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Exploration of Liuwei Dihuang Pill on periodontitis based on network pharmacology and molecular docking

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Abstract

This study explores the mechanism of Liuwei Dihuang Pill (LWDHP) in the treatment of periodontitis using network pharmacology and molecular docking. The active ingredients and targets of LWDHP were obtained from databases such as Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform. Databases such as GeneCards, OMIM, and DisGeNET were used to obtain the relevant targets related to periodontitis. The intersection of these 2 groups of targets was taken and imported into STRING to facilitate the acquisition of protein–protein interaction data, which was then imported into Cytoscape 3.10.2 to perform topological analysis to obtain the core targets. Gene ontology and Kyoto encyclopedia of genes and genomes bioinformatics enrichment analyses of the intersecting targets were performed using the DAVID database. Validation of molecular docking matching between key active ingredients with top 5 degree values and key targets with top 5 degree values in the treatment of periodontitis with LWDHP using AutoDockTools-1.5.6. A total of 69 active ingredients were discerned in LWDHP, implicating 198 periodontitis-relevant targets. Thirty-four core targets were obtained by protein–protein interaction network topology analysis, among which the key targets with the top 5 values of degree were tumor necrosis factor (TNF), serine/threonine protein kinase AKT1, sarcoma, epidermal growth factor receptor, and matrix metallopeptidase 9. Topological analysis revealed that the key active ingredients with the top 5 values of degree in LWDHP were Polyporenic acid C, Alisol B, Hydroxygenkwanin, Denudatin B, and Kadsurenone. The molecular docking results demonstrated that the binding energies of the above molecules with targets were all <-5 kcal/mol, indicating a good binding ability between these molecules. The gene ontology enrichment results indicated that the treatment of periodontitis by LWDHP was mainly related to the inflammatory response, positive regulation of phosphatidylinositol-3-kinase-Akt (PI3K-Akt) signal transduction and other processes. Analysis of the Kyoto encyclopedia of genes and genomes signaling pathway showed that the TNF signaling pathway, the PI3K-Akt signaling pathway, and so on are important signaling pathways. In conclusion, the mechanism of action of LWDHP in the treatment of periodontitis is characterized by multicomponents, multi-targets, and multi-pathways. TNF, serine/threonine protein kinase AKT1, sarcoma, epidermal growth factor receptor, and matrix metallopeptidase 9 are the key targets and the TNF signaling pathway, the PI3K-Akt signaling pathway are the key pathways. LWDHP treats periodontitis through actions such as anti-inflammatory and regulation of the balance between osteogenesis and bone destruction.

Abbreviations: AKT1 = AKT serine/threonine kinase 1, BP = biological processes, CC = cellular components, EGFR = epidermal growth factor receptor, GO = gene ontology, KEGG = Kyoto encyclopedia of genes and genomes, LWDHP = Liuwei Dihuang Pill, MF = molecular functions, MMP9 = matrix metallopeptidase 9, PI3K-Akt = phosphatidylinositol-3-kinase-Akt, PPI = protein–protein interactions, SRC = sarcoma, TCMSP = Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform, TNF = tumor necrosis factor.

Keywords: Liuwei Dihuang Pill, molecular docking, network pharmacology, periodontitis

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The authors have no conflicts of interest to disclose.

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

Because we use public databases, according to the ethics guidelines, neither informed consent nor approval of the ethics committee is required.

Supplemental Digital Content is available for this article.

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1. Introduction

Periodontitis is an inflammatory disease that occurs in the periodontal supporting tissues. It is one of the most common oral diseases and the leading cause of tooth loss in adults. Since subgingival plaque causes inflammatory and immune responses in the host, it will eventually lead to irreversible destruction of periodontal tissues in susceptible hosts.[1] Periodontal tissue destruction, including attachment loss and alveolar bone resorption, can lead to tooth loosening and loss,[2] seriously affecting the quality of life of patients.[3] In addition, periodontitis is also related to some other diseases. It includes cardiovascular diseases,[4] diabetes,[5] Alzheimer disease,[6] chronic liver disease,[7] etc. Therefore, the prevention and treatment of periodontitis has great clinical significance.

Although bacterial infection is a necessary condition for the development of periodontitis, the host immune response is critical for the development and tissue destruction of periodontitis.[8] Traditional treatment methods include mechanical removal of dental plaque and calculus, various types of periodontal surgery and local adjuvant antibiotics.[9] These treatments reduce bacterial infection but ignore the role of the host's immune response in periodontitis.In addition, long-term topical use of antibiotics may also cause the generation of drug-resistant bacteria,[10] which will destroy the normal oral microenvironment of flora. There are many biologically active ingredients with immunomodulatory functions in Chinese medicine compounds,[11] which can enhance the effectiveness of treatment of periodontitis by modulating the host's immune response to infection. Therefore, traditional Chinese medicine has become a new direction for the treatment of periodontitis[12] due to its immunomodulatory effects.

Liuwei Dihuang Pill is a kind of traditional Chinese medicine compound preparation, which contains 6 kinds of traditional Chinese medicine such as Dihuang (Rehmannia), Shanyao (Chinese yam), Shanzhuyu (Cornus officinalis), Fuling (Poria cocos), Zexie (Rhizoma alismatis), and Mudanpi (Peony peel). Its pharmacological effects mainly include antitumor, antioxidation, immune regulation, and metabolism regulation.[13] Liuwei Dihuang Pill (LWDHP) can be used to treat various types of diseases, including diabetes,[14] hypertension,[15] osteoporosis,[16] chronic kidney disease,[17] etc. Recently, it has been found that periodontal therapy combined with LWDHP can improve the efficacy of periodontitis,[18,19] and the efficacy of patients with chronic periodontitis combined with osteoporosis can be significantly improved.[20] Although there have been many clinical studies on LWDHP in the treatment of periodontitis, the molecular mechanism is still unclear.

Network pharmacology is an emerging discipline formed by the fusion of pharmacology and various disciplines, which can comprehensively reflect the molecular mechanism of drug action on the disease. This study will deeply excavate the potential target and mechanism of action of LWDHP on periodontitis through network pharmacology and molecular docking technology, to provide a theoretical basis for the clinical use of LWDHP in the treatment of periodontitis and to provide a reference for the development of new drugs for the treatment of periodontitis.

2. Materials and methods

2.1. Screening of active ingredients and targets of LWDHP

The oral bioavailability ≥30% and drug-like index ≥ 0.18 were set as screening conditions in the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP, https://old.tcmsp-e.com/tcmsp.php). To search the active ingredients of 6 kinds of traditional Chinese medicine (Dihuang, Shanyao, Shanzhuyu, Fuling, Zexie, and Mudanpi) contained in LWDHP. The SMILES of the active ingredient of the drug was searched in PubChem database (https://pubchem.ncbi.nlm.nih.gov/), and then was imported into SwissTargetPrediction database (http://www.swisstargetprediction.ch/). The species was selected as "Homo sapiens" to obtain the corresponding targets of the ingredient. The targets were merged and the duplicate values were removed to obtain the targets of LWDHP.

2.2. Targets acquisition for periodontitis

The targets of periodontitis were retrieved from GeneCards database (https://www.genecards.org), OMIM database (https://www.omim.org), and DisGeNET database (https://www.disgenet.org) with "periodontitis" as the keyword. The targets obtained from the 3 databases were integrated and the duplicate values were deleted to obtain periodontitis targets.

2.3. The intersection targets between LWDHP and periodontitis

The intersection targets of LWDHP and periodontitis were acquired from VENNY 2.1.0 (https://bioinfogp.cnb.csic.es/tools/venny/).

2.4. Construction of "Chinese Medicine–Ingredient–Target" network

The active ingredients of LWDHP and the obtained intersection targets were imported into the Cytoscape 3.10.2 software to construct a "Chinese Medicine–Ingredient–Target" network diagram, and topological analysis was carried out to screen the key active ingredients.

2.5. Construction of PPI network and acquisition of core targets

The intersection targets were imported into the STRING database (https://string-db.org), the protein type was set as homo sapiens, the minimum required interaction score was set as "medium confidence (0. 400)," and the protein–protein interaction (PPI) network was obtained and the relevant data were downloaded in TSV format and imported into Cytoscape 3.10.2 software. The core targets were screened and plotted according to the average value of "Degree," "Closeness," and "Betweenness."

2.6. GO and KEGG enrichment analysis

The intersection targets were imported into the DAVID database (https://david.ncifcrf.gov/) for gene ontology (GO) and Kyoto encyclopedia of genes and genomes (KEGG) enrichment analysis. GO function includes 3 parts: biological process (BP), cellular component (CC), and molecular function (MF). In the 3 items of BP, CC, and MF, the top 10 data were ranked by "gene ratio" and screened to make the GO functional analysis bubble chart. The top 20 pathways with the lowest *P* value in KEGG pathway enrichment analysis were plotted using horizontal bars.

2.7. Molecular docking analysis

The 3D structure of the required small molecule ligand was downloaded from the TCMSP database and saved as a "mol2" structure file. The ligand structure was optimized by AutoDockTools-1.5.6 and saved as a pdbqt file. The 3D structure of the target protein was obtained from the PDB database (https://www.rcsb.org) and the water molecules and ligands in the target protein were removed by PyMOL 3.0.4. Then the target protein was imported into AutoDockTools-1.5.6 for hydrogenated and saved as pdbqt files. The search parameters

of the docking were set to "Genetic AIgorithm" and the output was set to "Lamarckian GA(4.2)." The binding affinity was used as a reference to determine the binding activity of the ligand and target protein. The docking results were displayed in the form of heat map. Generally, it is believed that the docking score less than or equal to -5.0 kcal/mol indicates a strong affinity between the ligand and the target protein.[21] PyMOL 3.0.4 was used to visualize the molecular docking results.

3. Result

3.1. Active ingredients and targets of LWDHP

The active ingredients of 6 traditional Chinese medicines in LWDHP were retrieved from TCMSP database. Under the condition of oral bioavailability ≥30% and drug-like index ≥0.18, a total of 69 active ingredients were obtained, including 2 active ingredients from Dihuang, 16 active ingredients from Shanyao, 20 active ingredients from Shanzhuyu, 11 active ingredients from Mudanpi, 15 active ingredients from Fuling, and 10 active ingredients from Zexie (Supplemental Digital Content 01, Supplemental Digital Content, https://links.lww.com/MD/O917). The corresponding targets of active ingredients were predicted by SwissTargetPrediction database, and 707 targets of LWDHP were obtained after combined and deduplicated.

3.2. Acquisition of targets in periodontitis

Among 3238 periodontitis targets retrieved from GeneCards database, 1632 periodontitis targets were screened according to the median of Relevance score value (Median Relevance score = 0.818983614; selecting Relevance score > 0.81). Eight gene targets were obtained in the OMIM database. 682 periodontitis targets were obtained from the DisGeNET database. The periodontitis gene targets obtained from the 3 databases were merged and the duplicate values were removed, and 1902 periodontitis gene targets were finally obtained.

3.3. Acquisition of intersection targets of LWDHP and periodontitis

With VENNY 2.1.0, 707 targets of LWDHP were taken as intersections with 1902 targets of periodontitis, and finally 198 intersecting targets were obtained (Fig. 1, Supplemental Digital Content 02, Supplemental Digital Content, https://links.lww.com/MD/O918). These are the potential targets of action of LWDHP for the treatment of periodontitis.

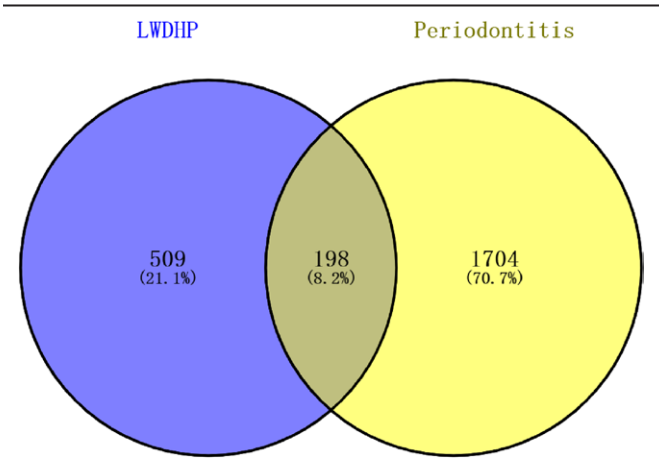

Figure 1. Venn diagram of the interactive targets of LWDHP and periodontitis. LWDHP = Liuwei Dihuang Pill.

3.4. Construction of "Chinese Medicine–Ingredient–Target" network

The active ingredients of LWDHP and the obtained intersection targets were imported into Cytoscape 3.10.2, and a network diagram of "Chinese Medicine–Ingredient–Target" was constructed (Fig. 2), and topological analyses were carried out to screen out the key active ingredients with the top 5 degree values (Table 1).

3.5. Construct PPI networks and acquire core targets

The 198 intersection targets of LWDHP and periodontitis were imported into the STRING database to obtain the PPI network diagram (Fig. 3). The results of STRING database were downloaded and imported into Cytoscape 3.10.2 software, and 34 core targets were obtained by screening based on the average values of degree, closeness, and betweenness indicators (Supplemental Digital Content 03, Supplemental Digital Content, https://links.lww.com/MD/O919). The PPI circle diagrams were produced according to the different degree values of the core targets (Fig. 4). According to Figure 4, tumor necrosis factor (TNF; TNF-α), serine/threonine protein kinase AKT1 (AKT1), sarcoma (SRC), epidermal growth factor receptor (EGFR), and matrix metallopeptidase 9 (MMP9) may be key targets for the treatment of periodontitis with LWDHP.

3.6. The enrichment analysis of GO and KEGG

The results of BP, CC, and MF in the GO functional enrichment analysis were each screened for the top 10 entries to make bubble diagrams (Fig. 5). Among them, BP mainly includes positive regulation of transcription by RNA polymerase II, signal transduction, phosphorylation, inflammatory response, positive regulation of gene expression, positive regulation of cell population proliferation, proteolysis, negative regulation of apoptotic process, positive regulation of phosphatidylinositol-3-kinase-Akt (PI3K-Akt) signal transduction, etc. CC mainly includes plasma membrane, cytoplasm, cytosol, nucleus, membrane, etc. MF mainly includes in protein binding, identical protein binding, ATP binding, metal ion binding, zinc ion binding, enzyme binding, integrin binding, etc. The top 20 KEGG signaling pathways were enriched into a horizontal bar chart (Fig. 6), and the main pathways included pathways in cancer, proteoglycans in cancer, EGFR tyrosine kinase inhibitor resistance, PI3K-Akt signaling pathway, lipid and atherosclerosis, Rap1 signaling pathway, Kaposi sarcoma-associated herpesvirus infection, AGE-RAGE (Advanced Glycation End Products Receptor) signaling pathway in diabetic complications, focal adhesion, human cytomegalovirus infection, apoptosis, prostate cancer, endocrine resistance, relaxin signaling pathway, TNF signaling pathway, etc.

3.7. Molecular docking

The key active ingredients in Table 1 were molecularly docked with the key target proteins with the top 5 degree values, and all the docking energies were <-5 kcal/mol, indicating a good binding ability between the molecules involved, and the molecular docking energy scores were shown in a heat map (Fig. 7). According to the docking results, it is known that the key components of LWDHP, Polyporenic acid C, Alisol B, Hydroxygenkwanin, Denudatin B, and Kadsurenone, were stably bound to the key targets TNF, AKT1, SRC, EGFR, and MMP9. Among them, the lowest binding energy to TNF, SRC and EGFR was Polyporenic acid C, and the lowest binding energy to AKT1 and MMP9 was

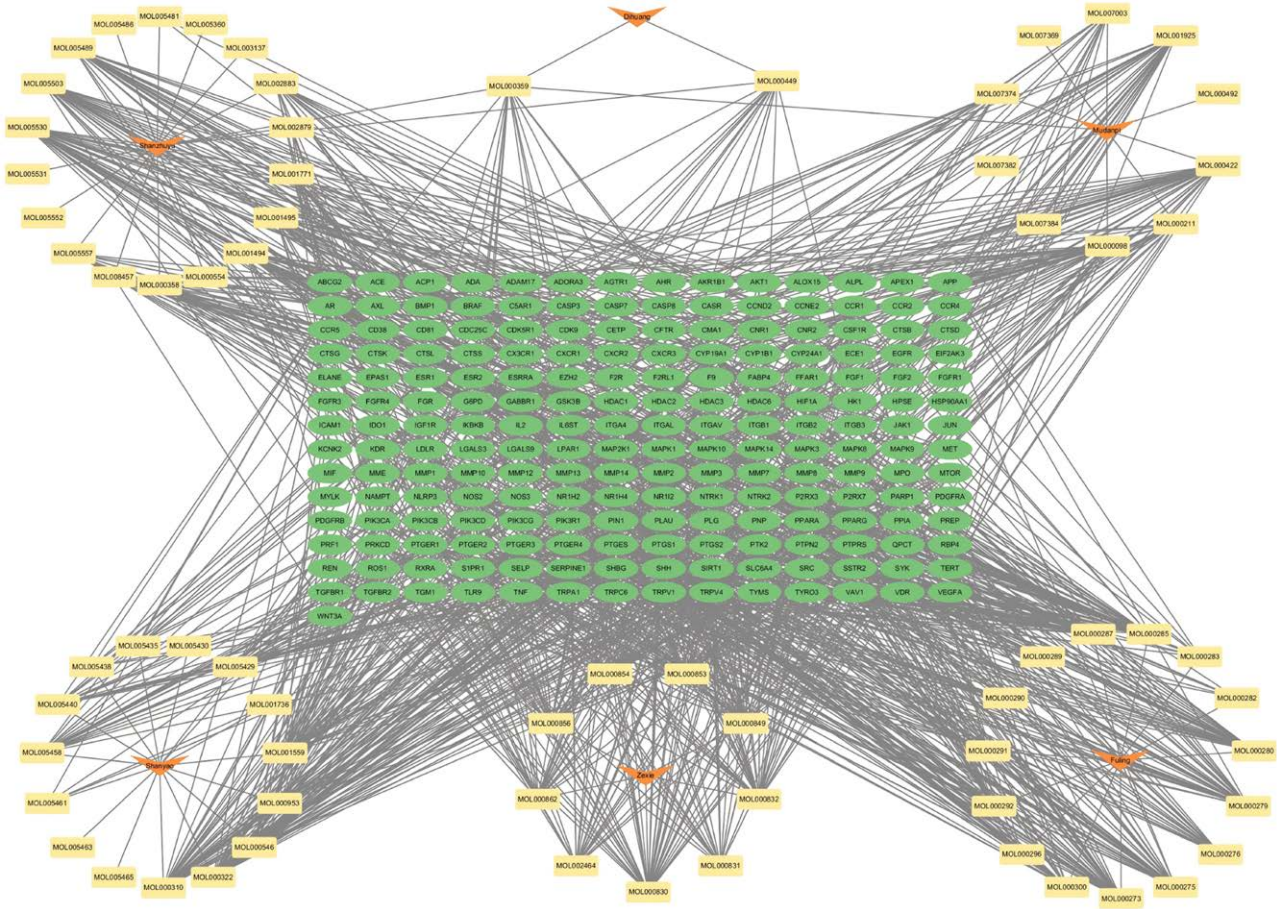

Figure 2. "Chinese Medicine–Ingredient–Target" network diagram. The orange nodes indicate the Chinese Medicine, the yellow nodes indicate ingredients, the green nodes indicate potential gene targets, and the edges indicate their interactions.

Table 1

The key active ingredients in the treatment of periodontitis with Liuwei Dihuang Pill.

MOL ID	Molecular name	Degree	Chinese medicine
MOL000285	Polyporenic acid C	44	Fuling
MOL000830	Alisol B	40	Zexie
MOL005530	Hydroxygenkwanin	38	Shanzhuyu
MOL000310	Denudatin B	37	Shanyao
MOL000322	Kadsurenone	37	Shanyao

Alisol B. The docking results of Polyporenic acid C with TNF, SRC and EGFR, and the docking results of Alisol B with AKT1 and MMP9 were imported into PyMOL 3.0.4 for visualization and analysis (Fig. 8). Polyporenic acid C binds to TNF to form 2 hydrogen bonds, to SRC to form 3 hydrogen bonds, and to EGFR to form 4 hydrogen bonds. Alisol B binds to AKT1 to form 2 hydrogen bonds and to MMP9 to form 5 hydrogen bonds. This indicates that the binding of the relevant molecules to the corresponding targets is relatively stable.

4. Discussion

LWDHP is a traditional Chinese medicine compound formula that can nourish the yin of the liver and kidney and is mainly used for treating dizziness and tinnitus, lumbar and knee soreness and loosening of teeth caused by kidney yin deficiency.[22] Clinical studies have shown that LWDHP has some therapeutic effects on periodontitis, but its molecular mechanism is still unclear, this paper explores the mechanism of action of LWDHP in treating periodontitis through network pharmacology and molecular docking technology.

The key active ingredients in the top 5 degree values of LWDHP for periodontitis are Polyporenic acid C, Alisol B, Hydroxygenkwanin, Denudatin B, and Kadsurenone. Polyporenic acid C is a triterpenoid derived from Fuling, and it has been documented that Polyporenic acid C exhibits an inhibitory effect on receptor activators of nuclear factor-κB ligand-induced osteoclastogenesis,[23] which may inhibit osteoclast formation during the progression of periodontitis, thereby delaying resorption of alveolar bone, and that Polyporenic acid C inhibits the release of NO from macrophages,[24] which plays an important role in the inflammatory response process, suggesting that it may inhibit the inflammatory response in periodontal tissues. Alisol B, a novel plant steroid derived from Zexie, inhibits RANKL-induced osteoclast formation and prevents bone loss in mice,[25] and may have a role in inhibiting periodontitis-induced alveolar bone resorption. Hydroxygenkwanin, derived from Shanzhuyu, which has significant anti-inflammatory, antitumor, antiproliferative,

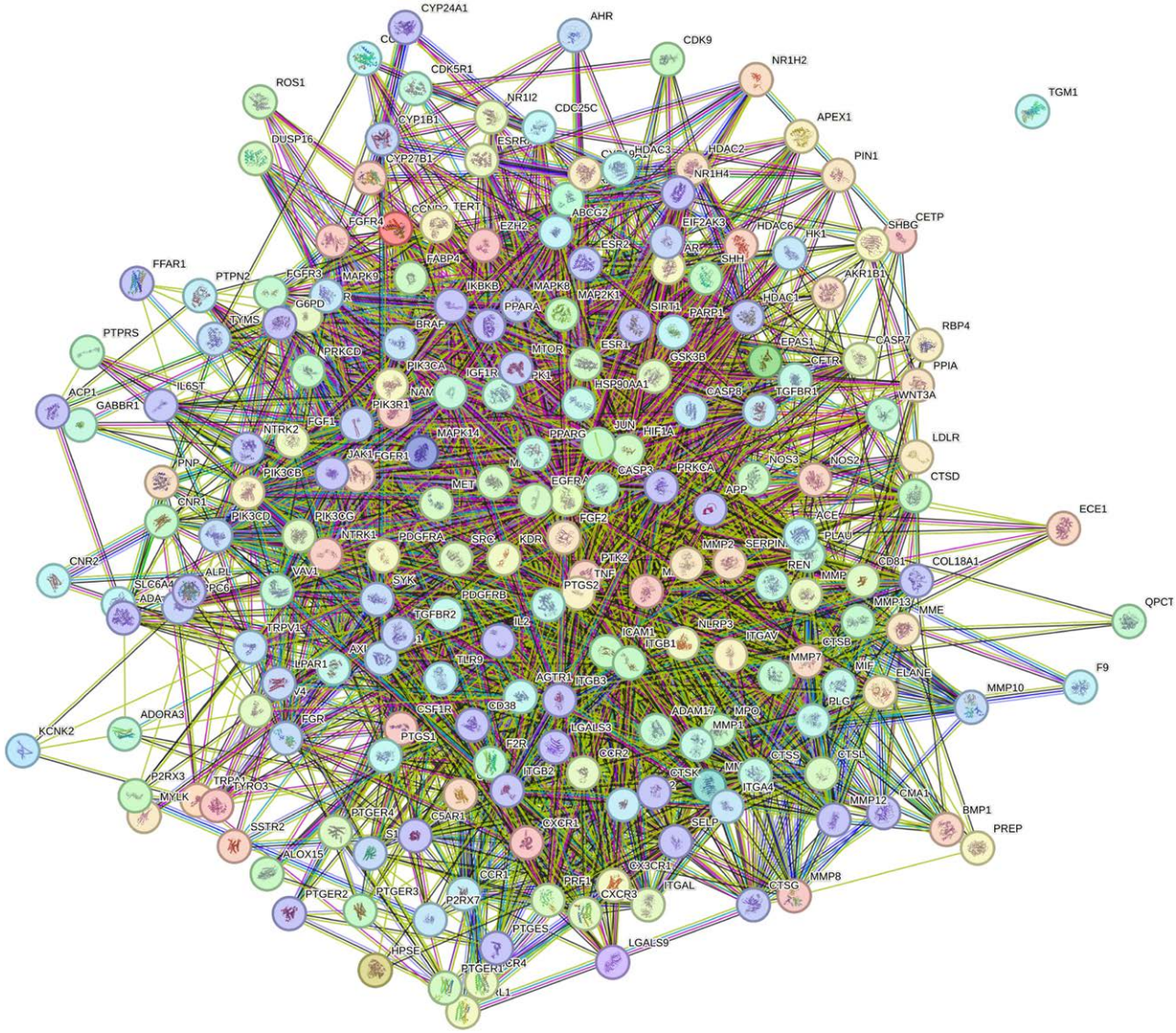

Figure 3. The PPI network of LWDHP in treating periodontitis. PPI = protein–protein interactions, LWDHP = Liuwei Dihuang Pill.

and anti-migratory effects,[26] may be able to reduce periodontal inflammation. Denudatin B and Kadsurenone, which are naturally derived from Shanyao, are platelet-activating factor antagonists, whose molecular functions have not been thoroughly investigated and whose role in the treatment of periodontitis is not clear.

The key targets of LWDHP for the treatment of periodontitis were identified from the database, which included TNF, AKT1, SRC, EGFR, and MMP9. The results of molecular docking showed that the key components of LWDHP for the treatment of periodontitis, Polyporenic acid C, Alisol B, Hydroxygenkwanin, Denudatin B, and Kadsurenone, were all able to bind stably to TNF, AKT1, SRC, EGFR, and MMP9. As the central cytokine of inflammatory response, TNF can directly or indirectly drive inflammatory response and biologics that neutralize TNF are among the most successful drugs for the treatment of chronic inflammation and autoimmune diseases.[27] GO enrichment analyses showed that the treatment of periodontitis by LWDHP was associated with inflammatory response, and KEGG enrichment analysis indicated that TNF signaling pathway was one of the major pathways. Therefore, LWDHP mainly act on the TNF signaling pathway by targeting the TNF target and thus inhibit the inflammatory response.

AKT1 is a target kinase in the PI3K-Akt signaling pathway, which is a typical pathway regulating inflammation,[28] and the PI3K-Akt signaling pathway has been shown to regulate osteoclast survival and differentiation[29] and enhance the proliferation of osteoblasts.[30] The PI3K-Akt signaling pathway not only maintains the balance between osteogenesis and bone destruction, but also effectively improves insulin sensitivity, alleviates insulin resistance and regulates glucose metabolism. It has been demonstrated that the PI3K-Akt signaling pathway is related to diabetes-associated bone disease, and activation of the PI3K-Akt pathway in human bone marrow mesenchymal stem cells cultured with high glucose levels can attenuate high glucose-induced osteoblast proliferation and differentiation.[30] GO enrichment analysis showed that the treatment of periodontitis by LWDHP was associated with positive regulation of PI3K-Akt signal transduction, and KEGG enrichment analysis indicated that the PI3K-Akt signaling pathway was one of the major pathways. Therefore, through the PI3K-Akt signaling pathway, LWDHP can not only control periodontal inflammation and regulate alveolar bone resorption and remodeling, but also play a therapeutic role in diabetes and osteoporosis.

SRC is a protein tyrosine kinase that plays a key role in cell growth, division, migration, and survival signaling pathways,[31] and SRC is essential for bone resorption by osteoclasts and inhibits bone formation by osteoblasts.[32] Therefore, the key active ingredient in LWDHP may regulate alveolar bone resorption and formation by targeting SRC.

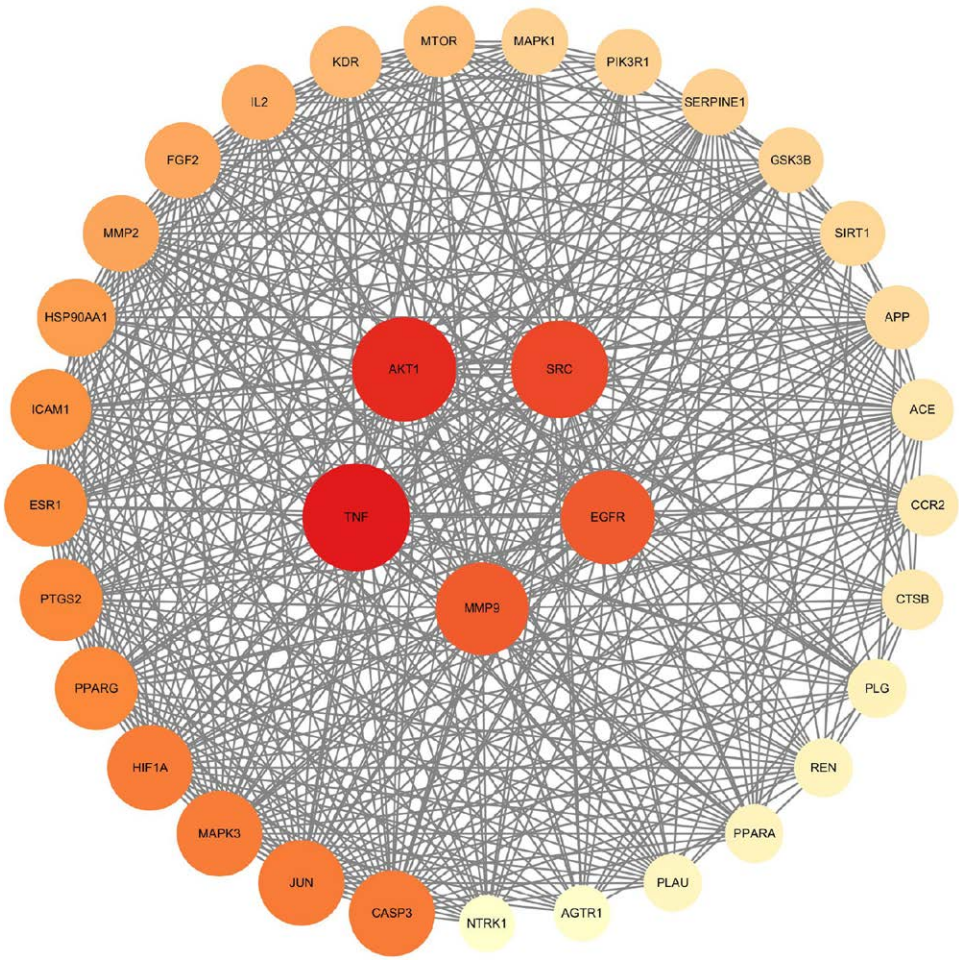

Figure 4. The PPI network of core targets. The nodes the redder and the bigger have the higher degree of interaction. PPI = protein–protein interactions.

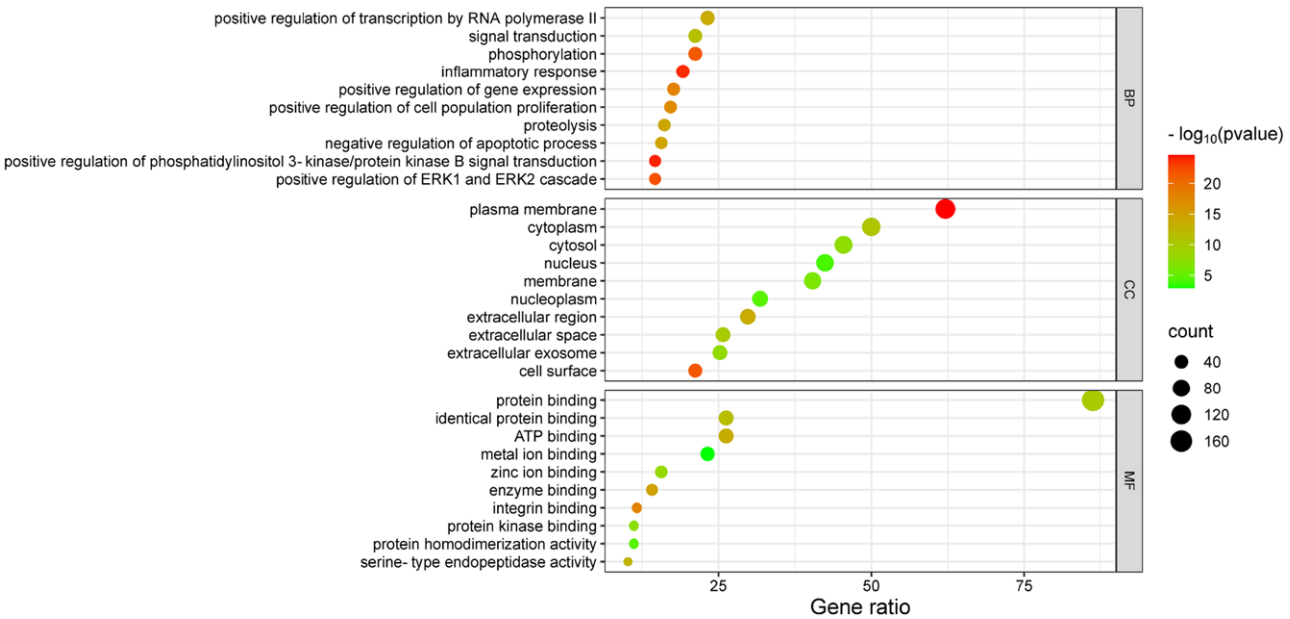

Figure 5. The top 10 results of BP, CC, and MF in the GO functional enrichment analysis. BP = biological processes, CC = cell composition, GO = gene ontology, MF = molecular function.

EGFR is a tyrosine kinase involved in cell proliferation, division, and cancer development.[33] In an experimental mouse model of periodontitis, inhibition of EGFR significantly reduced bone loss and periodontal inflammation,[34] suggesting that EGFR may be one of the key targets for the treatment of periodontitis.

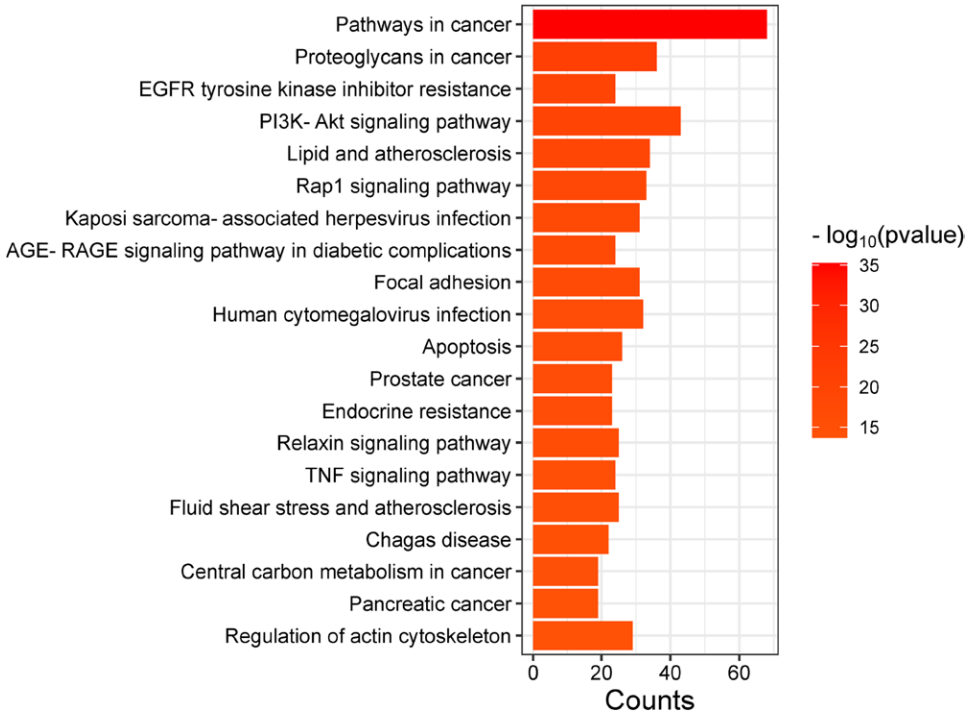

Figure 6. The horizontal bar chart of the top 20 KEGG signaling pathways. KEGG = Kyoto encyclopedia of genes and genomes.

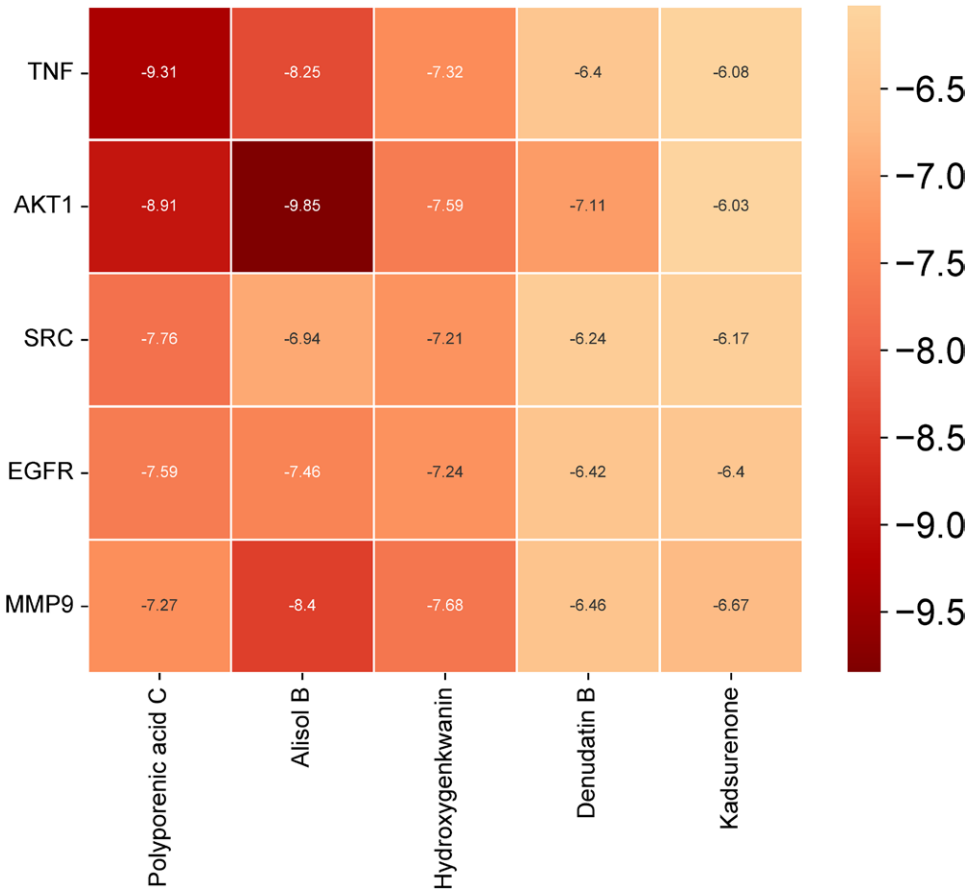

Figure 7. Heatmap of the molecular docking energy scores of the key ingredients with the key targets (kcal/mol). The lower the scores the redder the color.

MMP9 is a protease, mainly secreted by neutrophils and macrophages, that regulates inflammation in tissues.[35] It not only destroys collagen and promotes the secretion of inflammatory mediators, but its overexpression promotes the expression of osteoclast-specific genes.[36] Therefore, one of the mechanisms of LWDHP in treating periodontitis may be to reduce the destruction

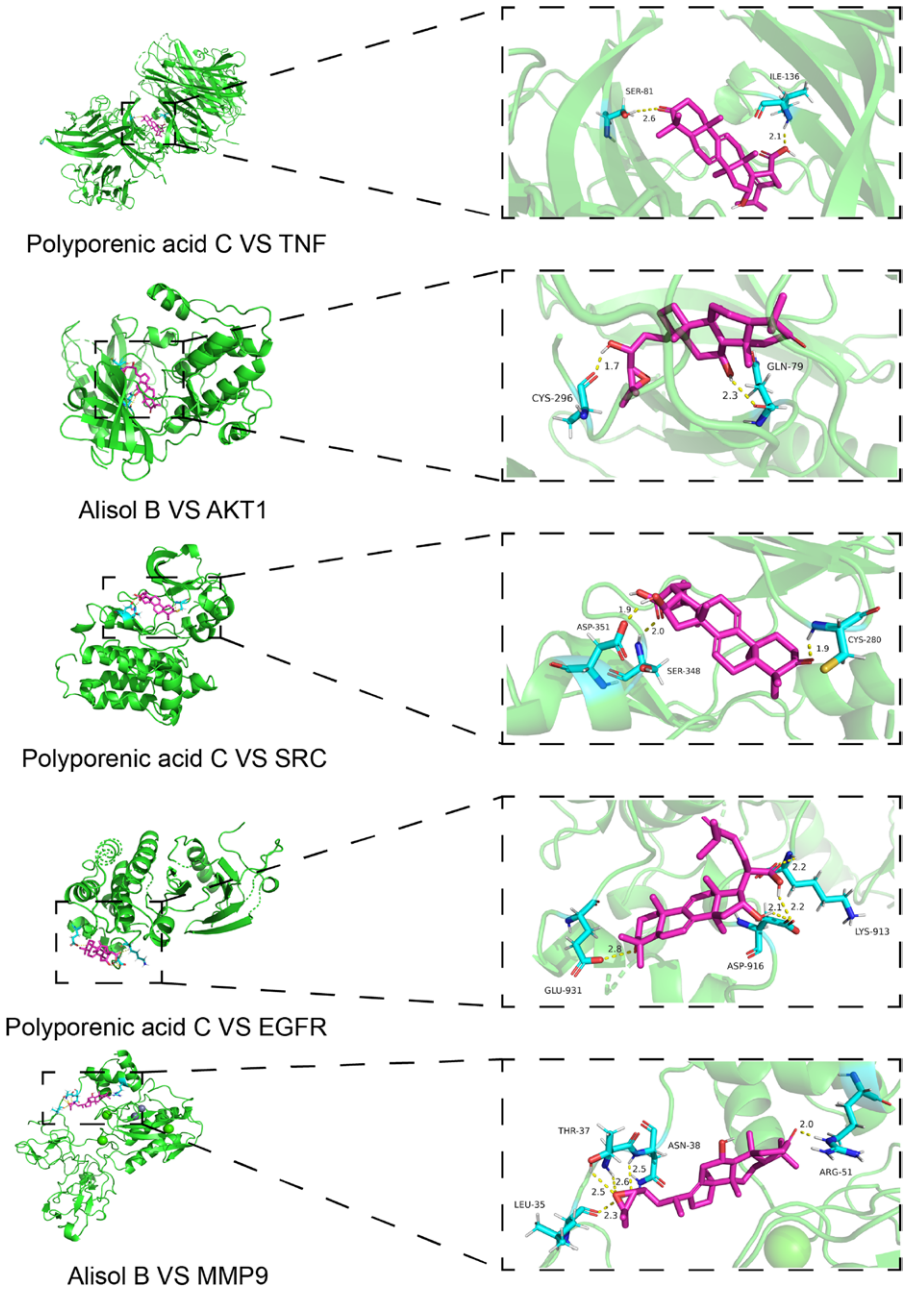

Figure 8. Diagram of molecular docking patterns. Green bands represent the protein receptors, purple conformations represent the ligands, yellow lines represent the hydrogen bonds, and blue conformations represent the amino acids in the protein receptors that forms hydrogen bonds with the ligands.

of periodontal tissues by targeting MMP9. Furthermore, sustained hyperglycemia induces the synthesis of MMP9, which mediates diabetic retinal neuropathy and vasculopathy and is associated with the severity of diabetic retinopathy.[37] By targeting MMP9, LWDHP has been shown to attenuate not only periodontal tissue destruction, but also diabetes-induced retinopathy.

LWDHP can control inflammation and regulate the balance between osteogenesis and bone destruction. Its treatment of periodontitis is multicomponent, multi-target and multi-pathway. In addition, it has therapeutic effects on diabetes and osteoporosis. It has good prospects for clinical application, but further experimental data are needed to confirm it.

5. Conclusion

In conclusion, the mechanism of action of LWDHP in the treatment of periodontitis is characterized by multicomponents, multi-targets, and multi-pathways. TNF, AKT1, SRC, EGFR, and MMP9 are the key targets and the TNF signaling pathway, the PI3K-Akt signaling pathway are the key pathways. LWDHP treats periodontitis through actions such as anti-inflammatory and regulation of the balance between osteogenesis and bone destruction.

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Adobe Illustrator 2020 was for modifying image formatting and layout only.

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Software: Jiaming Liu.

Visualization: Changyue Liu.
Writing – original draft: Yu Luo.
Writing – review & editing: Ying Liu.

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