MEDS Chinese Medicine (2026)
Clausius Scientific Press, Canada

DOI: 10.23977/medcm.2026.080102
ISSN 2616-1753 Vol. 8 Num. 1

# *Investigating the Therapeutic Mechanisms of Liuwei Dihuang Decoction for Osteoporosis via Network Pharmacology*

**Wei Tao[1,a], Zhang Yu[2,b,*]**

[1]*Chengdu University of Traditional Chinese Medicine, Chengdu, Sichuan, 610075, China*
[2]*Chengdu First People's Hospital, Chengdu, Sichuan, 610041, China*
[a]*3272770098@qq.com*, [b]*52032849@qq.com*
[*]*Corresponding author*

***Keywords:*** Osteoporosis; Liuwei Dihuang Decoction; Network Pharmacology; Mechanism of Action

***Abstract:*** This study aimed to systematically investigate the active ingredients, key targets, and potential molecular mechanisms of Liuwei Dihuang Decoction (LWDHD) in the treatment of osteoporosis (OP) using a network pharmacology approach. We identified the active ingredients and corresponding targets of LWDHD from the TCMSP database and collected OP-related targets from the GeneCards database. A protein-protein interaction (PPI) network was constructed using the STRING database to screen for key targets. Finally, Gene Ontology (GO) functional annotation and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were performed using the DAVID platform. Resultly, the results indicated that LWDHD likely exerts its therapeutic effects primarily through core active ingredients such as quercetin, kaempferol, and beta-sitosterol. These compounds act on key targets including TNF, IL6, and CASP3, modulating pathways such as the TNF signaling pathway and HIF-1 signaling pathway. The potential mechanisms involve anti-inflammatory and antioxidant effects, as well as the regulation of apoptosis. In conclusion, this study preliminarily reveals that LWDHD treats OP through a "multi-component, multi-target, multi-pathway" collaborative network, laying a theoretical foundation for subsequent experimental validation and clinical application.

## 1. Introduction

Osteoporosis (OP) is a systemic skeletal disorder characterized primarily by reduced bone mass and deterioration of bone microarchitecture, leading to increased bone fragility and a significantly elevated risk of fractures [1]. Its typical clinical manifestations include bone pain, changes in spinal morphology, and fragility fractures, with chronic low back pain being one of the most common symptoms [2]. Epidemiological studies indicate that the prevalence of OP in China exhibits a significant age-related correlation. The incidence is approximately 15%–50% in individuals under 50 years old, rising to about 56% in the elderly population over 60 [3]. The overall prevalence ranges from 6.6% to 19.3%, with an average of around 13% [4]. The pain and functional impairment caused

by OP and its resulting fractures severely compromise patients' quality of life, establishing OP as a major chronic disease threatening the health of middle-aged and older adults [5].

In traditional Chinese medicine (TCM), osteoporosis falls under the categories of "bone wilting" (Gu Wei) and "bone desiccation" (Gu Ku). Its pathogenesis is closely linked to deficiency of the liver and kidney. The kidney, considered the foundation of innate constitution, governs the bones and produces marrow. The liver governs the tendons and stores blood; furthermore, the liver and kidney share a common source. As women are considered to have the liver as their innate foundation, the liver-kidney yin deficiency occurring after menopause leads to the depletion of Tian Gui (a TCM concept roughly analogous to reproductive essence), resulting in insufficient essence and blood. This deficiency fails to nourish the bone marrow, and over time leads to bone desiccation and marrow reduction, manifesting as osteoporosis. Therefore, nourishing the liver and kidney, replenishing essence, and strengthening the bones constitute the core therapeutic principle for this type of OP.

Liuwei Dihuang Decoction (LWDHD), originating from Key to Diagnosis and Treatment of Children's Diseases, is a classic formula for nourishing yin and tonifying the kidney. It is composed of six medicinal ingredients: Rehmanniae Radix Praeparata (Shu Di Huang), Corni Fructus (Shan Zhu Yu), Dioscoreae Rhizoma (Shan Yao), Alismatis Rhizoma (Ze Xie), Moutan Cortex (Mu Dan Pi), and Poria (Fu Ling). Current clinical research demonstrates that LWDHD and its modified formulations can effectively alleviate symptoms in OP patients, such as bone pain and soreness/weakness of the lower back and knees, and improve bone mineral density, showing promising therapeutic potential [6, 7]. However, the material basis for its efficacy is complex, involving the synergistic effects of multiple components, targets, and pathways. The specific active ingredients and the systematic molecular mechanisms underlying its therapeutic effect on OP have not been fully elucidated. Therefore, this study aims to employ a network pharmacology approach to construct a multi-level network encompassing "components - targets - pathways - disease," in order to systematically reveal the potential active ingredients and mechanisms of action of LWDHD in treating OP, thereby providing a scientific basis for its clinical application.

## 2. Materials and Methods

### 2.1. Collection of LWDHD Active Ingredients and Target Prediction

The active ingredients and their corresponding targets for the six herbs comprising LWDHD (Rehmanniae Radix Praeparata, Corni Fructus, Dioscoreae Rhizoma, Alismatis Rhizoma, Moutan Cortex, and Poria) were predicted using the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP: http://tcmspw.com/tcmsp.php). Screening criteria were set as oral bioavailability (OB) ≥ 30% and drug-likeness (DL) ≥ 0.18 to identify potentially effective active ingredients and targets. After integrating and removing duplicates from all obtained compound targets, the UniProt database (https://www.uniprot.org/) was used to standardize the gene names.

### 2.2. Acquisition of Osteoporosis-Related Targets

The GeneCards database (https://www.genecards.org/) was comprehensively searched using the keyword "Osteoporosis." Targets related to the disease were screened by setting a threshold of Relevance score ≥ median value.

### 2.3. Identification of Intersection Targets between the Drug and Disease

The obtained LWDHD component targets and disease targets were mapped against each other. The Jvenn online platform (https://www.bioinformatics.com.cn/static/others/jvenn_en/example.html)

was used to identify the intersecting target genes.

### 2.4. Construction of the Herb-Active Ingredient-Disease-Target Network and Screening of Key Active Ingredients

Files named "network.xlsx" and "type.xlsx" containing the drug components and the aforementioned intersection targets were created and imported into Cytoscape 3.10.1 to construct an herb-active ingredient-disease-target network. Network topology analysis was performed, and key active ingredients of LWDHD for treating OP were screened based on their degree values.

### 2.5. Construction of Protein-Protein Interaction (PPI) Network and Screening of Key Targets

To further investigate protein-protein interactions involved in LWDHD's treatment of OP, the intersecting genes between the drug's core components and the disease were uploaded to the STRING database (https://string-db.org/) to construct a PPI network. The species was set to "Homo sapiens," the minimum interaction score was set to 0.4, and disconnected nodes were hidden in the network. Other parameters were kept at their default settings. The results were saved in TSV format. The TSV file was imported into Cytoscape 3.10.1, and the CytoHubba plugin was used to screen for hub genes using three algorithms: MCC, Degree, and Closeness. The intersection of genes identified by these three algorithms was taken as the core targets for the drug's therapeutic action on the disease.

### 2.6. GO and KEGG Pathway Enrichment Analysis

To gain deeper insights into the potential mechanisms of LWDHD in treating OP, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were performed on the intersecting targets between the drug and the disease. The intersection targets were uploaded to the DAVID database (https://david.ncifcrf.gov/summary.jsp) for analysis. The gene identifier was set to OFFICIAL_GENE_SYMBOL, and the species was specified as Homo sapiens. GO functional annotation was conducted in three categories: Biological Process (BP), Cellular Component (CC), and Molecular Function (MF), elucidating the roles of the target proteins in gene function. The top 10 terms for BP, CC, and MF were selected based on p-value. Additionally, the top 20 KEGG pathways most significantly associated with the disease were selected based on p-value as key signaling pathways, to predict the drug's mechanism of action. Finally, visualization analysis was performed using the bioinformatics online platform (http://www.bioinformatics.com.cn/).

## 3. Results

### 3.1. Collection of LWDHD Active Ingredients and Prediction of Drug and Disease Targets

Through the TCMSP database, a total of 58 active compounds were screened. Target gene names were standardized using the UniProt database, yielding 181 potential drug targets. A search of the GeneCards database identified 3655 OP-related targets. Intersection analysis between drug and disease targets identified 121 common targets (Figure 1), suggesting their potential role as therapeutic targets for LWDHD in treating OP.

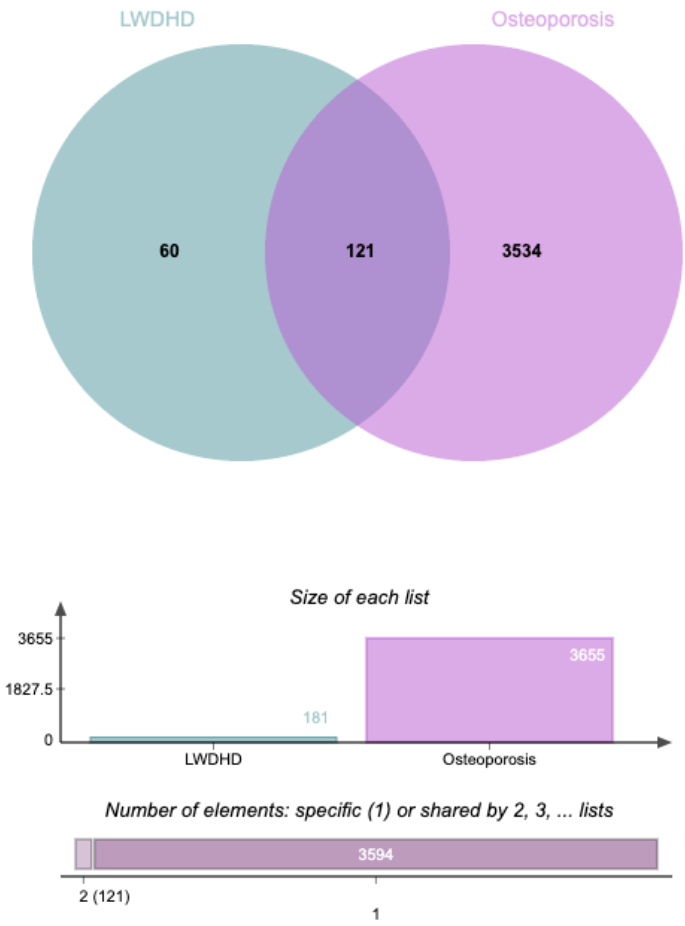


Figure 1: Venn Diagram of Intersecting Targets between Drugs and Diseases

### 3.2. Construction of the "Herb-Active Ingredient-Disease-Target" Network and Identification of Key Active Ingredients

Based on the corresponding relationships between active ingredients and intersecting targets, an interaction network comprising 187 nodes and 440 edges was constructed using Cytoscape 3.10.1 (Figure 2). Network topology analysis revealed that key components with a degree ≥ 9 included: quercetin, kaempferol, diosgenin, beta-sitosterol, Kadsurenone, and hancinone C. These components may play crucial roles in the mechanism of LWDHD for treating OP.

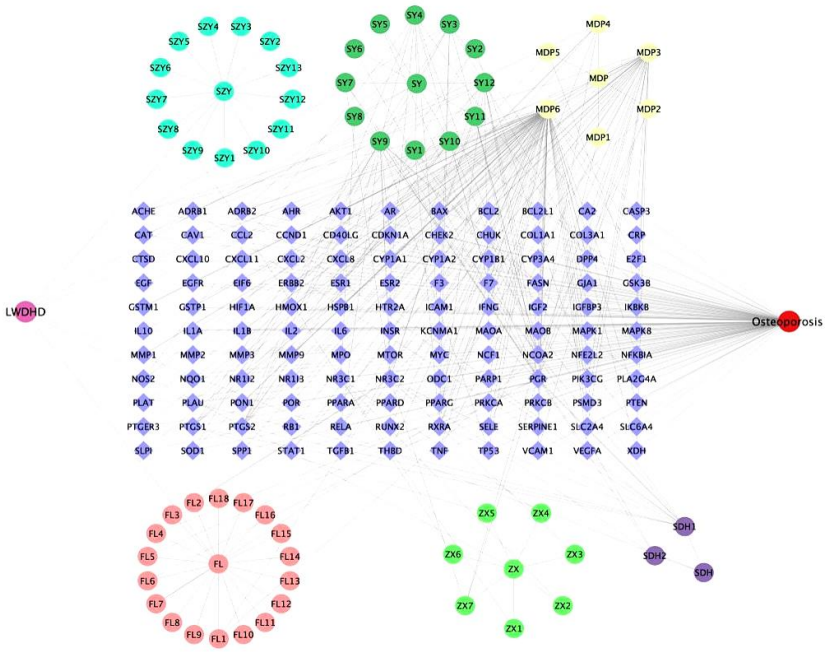


Figure 2: "Traditional Chinese Medicine–Active Component–Disease–Target" Network

Note: In the figure, "circles" represent traditional Chinese medicines, diseases, and active components, while "diamonds" represent the targets corresponding to the components.

### 3.3. Construction of Protein-Protein Interaction (PPI) Network and Screening of Key Targets

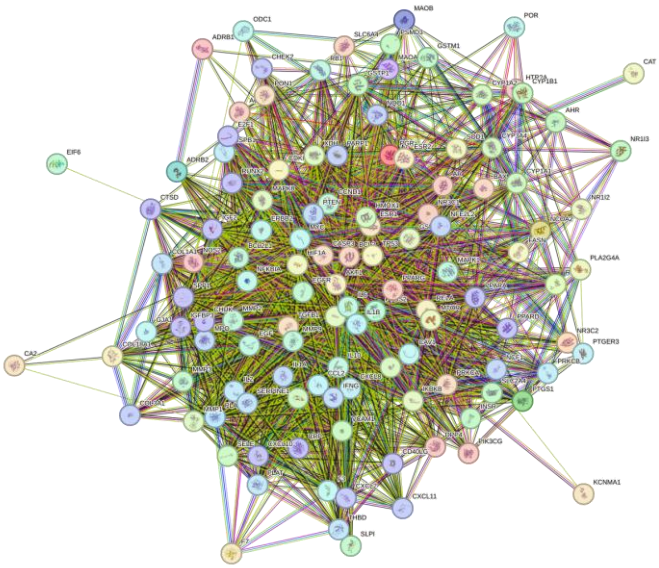

Figure 3: STRING Preliminary Protein-Protein Interaction Network

An initial PPI network was constructed for the 121 intersecting targets using the STRING database (Figure 3). After visualization with Cytoscape, the network contained 121 nodes and 2583 edges. The CytoHubba plugin within Cytoscape was used to perform calculations based on the MCC, Degree, and Closeness algorithms, yielding 10 hub targets from each method: MCC (Figure 4), Degree (Figure 5), and Closeness (Figure 6). Taking the intersection of these results identified 8 core targets: TNF, IL6, CASP3, XIAP, MMP2, RELA, PARP1, and KDR (Figure 7), which likely serve as hubs

within the regulatory network.

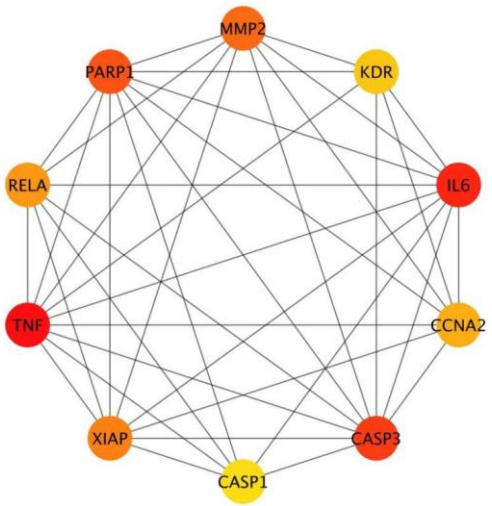


Figure 4: MCC Algorithm

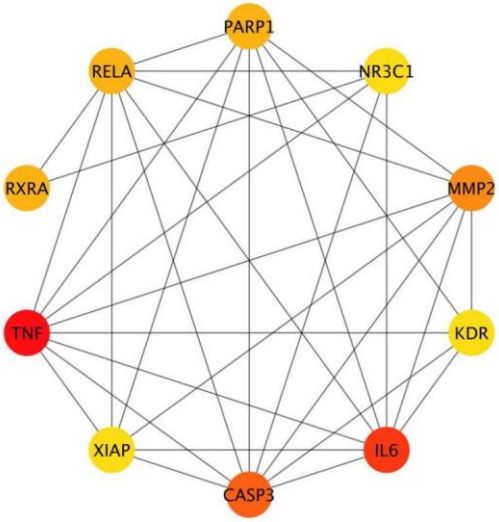


Figure 5: Degree Algorithm

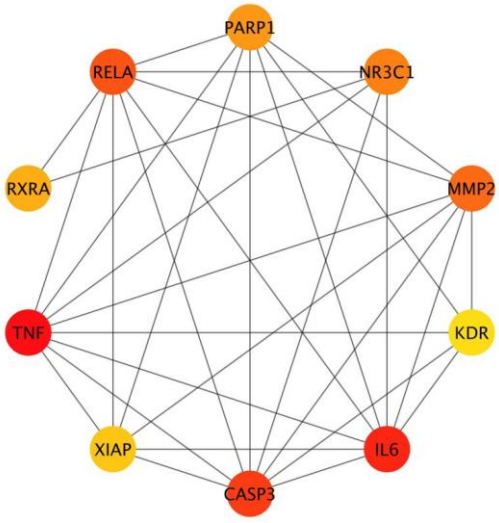


Figure 6: Closeness Centrality Algorithm

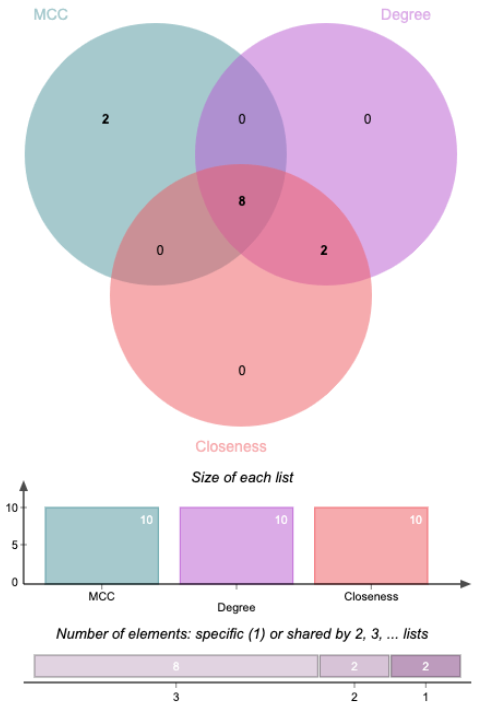


Figure 7: Venn Diagram of the Intersection among Three Algorithms

## 3.4. Biological Function Enrichment Analysis

### 3.4.1. GO Functional Enrichment Analysis

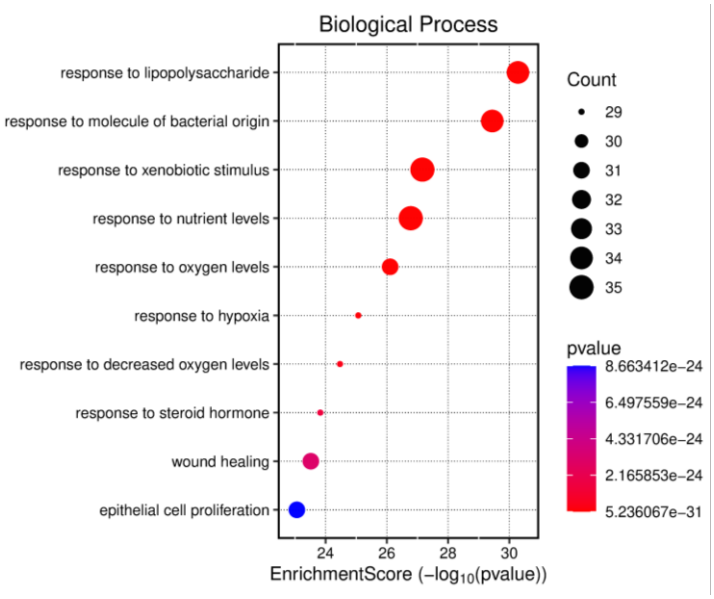


Figure 8: Bubble Chart of Top 10 BP Enrichment Analysis

GO analysis of the drug-disease intersecting genes identified 2559 significant terms ($P < 0.05$). These included 2337 terms in Biological Process (BP), primarily involved in response to lipopolysaccharide, response to molecule of bacterial origin, response to xenobiotic stimulus, response to nutrient levels, and response to oxygen levels (Figure 8). There were 61 terms in Cellular Component (CC), significantly enriched in structures such as membrane raft, membrane microdomain,

caveola, vesicle lumen, and plasma membrane raft (Figure 9). Furthermore, 161 terms were found in Molecular Function (MF), crucially involving nuclear receptor activity, ligand-activated transcription factor activity, DNA-binding transcription factor binding, RNA polymerase II-specific DNA-binding transcription factor binding, and steroid hormone receptor activity (Figure 10).

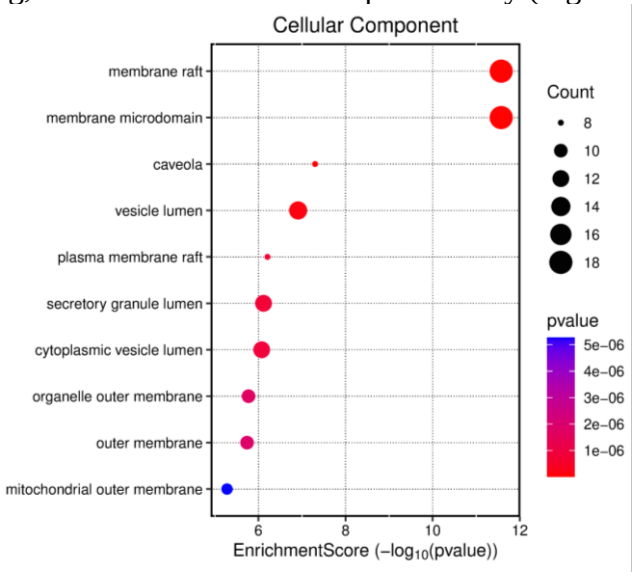


Figure 9: Bubble Chart of Top 10 CC Enrichment Analysis

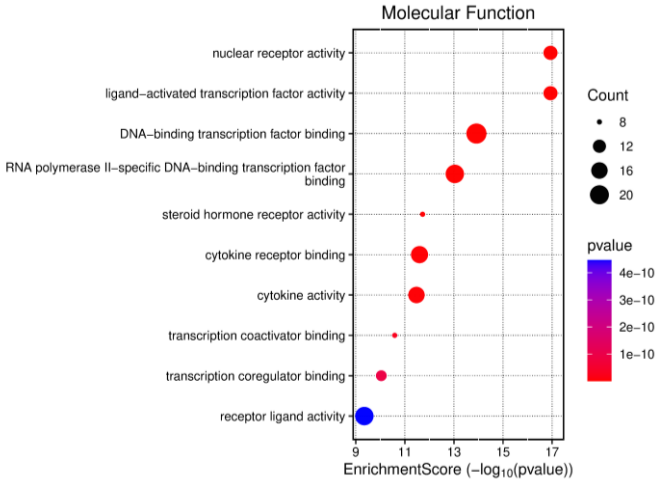


Figure 10: Bubble Chart of Top 10 MF Enrichment Analysis

### 3.4.2. KEGG Pathway Enrichment Analysis

KEGG analysis of the intersecting targets identified 161 significant pathways ($P < 0.05$). Figure 11 displays the top 20 core pathways ranked by P-value, including the IL-17 signaling pathway, TNF signaling pathway, HIF-1 signaling pathway, among others.

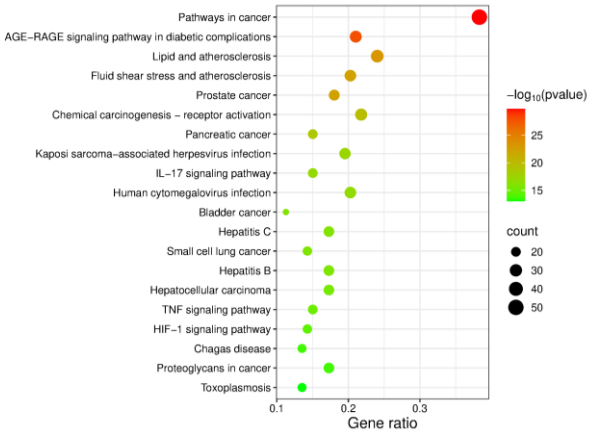


Figure 11: Top 20 KEGG Pathway Enrichment Analysis Chart

## 4. Discussion

With the acceleration of population aging in China, the incidence of OP continues to rise. Characterized by progressive decline in bone mineral density and bone quality, its clinical manifestations include low back pain, loss of height, and a significantly increased risk of fragility fractures. In traditional Chinese medicine (TCM), this disease falls under the categories of "bone wilting" (Gu Wei) and "bone desiccation" (Gu Ku). The core pathogenesis is considered to be deficiency of kidney essence, where the kidney's function of governing the bones and generating marrow declines, leading to a lack of nourishment for the bones, resulting in porosity and fragility. Targeting this pathogenesis, the classic formula LWDHD adheres to the principle of "three tonifications and three purgations." Rehmanniae Radix Praeparata (Shu Di Huang), Corni Fructus (Shan Zhu Yu), and Dioscoreae Rhizoma (Shan Yao) are used to tonify the deficiencies of kidney, liver, and spleen yin, constituting the "three tonifications." These are assisted by Alismatis Rhizoma (Ze Xie), Moutan Cortex (Mu Dan Pi), and Poria (Fu Ling) to purge kidney turbidity, clear deficient fire, and drain spleen dampness, constituting the "three purgations." The formula collectively achieves the effect of nourishing yin, tonifying the kidney, replenishing essence, and benefiting the marrow. This study employed a network pharmacology approach to systematically analyze the active ingredients, potential targets, and related signaling pathways of LWDHD in treating OP, aiming to elucidate its synergistic multi-component, multi-target mechanism at the molecular level.

With their multi-component, multi-target synergistic mechanism of action, TCM compound formulas can effectively intervene in key pathological processes such as anti-inflammation, antioxidation, tissue metabolism, and apoptosis by regulating multiple signaling pathways, thereby achieving therapeutic effects on OP [8]. Based on network pharmacology analysis, this study preliminarily identified components such as quercetin, kaempferol, diosgenin, beta-sitosterol, Kadsurenone, and hancinone C as potential core active substances of LWDHD for treating OP. Among these, quercetin, a natural flavonol, possesses significant anti-inflammatory, antioxidant, and antimicrobial properties. Research indicates that quercetin can inhibit osteoclastogenesis and reduce bone resorption by affecting the OPG/RANK/RANKL mechanism through suppression of RANKL activation, thereby stabilizing bone mass homeostasis [9, 10]. Furthermore, quercetin can effectively inhibit the premature senescence of osteoblast cell line MC3T3-E1; through this pathway, it effectively reduces bone loss in ovariectomized osteoporotic mouse models, exerting estrogen-like effects [11]. Kaempferol can activate estrogen receptor activity, enhance the proliferation and differentiation capacity of osteoblast cell line MG-63, and promote osteoblast mineralization [12]. Beta-sitosterol, widely found in various TCM ingredients, possesses physiological functions such as

anti-inflammatory, antioxidant, and anti-androgenic activities [13]. Studies have found that beta-sitosterol can promote and strengthen osteogenesis by increasing the osteoprotegerin/osteoclast differentiation factor (OPG/ODF) ratio in osteoblasts and stimulating the function of ovarian granulosa cells to differentiate estradiol [14].

Intersection analysis using multiple algorithms revealed that the key targets for LWDHD in treating OP primarily include TNF, IL6, CASP3, XIAP, MMP2, RELA, PARP1, and KDR. Among these, TNF plays a central role in bone homeostasis imbalance. It can directly promote the differentiation and maturation of osteoclast precursors, enhance bone resorption activity, and simultaneously inhibit the generation and function of osteoblasts, hindering bone formation [15]. Particularly in the estrogen-deficient state of postmenopause, serum TNF-α levels are significantly elevated and inversely correlated with bone mineral density, positioning it as a key inflammatory cytokine driving OP progression [16]. Secondly, IL6, another core inflammatory mediator, acts synergistically with TNF-α. Elevated IL-6 levels are not only associated with aging and estrogen decline but also amplify the inflammatory response, creating a favorable bone marrow microenvironment for osteoclast differentiation and activation by stimulating the expression of factors like RANKL [17, 18]. Clinical studies confirm that serum IL-6 levels are significantly higher in postmenopausal OP patients and positively correlate with the rate of bone loss [16]. CASP3 participates in the pathogenesis of OP from the perspective of cell apoptosis. As a key executor of apoptosis, Caspase-3 activation leads to the apoptosis of osteoblasts and osteocytes, directly weakening bone formation capacity and disrupting the osteocyte network, thereby impairing the bone's mechanosensing and repair functions [19, 20]. Therefore, these core targets likely profoundly influence the balance between osteogenesis and osteoclastogenesis primarily by regulating the bone-immune inflammatory microenvironment and cell apoptosis.

Further KEGG pathway enrichment analysis indicated that the key mechanisms of LWDHD in treating OP may involve pathways such as the IL-17 signaling pathway, TNF signaling pathway, and HIF-1 signaling pathway, exerting biological effects like anti-inflammation and antioxidation. The chronic inflammation-related bone remodeling process mediated by the TNF signaling pathway is an important avenue for OP treatment. Core genes within this pathway, such as TNF and IL-6, regulate the bone resorption-formation balance by facilitating signal transduction between the TNF and RANK/RANKL/OPG signaling pathways [21]. The HIF-1 signaling pathway, a core pathway regulating bone metabolism, plays a crucial role in maintaining the balance between bone formation and resorption. On one hand, it can promote the osteogenic and angiogenic differentiation of bone marrow mesenchymal stem cells in hypoxic environments through the osteogenesis-angiogenesis coupling mechanism, coordinating bone regeneration [22]. On the other hand, the activation of HIF-1 can directly promote osteoclast differentiation and function, and pathological states like estrogen deficiency can weaken the physiological inhibition of its activation, thereby exacerbating bone resorption [23]. Research confirms that inhibiting this pathway can effectively delay the progression of postmenopausal OP, highlighting its significance in the pathological process [24]. Therefore, it is hypothesized that LWDHD may treat OP by targeting TNF, IL6, CASP3, and other key targets to regulate the TNF signaling pathway, HIF-1 signaling pathway, and other pathways, thereby exerting anti-inflammatory, antioxidant, and apoptosis-regulating effects.

## 5. Conclusion

In summary, this study utilized network pharmacology to demonstrate that the effective active ingredients of LWDHD can act on OP through multiple targets and pathways. The specific molecular mechanism involves core components in LWDHD, such as quercetin, kaempferol, and beta-sitosterol, potentially targeting TNF, IL6, CASP3, and others, and regulating signaling pathways including the

TNF signaling pathway and HIF-1 signaling pathway. This results in anti-inflammatory, antioxidant, and apoptosis-modulating effects, ultimately achieving the therapeutic goal for OP. This provides a theoretical basis for the clinical application of this formula. However, this study has certain limitations, and further experimental validation of the related core targets and pathways is needed to explore the specific mechanisms of action.

## References


*[1] Amin U, McPartland A, O'Sullivan M, Silke C. An overview of the management of osteoporosis in the aging female population. Womens Health (Lond). 2023;19:17455057231176655.*
*[2] Ensrud KE, Crandall CJ. Osteoporosis. Ann Intern Med. 2017;167(3):ITC17-ITC32.*
*[3] Mart ń-Merino E, Petersen I, Hawley S, et al. Risk of venous thromboembolism among users of different anti-osteoporosis drugs: a population-based cohort analysis including over 200,000 participants from Spain and the UK. Osteoporos Int. 2018;29(2):467-478.*
*[4] Li C, Lyu WH, Wang TT, et al. An epidemiological study on osteoporosis among 1088 medical staff. Chin J Osteoporos. 2015;21(10):1217-1220.*
*[5] Qaseem A, Forciea MA, McLean RM, et al. Treatment of low bone density or osteoporosis to prevent fractures in men and women: A clinical practice guideline update from the American College of Physicians. Ann Intern Med. 2017;166(11):818-839.*
*[6] Li LX, Wu LG, Liang BC, et al. Clinical efficacy evaluation of Liuwei Dihuang Pill in preventing and treating postmenopausal osteoporosis of kidney-yin deficiency type. Chin J Gen Pract. 2022;20(8):1392-1395.*
*[7] Zhang AQ, Cai XY, Xie YX. Analysis of the clinical efficacy of Liuwei Dihuang Pill in treating primary osteoporosis of liver-kidney yin deficiency type. Heilongjiang Med J. 2023;36(4):870-873.*
*[8] Zhang H, Zhou C, Zhang Z, et al. Integration of network pharmacology and experimental validation to explore the pharmacological mechanisms of Zhuanggu Busui Formula against osteoporosis. Front Endocrinol (Lausanne). 2022;12:841668.*
*[9] Zhang X, Zhang GQ, Gu BL, et al. Research progress on the effects of Salvia miltiorrhiza and its active components on bone metabolism. Chin J Osteoporos. 2015;21(1):112-116.*
*[10] Jian H, Yuan L, Wang X, et al. Icaritin and its glycosides enhance osteoblastic, but suppress osteoclastic, differentiation and activity in vitro. Life Sci. 2007;81(10):832-840.*
*[11] Gu YQ, Fu JY, Wu WQ, et al. Quercetin alleviates estrogen deficiency-induced osteoporosis through anti-senescence effect on bone cells. J Tongji Univ (Med Sci). 2019;40(3):274-280.*
*[12] Zeng YR, Zeng JC, Fan YG, et al. Effect of kaempferol on proliferation and differentiation of osteoblast MG-63. Lishizhen Med Mater Med Res. 2012;23:1463-1465.*
*[13] Rajavel T, Packiyaraj P, Suryanarayanan V, et al. β-Sitosterol targets Trx/Trx1 reductase to induce apoptosis in A549 cells via ROS mediated mitochondrial dysregulation and p53 activation. Sci Rep. 2018;8(1):584.*
*[14] Lin MZ, Zhao Y, Cai EB, et al. Antitumor effect of β-sitosterol on H22 tumor-bearing mice in vivo. Chin J Public Health. 2017;33(12):1797-1800.*
*[15] Wang S, Zhang H, Zhu Y, et al. Progranulin protects against osteoporosis by regulating osteoclast and osteoblast balance via TNFR pathway. J Cell Mol Med. 2025;29(3):e70385.*
*[16] He Y, Liu SQ, Wang CJ, et al. Role of IL-6 and TNF-α in the pathogenesis of postmenopausal osteoporosis. Chin J Clin Healthc. 2007;(1):41-43.*
*[17] Srivastava RK, Sapra L. The rising era of "immunoporosis": role of immune system in the pathophysiology of osteoporosis. J Inflamm Res. 2022;15:1667-1698.*
*[18] Yang XF, Hao YM, Lu K, et al. Mechanism of inflammatory bone marrow microenvironment affecting the occurrence and development of osteoporosis. Chin J Osteoporos. 2022;28(11):1614-1619.*
*[19] Ru JY, Wang YF. Osteocyte apoptosis: the roles and key molecular mechanisms in resorption-related bone diseases. Cell Death Dis. 2020;11(10):846.*
*[20] Djavaheri-Mergny M, Maiuri MC, Kroemer G. Cross talk between apoptosis and autophagy by caspase-mediated cleavage of Beclin 1. Oncogene. 2010;29(12):1717-1719.*
*[21] Ji YF, Jiang X, Li W, et al. Impact of interleukin-6 gene polymorphisms and its interaction with obesity on osteoporosis risk in Chinese postmenopausal women. Environ Health Prev Med. 2019;24(1):48.*
*[22] Wang Y, Wan C, Deng L, et al. The hypoxia-inducible factor α pathway couples angiogenesis to osteogenesis during skeletal development. J Clin Invest. 2007;117(6):1616-1626.*
*[23] Chen W, Wu P, Yu F, Luo G, Qing L, Tang J. HIF-1α regulates bone homeostasis and angiogenesis, participating in the occurrence of bone metabolic diseases. Cells. 2022;11(22):3552.*
*[24] Zhong H, Cao C, Yang J, et al. Study on the relationship between HIF-1 signaling pathway and postmenopausal osteoporosis. J Sichuan Univ (Med Sci). 2017;48(6):862-868.*