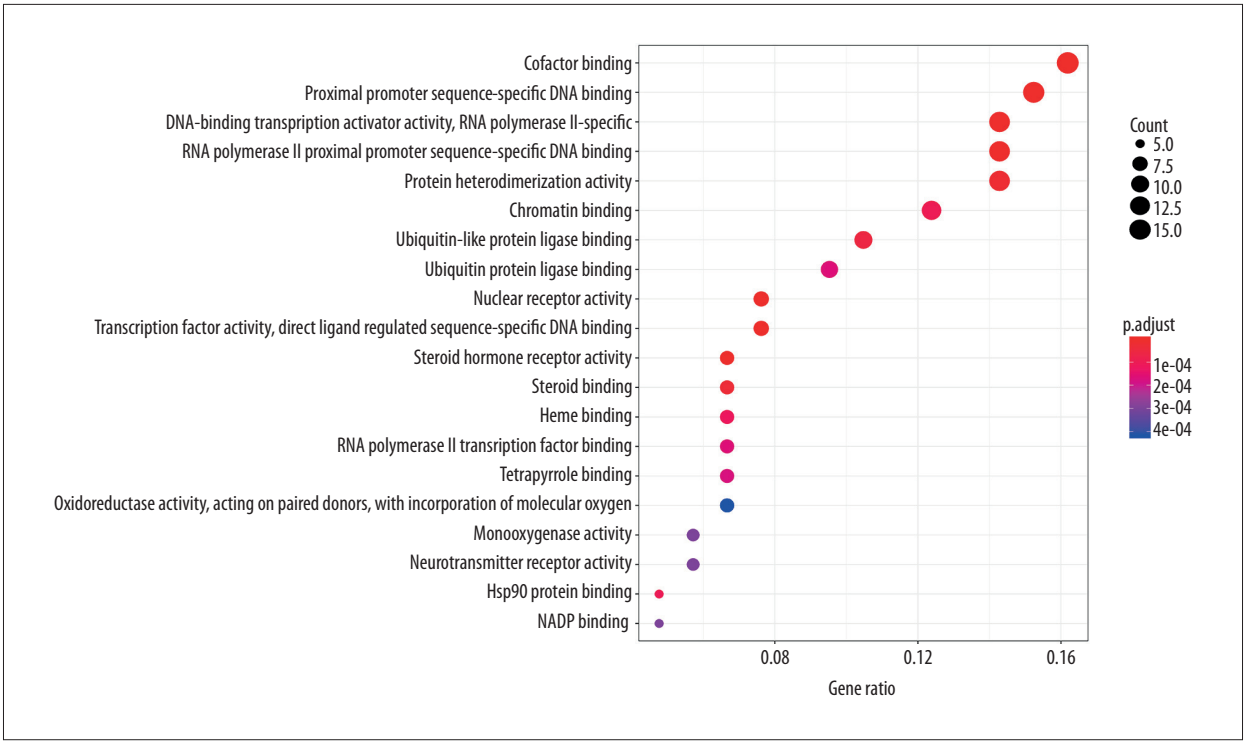

Figure 6. A dot plot to describe *P*-adjust value range of top 20 targets.

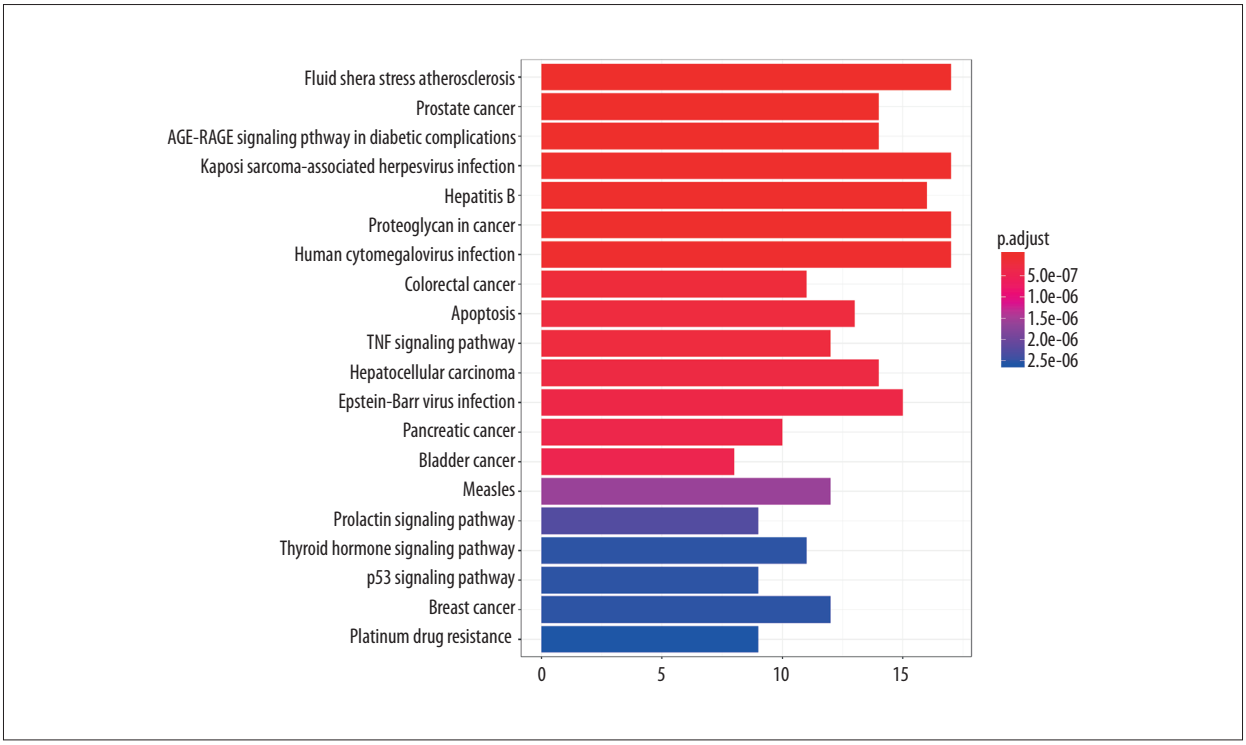

Figure 7. Top 20 pathways from Kyoto Encyclopedia of Genes and Genomes (KEGG). (*P*-adjust value <0.01).

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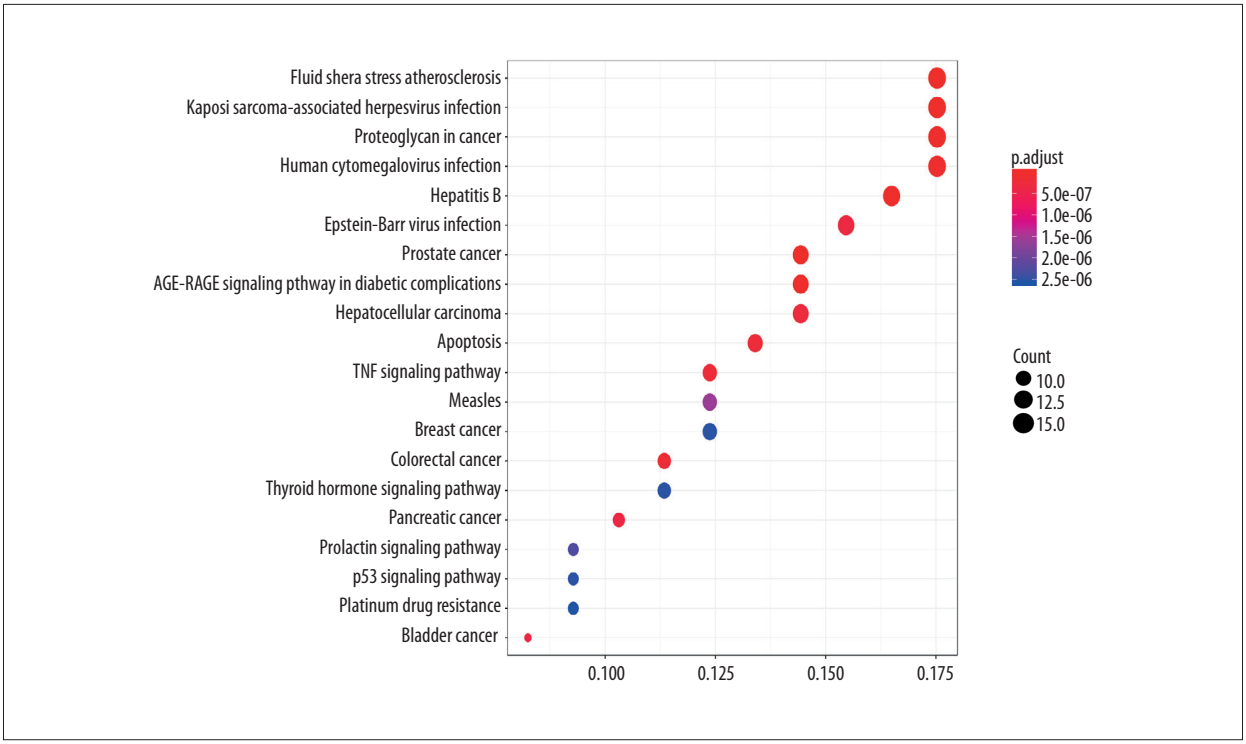

Figure 8. A dot plot to describe *P*-adjust value range of top 20 pathways.

Table 2. Binding free energy of 11 small molecules.

Affinity (kcal/mol)	CCND1	EGFR	IL6	MAPK8	VEGFA
Sesamin	–7.1	–9.3	–7.3	–8.7	–6.9
Isorhamnetin	–6.6	–8.4	–6.2	–7.6	–6.0
CLR	–6.2	–8.2	–6.9	–8.0	–5.6
Sophranol	–6.6	–8	–5.7	–7.9	–5.4
Matrine	–6.5	–7.8	–6.1	–7.5	–5.4
Kaempferol	–6.7	–8.2	–6.2	–7.6	–6.2
Isofucosterol	–6.3	–7.4	–6.6	–8.8	–6.0
Campest-5-en-3beta-ol	–6.5	–7.8	–6.2	–8.3	–5.6
NSC63551	–6.6	–8.0	–6.4	–7.9	–6.3
Quercetin	–7.1	–8.0	–6.0	–7.0	–6.9
beta-Sitosterol	–6.2	–7.1	–6.2	–8.1	–5.8

Sesamin, also known as flax and flax oil, originated in the western regions of ancient China and belongs to the flax seed family [24]. It is mainly distributed in tropics worldwide. A previous study reported the protective effect of sesame oil against bone loss in an ovariectomized (OVX) rat model [25]. Orawan et al. indicated that after treatment with sesamin of human fetal osteoblasts and human adipose-derived stem cells, osteoblast differentiation could be activated through the p38 and ERK/MAPK pathways [26]. Moreover, Ma et al. reported that sesamin had the ability to promote the osteoblastic differentiation of BMSCs by regulating the Wnt/β-catenin pathway [27]. Tsuji et al. verified the inhibitory effect of quercetin on bone loss in an ovariectomized mouse model [28]. *In vivo* experiments also indicated that quercetin could improve BMSC activity and osteogenic differentiation ability [29]. A study by Adhikary et al. showed that supplementation with kaempferol could increase the speed of the healing of fractures caused by glucocorticoids and minimize bone loss in rats [30].

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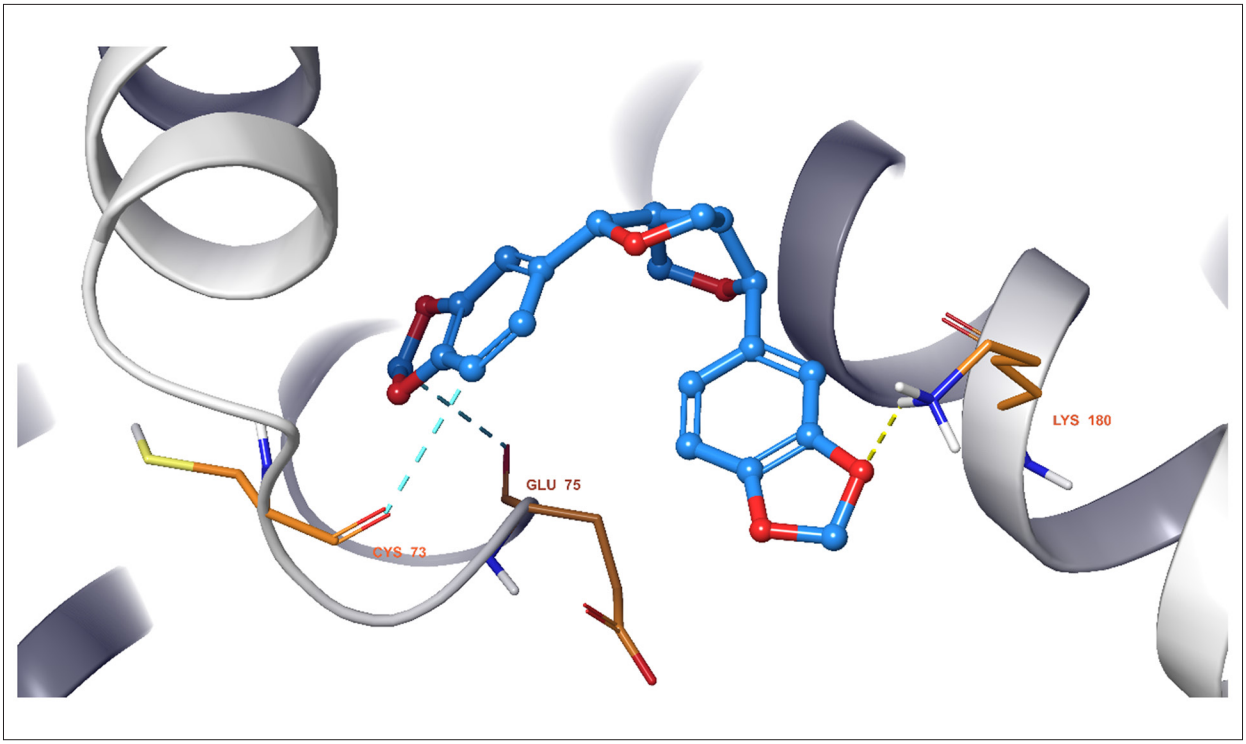

Figure 9. Sesamin bound to the active pocket of cyclin D1 (CCND1).

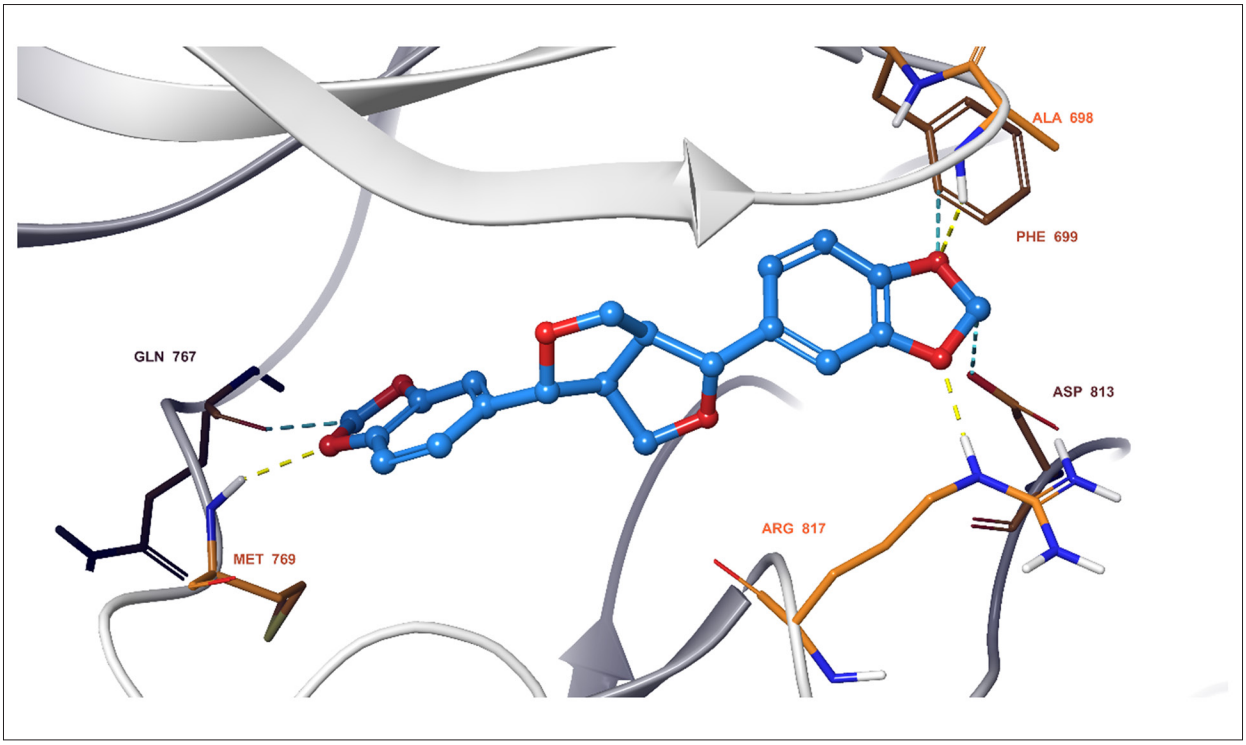

Figure 10. Sesamin bound to the active pocket of epidermal growth factor receptor (EGFR).

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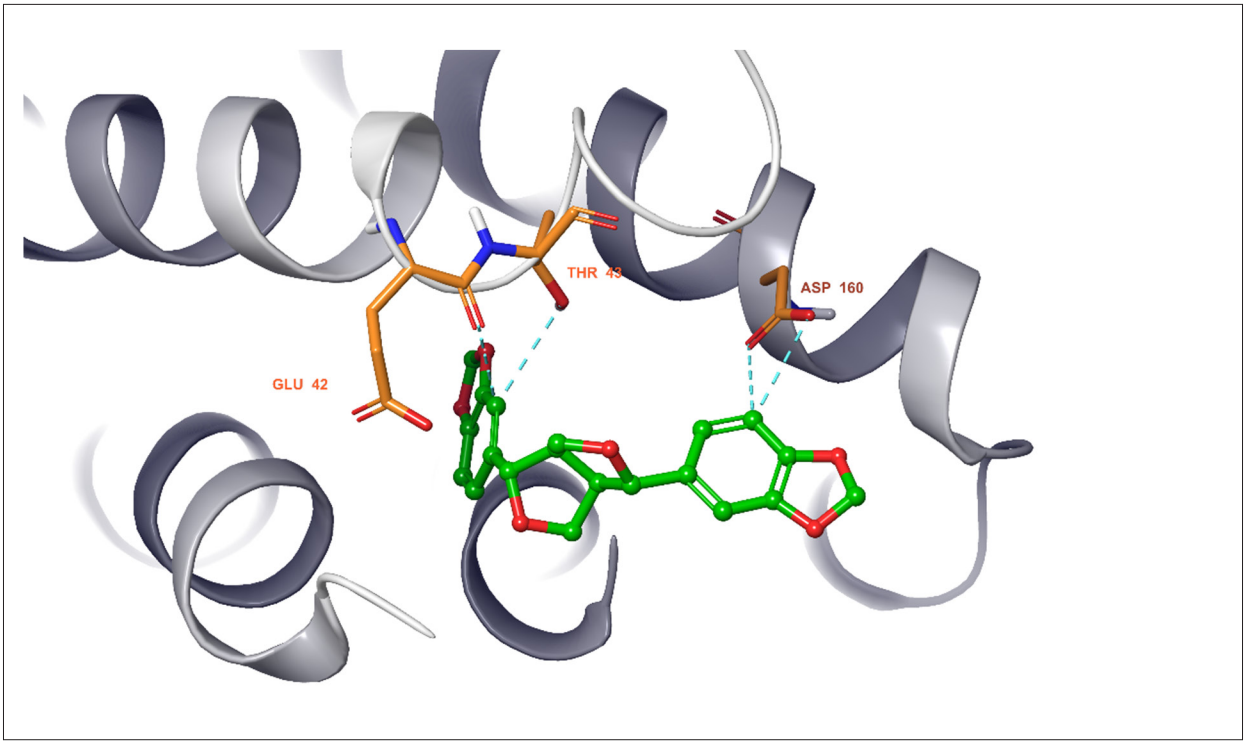

Figure 11. Sesamin bound to the active pocket of interleukin-6 (IL6).

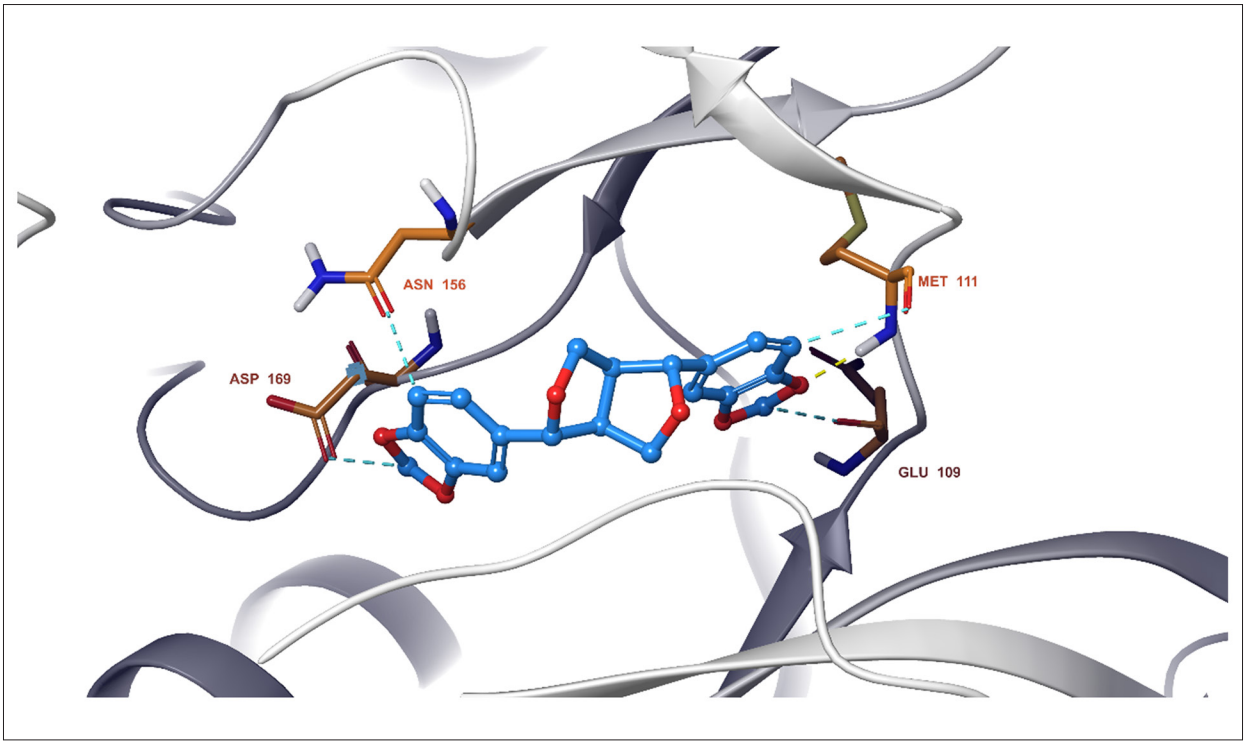

Figure 12. Sesamin bound to the active pocket of mitogen-activated protein kinase 8 (MAPK8).

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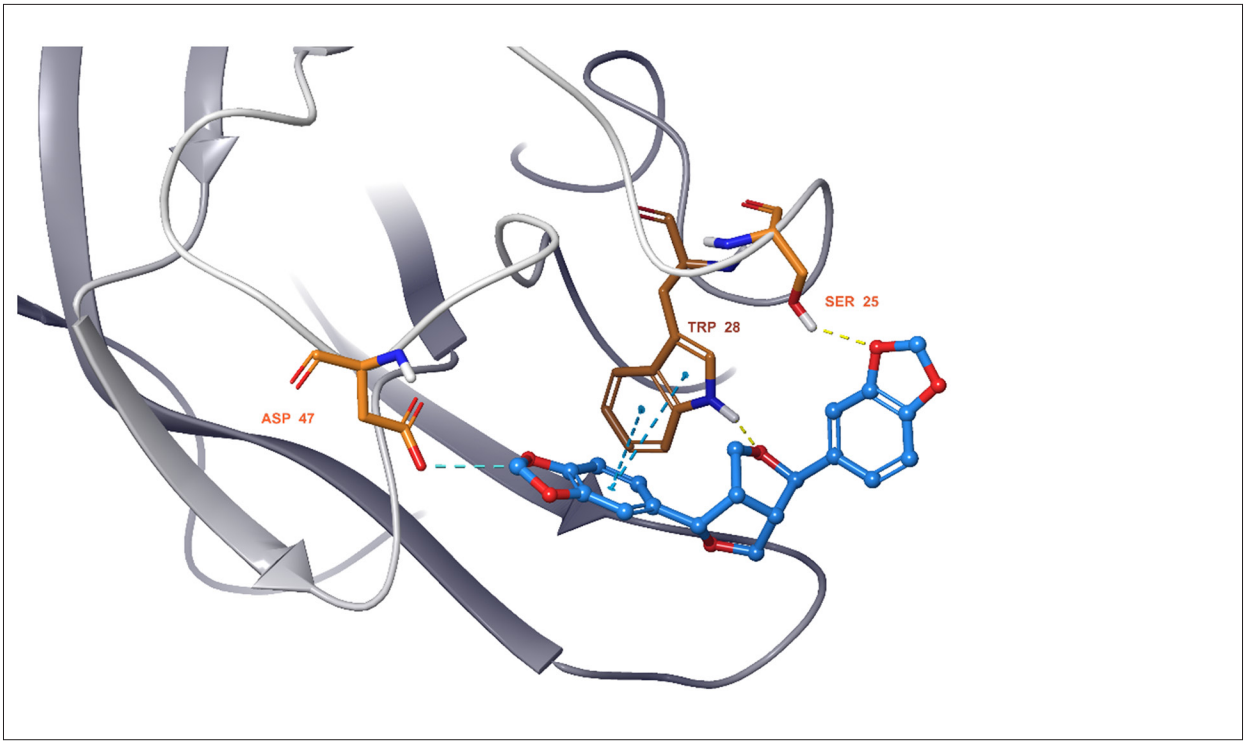

Figure 13. Sesamin bound to the active pocket of vascular endothelial growth factor A (VEGFA).

In this study, 107 common targets were analyzed via GO and KEGG enrichment analyses, and certain biological functions and signal pathways that had relationships with the ingredients and OP were determined. Based on the results, we speculated that the main biological functions involved DNA binding and protein or cofactor binding. Through these fundamental processes, CS may act on the relevant targets and thus affect the associated signaling pathways to exert drug effects. These pathways mainly play a role in certain cancers, TNF signaling pathways and stress signaling pathways. In a previous study, fluid shear stress (FSS) played a vital role in facilitating the proliferation and differentiation of osteoblasts [31] and reducing apoptosis [32]. As indicated by the bioinformatic findings of this study, the top 5 potential targets that were selected all had connections with OP.

MAPK8 performs the specific phosphorylation of the transcription factor c-Jun in the nucleus and is the kinase of c-Jun. Thus, MAPK8 is also called c-Jun amino-terminal kinase (JNK) [33]. JNK is one of most critical pathways related to osteoclastogenesis [34]. Some studies have confirmed this finding. Lee et al. used methylglyoxal to treat RAW264.7 macrophages and discovered that JNK was the most likely factor involved in activating osteoclasts [35]. Additionally, in another study, after treatment with a JNK inhibitor, researchers found that both the expression of mature osteoblast markers and mineralization of osteoblasts declined; however, upon the overexpression of JNK, osteoblast differentiation was enhanced [36]. Cyclin D1 (CCND1) plays an important role in regulating cell proliferation. A study indicated that chlorogenic acid exerted positive effects on BMSCs by activating cyclin D1 [37]. Other studies have also shown that enhanced cyclin D1 levels are associated with bone anabolism and anti-apoptosis [38–40]. Sato et al. focused on glucocorticoid-induced OP and demonstrated that when cyclin D1 was downregulated, bone formation was inhibited [41]. Interleukin (IL)-6 is a potent bone resorption factor that induces osteocyte differentiation and promotes osteoclast formation [42,43]. A previous study showed that IL-6 was associated with increased activity of osteoclasts during postmenopausal OP [44]. In addition, animal experiments also revealed that knockout of the mouse IL-6 gene could prevent bone loss after ovariectomy (OVX) [45]. Therefore, IL-6 plays an important role in the pathogenesis of OP [46]. Vascular endothelial growth factor A (VEGFA) is a homologue of the VEGF family. It has been confirmed that there are functional VEGF receptors in primary osteoblasts, which allow VEGF to play a role in promoting osteoblast proliferation and bone remodeling [47]. A recent study also indicated that in mesenchymal stem cells (MSCs), VEGFA overexpression could enhance cell vitality and proliferation, and the expression levels of type I and type II collagen were evidently upregulated [48]. Meanwhile, a study by Min et al. showed that VEGFA also functions in osteoclast differentiation [49]. Epidermal growth factor receptor (EGFR) is the receptor of EGF, which affects osteoprogenitor maintenance and new bone formation [50]. At the same time, a study by Liu et al. revealed an interesting phenomenon in

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which the expression of p-EGFR on the endosteal surface of cortical bone was decreased in 15-month-old mice compared with that in 3-month-old mice [51]. This finding indicated that EGFR had a relationship with age in bone metabolism. Moreover, knockdown of EGFR in osteoblastic cells led to bone loss due to a decreased number of bone marrow mesenchymal progenitors [52]. In addition, it has also been reported that the mechanism of the regulation by EGFR of bone development involves the negative regulation of mTOR signaling during the process of osteoblastic differentiation [53]. As Table 2 indicates, the lower the docking score, the higher the affinity of the docking molecule and the target was. Therefore, according to all the docking results, sesamin bound well to all 5 proteins. At the same time, based on the docking structure, we presume that the binding can be further improved by increasing the number of hydrogen bond interactions to enhance activity, which may allow sesamin to become a crucial agent for treating OP.

Conclusions

By using a novel analysis approach, we tested the hypothesis that the Chinese herbal medicine, *Cuscutae semen* (CS), had a positive influence on the treatment and prevention of OP. Accordingly, this study further revealed the CS pharmacodynamic basis and mechanism of action involved in the treatment of OP at the systemic level. The most active ingredient in CS that produces its effect was predicted.

However, because this study depended on database and statistical code analysis to make predictions about the effectiveness of drugs, some limitations should be considered. Hence, our future research will focus on experimental studies to verify these hypotheses.

Conflicts of Interest

None.

Supplementary Data

Supplementary Table 1. Potential targets and corresponding symbols of *Cuscutae semen* (CS) active components.

Mol Id	Mol name	Target	Symbol
MOL001558	Sesamin	G1/S-specific cyclin-D1	CCND1
MOL001558	Sesamin	Fatty acid synthase	FASN
MOL001558	Sesamin	Acetyl-CoA carboxylase 1	ACACA
MOL001558	Sesamin	Nitric oxide synthase, endothelial	NOS3
MOL001558	Sesamin	Endothelin-converting enzyme 1	ECE1
MOL001558	Sesamin	Cytochrome P450 2B6	CYP2B6
MOL001558	Sesamin	Sterol regulatory element-binding protein 1	SREBF1
MOL001558	Sesamin	NADPH oxidase 1	NOX1
MOL001558	Sesamin	Peroxisomal acyl-coenzyme A oxidase 1	ACOX1
MOL001558	Sesamin	Peroxisomal bifunctional enzyme	EHHADH
MOL001558	Sesamin	Trifunctional enzyme subunit beta, mitochondrial	HADHB
MOL001558	Sesamin	2,4-dienoyl-CoA reductase, mitochondrial	DECR1
MOL000184	NSC63551	Progesterone receptor	PGR
MOL000354	Isorhamnetin	Prostaglandin G/H synthase 1	PTGS1
MOL000354	Isorhamnetin	Estrogen receptor	ESR1
MOL000354	Isorhamnetin	Androgen receptor	AR
MOL000354	Isorhamnetin	Peroxisome proliferator activated receptor gamma	PPARG
MOL000354	Isorhamnetin	Estrogen receptor beta	ESR2
MOL000354	Isorhamnetin	Glycogen synthase kinase-3 beta	GSK3B

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Mol Id	Mol name	Target	Symbol
MOL000354	Isorhamnetin	Trypsin-1	PRSS1
MOL000354	Isorhamnetin	Nuclear receptor coactivator 2	NCOA2
MOL000354	Isorhamnetin	Serine/threonine-protein kinase Chk1	CHEK1
MOL000354	Isorhamnetin	Aldose reductase	AKR1B1
MOL000354	Isorhamnetin	Nuclear receptor coactivator 1	NCOA1
MOL000354	Isorhamnetin	Coagulation factor VII	F7
MOL000354	Isorhamnetin	Acetylcholinesterase	ACHE
MOL000354	Isorhamnetin	Gamma-aminobutyric acid receptor subunit alpha-1	GABRA1
MOL000354	Isorhamnetin	Glutamate receptor 2	GRIA2
MOL000354	Isorhamnetin	Transcription factor p65	RELA
MOL000354	Isorhamnetin	Oxidized low-density lipoprotein receptor 1	OLR1
MOL000358	beta-Sitosterol	Progesterone receptor	PGR
MOL000358	beta-Sitosterol	Nuclear receptor coactivator 2	NCOA2
MOL000358	beta-Sitosterol	Prostaglandin G/H synthase 1	PTGS1
MOL000358	beta-Sitosterol	Muscarinic acetylcholine receptor M3	CHRM3
MOL000358	beta-Sitosterol	Muscarinic acetylcholine receptor M1	CHRM1
MOL000358	beta-Sitosterol	Muscarinic acetylcholine receptor M4	CHRM4
MOL000358	beta-Sitosterol	Alpha-1A adrenergic receptor	ADRA1A
MOL000358	beta-Sitosterol	Muscarinic acetylcholine receptor M2	CHRM2
MOL000358	beta-Sitosterol	Neuronal acetylcholine receptor subunit alpha-2	CHRNA2
MOL000358	beta-Sitosterol	Gamma-aminobutyric acid receptor subunit alpha-1	GABRA1
MOL000358	beta-Sitosterol	Apoptosis regulator Bcl-2	BCL2
MOL000358	beta-Sitosterol	Caspase-9	CASP9
MOL000358	beta-Sitosterol	Caspase-3	CASP3
MOL000358	beta-Sitosterol	Caspase-8	CASP8
MOL000358	beta-Sitosterol	Protein kinase C alpha type	PRKCA
MOL000358	beta-Sitosterol	Serum paraoxonase/arylesterase 1	PON1
MOL000422	Kaempferol	Prostaglandin G/H synthase 1	PTGS1
MOL000422	Kaempferol	Androgen receptor	AR
MOL000422	Kaempferol	Peroxisome proliferator activated receptor gamma	PPARG
MOL000422	Kaempferol	Nuclear receptor coactivator 2	NCOA2
MOL000422	Kaempferol	Trypsin-1	PRSS1
MOL000422	Kaempferol	Progesterone receptor	PGR
MOL000422	Kaempferol	Muscarinic acetylcholine receptor M1	CHRM1
MOL000422	Kaempferol	Acetylcholinesterase	ACHE
MOL000422	Kaempferol	Muscarinic acetylcholine receptor M2	CHRM2

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Mol Id	Mol name	Target	Symbol
MOL000422	Kaempferol	Gamma-aminobutyric acid receptor subunit alpha-1	GABRA1
MOL000422	Kaempferol	Coagulation factor VII	F7
MOL000422	Kaempferol	Transcription factor p65	RELA
MOL000422	Kaempferol	Inhibitor of nuclear factor kappa-B kinase subunit beta	IKBKB
MOL000422	Kaempferol	Apoptosis regulator Bcl-2	BCL2
MOL000422	Kaempferol	Activator of 90 kDa heat shock protein ATPase homolog 1	AHSA1
MOL000422	Kaempferol	Caspase-3	CASP3
MOL000422	Kaempferol	Mitogen-activated protein kinase 8	MAPK8
MOL000422	Kaempferol	Peroxisome proliferator-activated receptor gamma	PPARG
MOL000422	Kaempferol	Cytochrome P450 3A4	CYP3A4
MOL000422	Kaempferol	Cytochrome P450 1A1	CYP1A1
MOL000422	Kaempferol	Intercellular adhesion molecule 1	ICAM1
MOL000422	Kaempferol	E-selectin	SELE
MOL000422	Kaempferol	Vascular cell adhesion protein 1	VCAM1
MOL000422	Kaempferol	Cytochrome P450 1B1	CYP1B1
MOL000422	Kaempferol	Arachidonate 5-lipoxygenase	ALOX5
MOL000422	Kaempferol	Glutathione S-transferase P	GSTP1
MOL000422	Kaempferol	Aryl hydrocarbon receptor	AHR
MOL000422	Kaempferol	26S proteasome non-ATPase regulatory subunit 3	PSMD3
MOL000422	Kaempferol	Solute carrier family 2, facilitated glucose transporter member 4	SLC2A4
MOL000422	Kaempferol	Nuclear receptor subfamily 1 group I member 3	NR1I3
MOL000422	Kaempferol	Type I iodothyronine deiodinase	DIO1
MOL000422	Kaempferol	Glutathione S-transferase Mu 1	GSTM1
MOL000422	Kaempferol	Glutathione S-transferase Mu 2	GSTM2
MOL000422	Kaempferol	Aldo-keto reductase family 1 member C3	AKR1C3
MOL005043	Campest-5-en-3beta-ol	Progesterone receptor	PGR
MOL005440	Isofucosterol	Progesterone receptor	PGR
MOL005440	Isofucosterol	Mineralocorticoid receptor	NR3C2
MOL005440	Isofucosterol	4-aminobutyrate aminotransferase, mitochondrial	ABAT
MOL005440	Isofucosterol	Gamma-aminobutyric acid receptor subunit alpha-1	GABRA1
MOL005440	Isofucosterol	Alcohol dehydrogenase 1B	ADH1B
MOL005440	Isofucosterol	Nuclear receptor coactivator 2	NCOA2
MOL005944	Matrine	Transcription factor p65	RELA
MOL005944	Matrine	Interleukin-6	IL6
MOL005944	matrine	Caspase-3	CASP3

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Mol Id	Mol name	Target	Symbol
MOL005944	Matrine	Myc proto-oncogene protein	MYC
MOL005944	Matrine	Intercellular adhesion molecule 1	ICAM1
MOL005944	Matrine	Heparanase	HPSE
MOL005944	Matrine	Immediate early response 3-interacting protein 1	IER3IP1
MOL005944	Matrine	CD44 antigen	CD44
MOL000953	CLR	Progesterone receptor	PGR
MOL000953	CLR	Mineralocorticoid receptor	NR3C2
MOL000953	CLR	Nuclear receptor coactivator 2	NCOA2
MOL000098	Quercetin	Prostaglandin G/H synthase 1	PTGS1
MOL000098	Quercetin	Androgen receptor	AR
MOL000098	Quercetin	Peroxisome proliferator activated receptor gamma	PPARG
MOL000098	Quercetin	Nuclear receptor coactivator 2	NCOA2
MOL000098	Quercetin	Aldose reductase	AKR1B1
MOL000098	Quercetin	Trypsin-1	PRSS1
MOL000098	Quercetin	Coagulation factor VII	F7
MOL000098	Quercetin	Acetylcholinesterase	ACHE
MOL000098	Quercetin	Gamma-aminobutyric acid receptor subunit alpha-1	GABRA1
MOL000098	Quercetin	Transcription factor p65	RELA
MOL000098	Quercetin	Epidermal growth factor receptor	EGFR
MOL000098	Quercetin	Vascular endothelial growth factor A	VEGFA
MOL000098	Quercetin	G1/S-specific cyclin-D1	CCND1
MOL000098	Quercetin	Apoptosis regulator Bcl-2	BCL2
MOL000098	Quercetin	Proto-oncogene c-Fos	FOS
MOL000098	Quercetin	Eukaryotic translation initiation factor 6	EIF6
MOL000098	Quercetin	Caspase-9	CASP9
MOL000098	Quercetin	Urokinase-type plasminogen activator	PLAU
MOL000098	Quercetin	Retinoblastoma-associated protein	RB1
MOL000098	Quercetin	Interleukin-6	IL6
MOL000098	Quercetin	Activator of 90 kDa heat shock protein ATPase homolog 1	AHSA1
MOL000098	Quercetin	Caspase-3	CASP3
MOL000098	Quercetin	Cellular tumor antigen p53	TP63
MOL000098	Quercetin	ETS domain-containing protein Elk-1	ELK1
MOL000098	Quercetin	NF-kappa-B inhibitor alpha	NFKBIA
MOL000098	Quercetin	NADPH--cytochrome P450 reductase	POR
MOL000098	Quercetin	Caspase-8	CASP8
MOL000098	Quercetin	RAF proto-oncogene serine/threonine-protein kinase	RAF1

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Mol Id	Mol name	Target	Symbol
MOL000098	Quercetin	Protein kinase C alpha type	PRKCA
MOL000098	Quercetin	Hypoxia-inducible factor 1-alpha	HIF1A
MOL000098	Quercetin	Protein CBFA2T1	RUNX1T1
MOL000098	Quercetin	Receptor tyrosine-protein kinase erbB-2	ERBB2
MOL000098	Quercetin	Peroxisome proliferator-activated receptor gamma	PPARG
MOL000098	Quercetin	Acetyl-CoA carboxylase 1	ACACA
MOL000098	Quercetin	Cytochrome P450 3A4	CYP3A4
MOL000098	Quercetin	Caveolin-1	CAV1
MOL000098	Quercetin	Myc proto-oncogene protein	MYC
MOL000098	Quercetin	Cytochrome P450 1A1	CYP1A1
MOL000098	Quercetin	Intercellular adhesion molecule 1	ICAM1
MOL000098	Quercetin	E-selectin	SELE
MOL000098	Quercetin	Vascular cell adhesion protein 1	VCAM1
MOL000098	Quercetin	Prostaglandin E2 receptor EP3 subtype	PTGER3
MOL000098	Quercetin	Baculoviral IAP repeat-containing protein 5	BIRC5
MOL000098	Quercetin	Dual oxidase 2	DUOX2
MOL000098	Quercetin	Nitric oxide synthase, endothelial	NOS3
MOL000098	Quercetin	Heat shock protein beta-1	HSPB1
MOL000098	Quercetin	Maltase-glucoamylase, intestinal	MGAM
MOL000098	Quercetin	Cytochrome P450 1B1	CYP1B1
MOL000098	Quercetin	G2/mitotic-specific cyclin-B1	CCNB1
MOL000098	Quercetin	Arachidonate 5-lipoxygenase	ALOX5
MOL000098	Quercetin	Glutathione S-transferase P	GSTP1
MOL000098	Quercetin	Nuclear factor erythroid 2-related factor 2	NFE2L2
MOL000098	Quercetin	NAD(P)H dehydrogenase [quinone] 1	NQO1
MOL000098	Quercetin	Poly [ADP-ribose] polymerase 1	PARP1
MOL000098	Quercetin	Aryl hydrocarbon receptor	AHR
MOL000098	Quercetin	26S proteasome non-ATPase regulatory subunit 3	PSMD3
MOL000098	Quercetin	Solute carrier family 2, facilitated glucose transporter member 4	SLC2A4
MOL000098	Quercetin	Collagen alpha-1(III) chain	COL3A1
MOL000098	Quercetin	DDB1- and CUL4-associated factor 5	DCAF5
MOL000098	Quercetin	Nuclear receptor subfamily 1 group I member 3	NR1I3
MOL000098	Quercetin	Serine/threonine-protein kinase Chk2	CHEK2
MOL000098	Quercetin	Heat shock factor protein 1	HSF1
MOL000098	Quercetin	C-reactive protein	CRP
MOL000098	Quercetin	Runt-related transcription factor 2	RUNX2

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Mol Id	Mol name	Target	Symbol
MOL000098	Quercetin	Ras association domain-containing protein 1	RASSF1
MOL000098	Quercetin	Cathepsin D	CTSD
MOL000098	Quercetin	Insulin-like growth factor-binding protein 3	IGFBP3
MOL000098	Quercetin	Insulin-like growth factor II	IGF2
MOL000098	Quercetin	Interferon regulatory factor 1	IRF1
MOL000098	Quercetin	Receptor tyrosine-protein kinase erbB-3	ERBB3
MOL000098	Quercetin	Serum paraoxonase/arylesterase 1	PON1
MOL000098	Quercetin	Type I iodothyronine deiodinase	DIO1
MOL000098	Quercetin	Puromycin-sensitive aminopeptidase	NPEPPS
MOL000098	Quercetin	Hexokinase-2	HK2
MOL000098	Quercetin	Ras GTPase-activating protein 1	RASA1
MOL000098	Quercetin	Glutathione S-transferase Mu 1	GSTM1
MOL000098	Quercetin	Glutathione S-transferase Mu 2	GSTM2

Supplementary Table 2. Potential targets of osteoporosis.

Supplementary Table 2 available from the corresponding author on request.

Supplementary Table 3. 107 common genes of ingredients and osteoporosis.

CCND1	NCOA2	PRKCA	AKR1C3
FASN	CHEK1	PON1	NR3C2
ACACA	AKR1B1	IKBKB	ABAT
NOS3	NCOA1	AHSA1	ADH1B
ECE1	F7	MAPK8	IL6
CYP2B6	ACHE	CYP3A4	MYC
SREBF1	GABRA1	CYP1A1	HPSE
NOX1	GRIA2	ICAM1	IER3IP1
ACOX1	RELA	SELE	CD44
EHHADH	OLR1	VCAM1	EGFR
HADHB	CHRM3	CYP1B1	VEGFA
DECR1	CHRM1	ALOX5	FOS
PGR	CHRM4	GSTP1	EIF6
PTGS1	ADRA1A	AHR	PLAU
ESR1	CHRM2	PSMD3	RB1
AR	CHRNA2	SLC2A4	TP63
PPARG	BCL2	NR1I3	ELK1
ESR2	CASP9	DIO1	NFKBIA
GSK3B	CASP3	GSTM1	POR
PRSS1	CASP8	GSTM2	RAF1

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HIF1A	HSPB1
RUNX1T1	CCNB1
ERBB2	NFE2L2
CAV1	NQO1
PTGER3	PARP1
BIRC5	COL3A1
DUOX2	DCAF5

CHEK2	IGF2
HSF1	IRF1
CRP	ERBB3
RUNX2	NPEPPS
RASSF1	HK2
CTSD	RASA1
IGFBP3	

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