Research on the Potential Mechanism of Rhizoma Drynariae in the Treatment of Periodontitis Based on Network Pharmacology

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Abstract. Objective: To explore the potential mechanism of compound Rhizoma Drynariae on periodontitis. Methods: the main compounds and corresponding targets of Rhizoma Drynariae were got from the Chinese Medicine System Pharmacology Database and Analysis Platform (TCMSP) database, etc. Periodontitis-related targets were obtained from Gene Cards, DrugBank, OMIM, PharmGkb, TTD databases. Intersect the targets related to the active ingredients of Rhizoma Drynariae and the periodontitis-related genes to obtain the potential therapeutic targets of Rhizoma Drynariae for periodontitis. Cytoscape constructed the compound Chinese medicine target disease network; target protein interaction was analyzed via STRING for the network's topology to screen the core targets; GO Metascape and ClueGO visualized analysis and KEGG pathway enrichment. Finally, the AutoDock software is used to verify the binding potential of the key components with the core targets in the network. Results: A total of 75 active components from Rhizoma Drynariae were retrieved, with 304 potential targets, 2252 targets related to periodontitis, and 14 key targets were analyzed. GO enrichment items ($P < 0.01$) were determined, and most of these targets were inflammation-related mediators, Cell cycle regulatory factors; KEGG enrichment pathway analysis yields 13 pathways, most of which are related to viral and bacterial infections and endocrine function regulation. The docking results indicated that the main active components of Rhizoma Drynariae had relatively stable binding activity to the core target. The study revealed the mechanism of Drynariae for the treatment of periodontitis, mainly through anti-infection, inhibition of inflammation, immune regulation, endocrine function regulation to play a synergistic effect, providing a theoretical basis for rational clinical application.

Keywords: Rhizoma drynariae · Network pharmacology · Periodontal disease · Molecular docking

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1 Introduction

At present, for the treatment of periodontal disease, people have begun to focus more research into periodontal tissue regeneration engineering [1]. Periodontal ligament stem cells (PDLSC) are a kind of mesenchymal stem cell (MSC) with self-renewal and multi-differentiation potential, which can differentiate into cementum in the body- Periodontal ligament-alveolar bone-like structure, involved in the maintenance, reconstruction, regeneration, and fixation of periodontal tissue [2]. PDLSC is an essential part of periodontal tissue engineering; its actual clinical regeneration effect is unpredictable and not as good as in vitro, probably caused by the unsuitable stem cell microenvironment in the diseased tissue [3]. Studies have shown that the differentiation and regeneration ability of PDLSC derived from the inflammatory microenvironment is significantly inhibited, and the inflammatory environment induced by lipopolysaccharide also has a negative impact on the self-renewal and differentiation potential of PDLSC [4]. With the continuous development of traditional Chinese medicine, good results have been achieved in the prevention and treatment of periodontitis, etc. [5]. The mechanism of Rhizoma Drynariae for periodontitis gradually attract the researcher's interests [6]. However, Rhizoma Drynariae has many active ingredients. The targets are abundant, and the molecular mechanism is not precise. This research uses Network pharmacology and bioinformatics to explore Rhizoma Drynariae from the molecular level. The specific mechanism of Rhizoma Drynariae in the periodontitis treatment is expected to be the next step into the research to provide direction and reference.

2 Material and Method

2.1 Screening of the Active Ingredients of Rhizoma Drynariae and Corresponding Targets

Using Rhizoma Rhizoma Drynariae as the search term and the relevant parameters of pharmacokinetics as the search term, the oral bioavailability value is set to (oral bioavailability, OB) $>30\%$, and the drug-like property is set to (drug-likeness, DL) >0.18 Screen the active ingredients and related targets of Rhizoma Drynariae from TCMSP; finally, the target protein was converted to the official name through the Unipro database and further corrected.

2.2 Periodontitis Related Targets Retrieval

The periodontal disease-related targets can be obtained through Gene Cards, DrugBank, OMIM, PharmGkb, and TTD databases. Periodontitis target prediction With "periodontal disease" and "periodontitis" as search terms, and the local condition is "Homo sapiens".

2.3 Common Targets of Rhizoma Drynariae and Periodontitis

Rhizoma Drynariae is a potential target for the treatment of periodontitis. With the aid of the R language, the relevant targets of the active ingredients in Rhizoma Drynariae and those related to periodontitis are intersected, and the Venn diagram is drawn to obtain the potential targets of Rhizoma Drynariae for the treatment of periodontitis.

2.4 Network of Rhizoma Drynariae Active Ingredient and Periodontal Disease Target

Composition-construction of target network diagram Import the file containing the active ingredients, the potential targets of the active ingredients for periodontitis, and the properties of the interaction relationship into Cytoscape 3. 7. 2 Software to construct a network diagram of "Rhizoma Drynariae-Active Ingredient-Periodontal Disease-Target".

2.5 Protein-Protein Interaction (PPI) Network

Construction of protein-protein interaction (PPI) network and screening of critical targets Import the target of Rhizoma Drynariae's potential treatment of periodontitis to the STRING database, and set the species as "Homo sapiens," If the interaction score is greater than 0.7. The non-interaction targets are hidden; download the PPI file in tsv format. Import the file into Cytoscape 3.8.0 software, perform topology analysis through CytoNCA, and use R language to filter according to the six parameter values of "Betweenness," "Closeness," "Degree," "Eigenvector," "LAC," and "Network" Key target.

2.6 GO and KEGG Pathway Analysis

Go enrichment analysis of crucial target proteins through Metascape website ($P<0.01$), analysis of KEGG pathway through R language ($P<0.05$), and visual processing of the pathways in R language to predict the pairs of Rhizoma Drynariae and periodontitis.

3 Results

3.1 Active Compounds and Corresponding Targets in Rhizoma Drynariae

In this study, through TCMSP, OB $>30\%$ and DL >0.18 were used as the active ingredient screening conditions, and 17 active compounds were screened. The database collected 169 predictive targets for the active ingredients of Rhizoma Drynariae. See the number of active compounds and related targets in the Table 1.

Table 1. Active compounds screened

Name	OB	DL	Targets
Aureusidin	53.42	0.24	17
Eriodyctiol	41.35	0.24	17
Stigmasterol	43.83	0.76	31
Beta-sitosterol	36.91	0.75	38
Kaempferol	41.88	0.24	63
naringenin	59.29	0.21	37
(+)-Catechin	54.83	0.24	11
Eriodictyol	71.79	0.24	9
Digallate	61.85	0.26	3
Luteolin	36.16	0.25	57
22-Stigmasten-3-one	39.25	0.76	1
Cycloartenone	40.57	0.79	1
Davallioside	62.25	0.51	6
Cyclolaudenol	41.66	0.79	0
Cyclolaudenol	39.05	0.79	0
Marioside	70.79	0.19	0

3.2 GO and KEGG Pathway Analysis

Go enrichment analysis of crucial target proteins through Metascape website ($P<0.01$), analysis of KEGG pathway through R ($P<0.05$), and visual processing of the pathways in R language to predict the pairs of Rhizoma Drynariae and periodontitis.

3.3 The Potential Targets of Drynaria for the Treatment of Periodontitis

A total of 2,252 targets related to periodontitis were obtained through Gene Cards, DrugBank, OMIM, PharmGkb, and TTD databases; input the active ingredients of Rhizoma Drynariae and the corresponding targets of periodontitis into Venny 2.0.1 In the online drawing tool, the potential targets of Drynaria for the treatment of periodontitis are obtained. As shown in Fig. 1, there are 95 potential targets for Drynaria for the treatment of periodontitis.

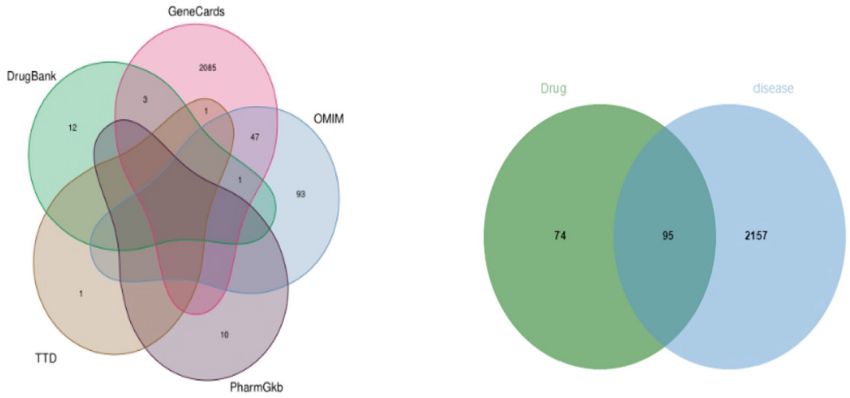

Fig. 1. a) Potential targets from databases and b) Venn diagram of drug and disease genes

3.4 Active Ingredient-Target Network Diagram

"component-target" diagram was drawn via Cytoscape3. 8. 0 to illustrate the compound, disease, and the interaction target between the compound and the disease as shown in Fig. 2. The larger the node, the greater the Degree value. The top 5 ingredients with the Degree value are luteolin, kaempferol, naringenin, beta-sitosterol, β-sitosterol, stigmasterol.

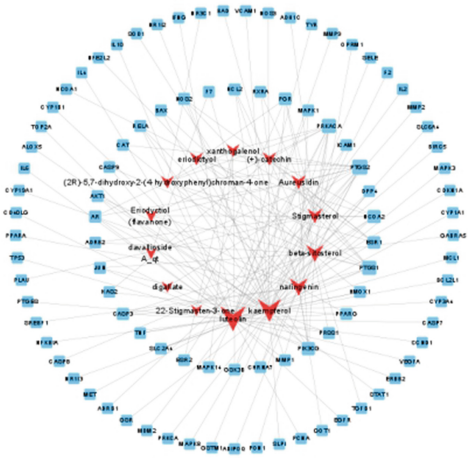

Fig. 2. Component-target diagram.

3.5 Establishment of PPI Network Diagram and Selection of Core Targets

Import the interactive target protein of Rhizoma Drynariae and periodontitis to the STRING database to obtain the PPI network file (.tsv format), and import the file into Cytoscape3. 8.0, keep the data of "node1", "node2", and "combination score," select CytoNCA in the tool to perform topology analysis on the network, get the PPI network

diagram, and compare the "Betweenness" "Closeness" "Degree" "Eigenvector" of the target point. "LAC" and "Network" are also calculated. Then the key targets are screened twice according to the value of the six parameters through the R language. The screening criterion is that the values of the six parameters are more significant than the median value of all genetic parameters. The results of the first target screening are shown in Fig. 3a, and 28 targets are obtained; the second screening process is shown in Fig. 3b and 3c, and finally, 14 targets are obtained(Table 2), which are defined as critical targets, namely MAPK1 AKT1, JUN, EGFR, STAT1, TNF, IL6, MAPK8, CASP3, TP53, MAPK3, MAPK14, NFKBIA, RELA, there are 141.5 edges, the average node degree is 20.21, the PPI network diagram of core targets is shown in Table 2.

Table 2. Pivotal targets after PPI network screening

Core targets	Betweenness	Closeness	Degree	Eigenvector	LAC	Network
AKT1	44.77	0.93	25	0.26	13.12	23.01
JUN	38.33	0.93	25	0.27	14	23.9
TP53	30.37	0.9	24	0.26	14	22.69
TNF	27.62	0.84	22	0.24	12.64	19.26
MAPK3	20.91	0.84	22	0.25	13.82	19.86
MAPK14	19.29	0.79	20	0.23	12.5	16.88
RELA	21.49	0.79	20	0.22	11.8	16.75
EGFR	14.12	0.77	19	0.22	12.32	16
IL6	14.86	0.77	19	0.22	12.32	16.2
MAPK1	11.8	0.75	18	0.21	11.78	14.67
CASP3	10.6	0.75	18	0.21	12.33	15.12
STAT1	8.91	0.73	17	0.21	11.76	13.69
MAPK8	13.57	0.73	17	0.19	10.35	13.19
NFKBIA	10.29	0.73	17	0.2	11.41	13.77

Table 3. Molecular docking of components toward targets

Components	AKT1	IL6	MAPK14	MMP1
Luteolin	– 9.4	– 7.1		– 5.7
Kaempferol	– 9.5			– 5.1
Naringenin	– 9.7			
Aureusidin			– 7.3	
Xanthogalenol			– 6.9	

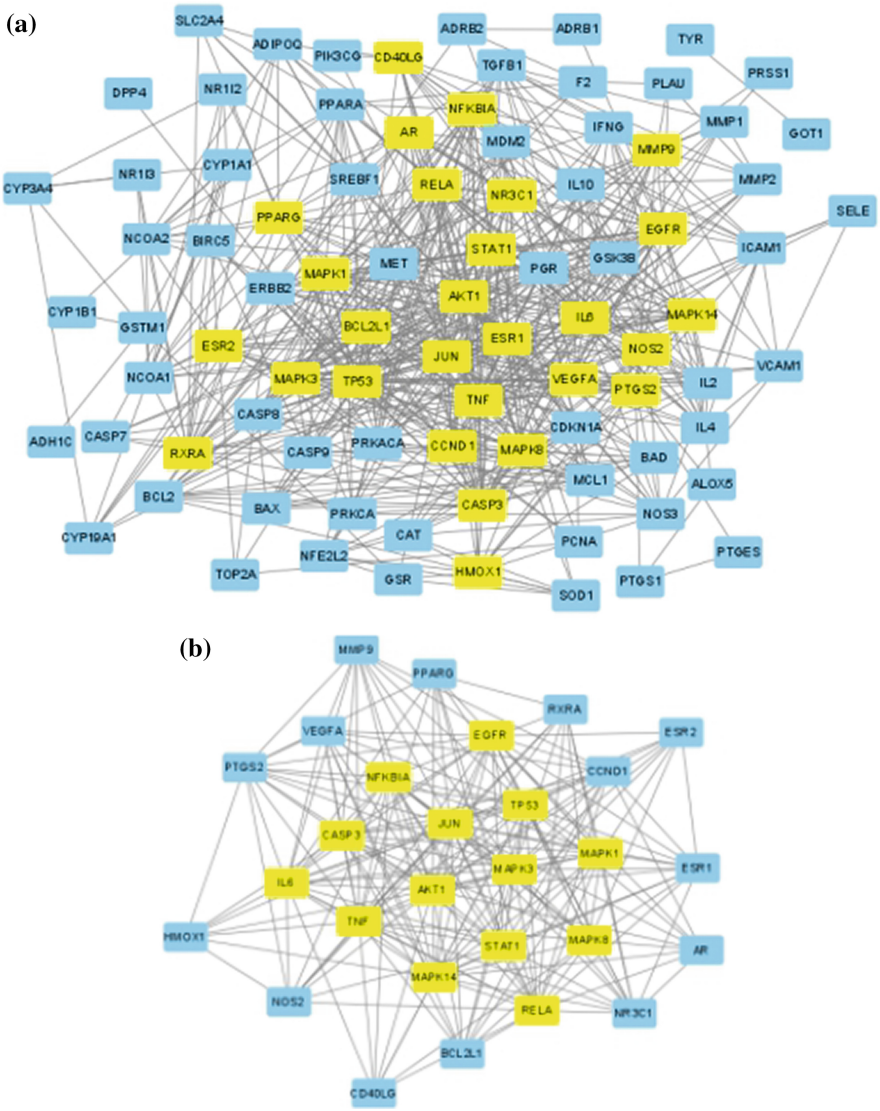

Fig. 3. (a) Filter1 betweenness > 38.61936036, closeness >0.245014245, degree >13, Eigen-vector >0.079680048, LAC >6.533333333, network >7.72106676, (b) filter betweenness >7.495325508, closeness >0.702364865, degree >15.5, Eigen-vector >0.176887855, LAC >10.330316745, network >12.38486444 (c) PPI network diagram of key targets

3.6 GO and KEGG Pathway Analysis

Enrichment analysis has done for the key targets using the Metascape database, and the GO analysis items included 537 biological processes (BP), 14 cell components (CC), 29 molecular functions (MF), and 209 KEGG pathways. At the same time, select $P<0.05$. As shown in the Fig. 4, BP includes 18 items, mainly related to lipopolysaccharide response, reactive oxygen species response, tumor necrosis factor response, mechanical

stimulation response, muscle cell proliferation, lipopolysaccharide regulation signaling pathway, aging, growth factor response, cellular abiotic stimulation response, stress response regulation. CC includes five processes, mainly involved in forming lipid rafts, cysts, transcriptional regulatory complexes, dendrites, and plasma membrane protein complexes. MF includes three processes: RNA polymerase II specific DNA binding transcription, and Tumor necrosis factor receptor family binding.

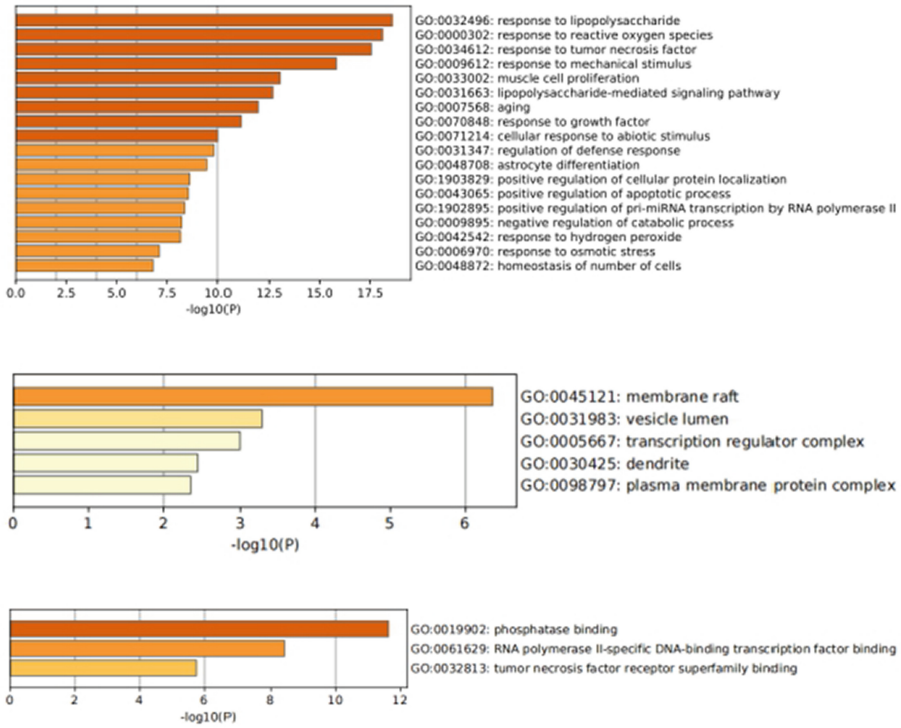

Fig. 4. GO analysis, BP, CC

157 pathways ($P<0.05$) were enriched. After sorting enriched genes, the first 20 signal pathways were visualized as shown in the Fig. 6, and then the AGE-RAGE signal was transferred through the R language. The path is visualized and mapped, as shown in the figure. The pathway's name is displayed on the Y-axis, and its percentage is displayed on the X-axis. The bubble area shows the number of enriched genes of the pathway, and the P-value is shown by the color of the bubble. Enrichment analysis results show that immune inflammation-related pathways involve TNF, AGE-RAGE, Toll-like receptor C-type lectin receptorand T cell receptor signaling pathway, etc.; Regulating cell cycle-related pathways involves MAPK signaling pathways, apoptosis, etc.; viral infection-related pathways involve diseases such as hepatitis B and human cytomegalovirus HCMV; bacterial infection-related pathways involve Shigella, Salmonella infections, and other related diseases; metabolism-related The pathway involves atherosclerosis caused by lipid metabolism disorders. The results show that Drynaria exerts therapeutic

effects through immunity, inflammatory factor level regulation, cell cycle regulation, antiviral and other aspects (Fig. 5).

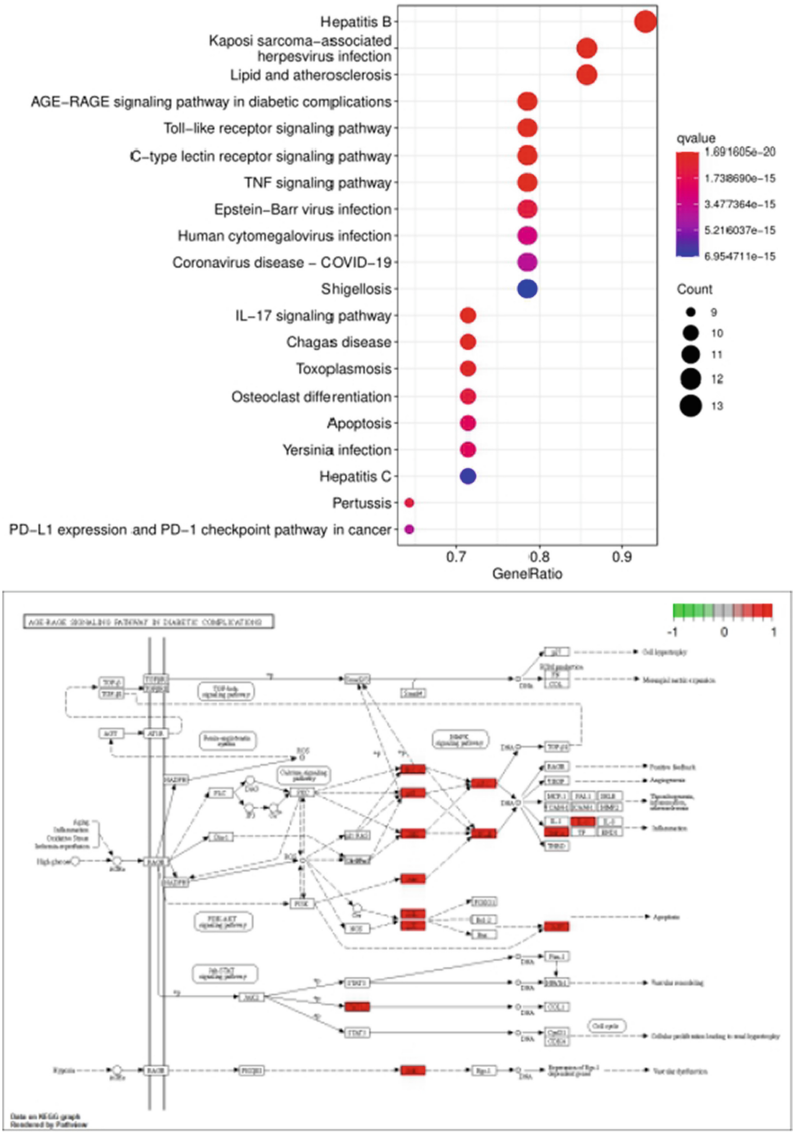

Fig. 5. Bubble chart of KEGG pathway enrichment analysis and enriched diabetes pathway

3.7 Drug Component-Core Target Molecular Docking Verification Analysis

In this study, as Table 3 shows, the top 5 critical medicinal substances in the effective active ingredients and core targets related to the regulation of cell cycle, regulation of inflammatory factors, and immune activity were screened for molecular docking. It is

believed that a docking score value of less than –4.25 kcal·mol^{-1} indicates that there is a specific binding activity between the two, and less than -5.0 kcal·mol^{-1} indicates a good binding activity (Table 3). Less than –7.0 kcal mol^{-1} means strong binding activity. The bind mode like Fig. 6 shows.

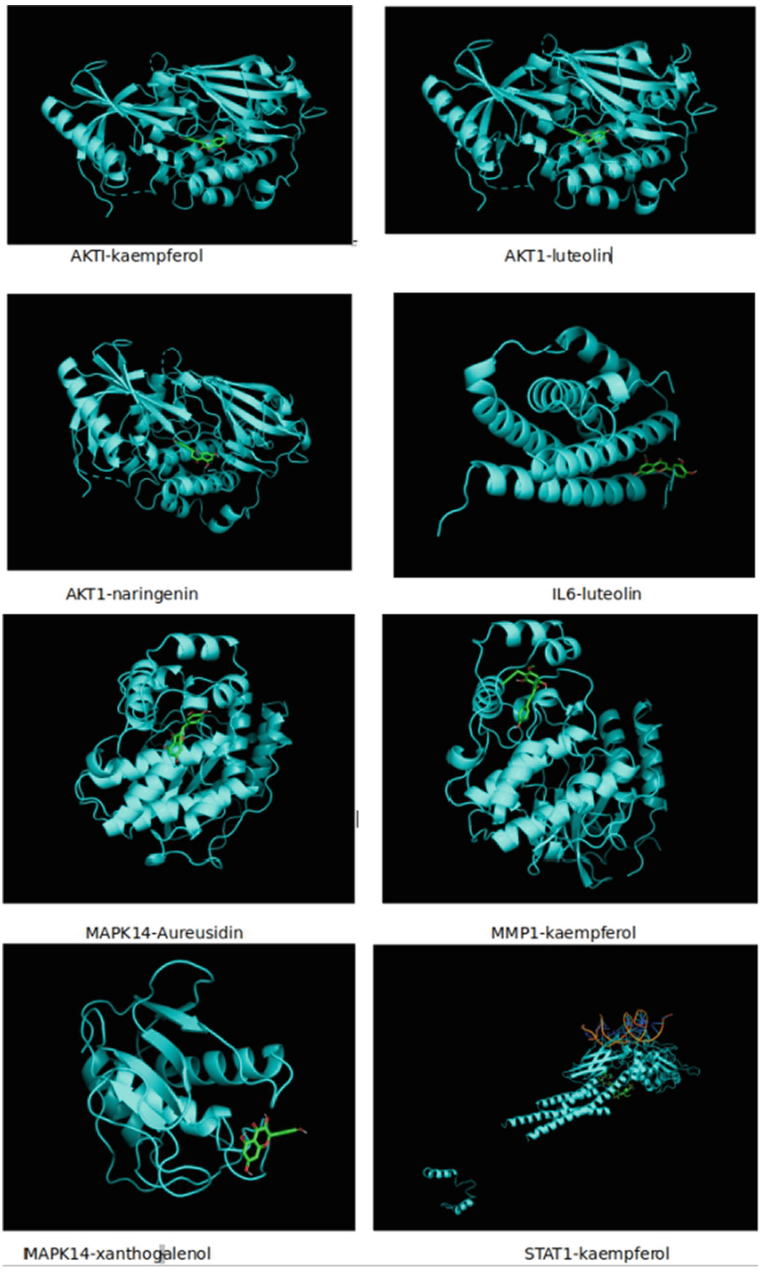

Fig. 6. Molecular docking

4 Discussion

A total of 75 active Rhizoma Drynariae were obtained, with 304 potential targets, 2252 targets related to periodontitis, and 14 key targets were analyzed. Naringenin is the aglycone of Naringin, and Naringin is a di-hydroflavonoid glycoside compound. After Naringin enters the blood circulation, most of it will be metabolized to naringenin and then absorbed into by passive diffusion [9]. Blood. At present, many experimental results on Naringin show that Naringin has a variety of biological activities, including anti-oxidant and inflammation, improving glucose and lipid metabolism, neuroprotection, anti-liver injury, and anti-osteoporosis. Jiang Junqiang used cell culture technology to measure and observe the effects of Rhizoma Drynariae naringin on periodontal ligament cell proliferation, alkaline phosphatase activity, and ultrastructure [9]. The study found within a certain mass concentration range, Naringin has the effect of promoting cell proliferation and cell function on hPDLC. Kaempferol is a flavonoid compound [9]. Balli Umut measured matrix metalloproteinase-1 (MMP-1), MMP-8, and TIMP-2 in the periodontal tissue of experimental periodontitis rats by the histomorphometric method. The application of Kaempferol may help reduce alveolar bone resorption, loss of attachment, and the production of MMP-1 and -8 in experimental periodontitis [10]. Eun Young Choi found luteolin may reduce the production of NO and IL-6 by macrophages and neutral polymorphonuclear leukocytes activated by lipopolysaccharide by inhibiting the STAT pathway and the NF-kB pathway, thereby reducing production of NO and IL-6. Inflammatory reaction damages the host [11]. Samuel Sampath found the combination therapy (MSC-CM plus stigmasterol) inhibited IL-1β-induced p65. The activation of NF-kB produced by phosphorylation of the IκBα subunit and the phosphorylation of the IkBα subunit exerts a special anti-inflammatory/anti-metabolism effect [12]. KEGG enrichment pathway analysis yields 13 pathways, most of which are related to viral and bacterial infections and endocrine function regulation. It is suggested that AKT is expressed in chronic periapical periodontitis in mice, and the expression is increased; AKT participates in the disease process of chronic periapical periodontitis and may promote bone destruction in periapical periodontitis [13]. Caspases (CASP) are intracellular proteases that mediate apoptosis. The activation of caspase three is enhanced in chronic periodontitis. Kang evaluated 51 patients and 33 control subjects and found that the CASP3 gene polymorphism may be related to the susceptibility of the Korean population to periodontal disease [14]. Diabetes is a common endocrine and metabolic disease and one of the risk factors for periodontal disease [7]. Some scholars have proposed that periodontitis is the sixth complication of diabetes [15]. In recent years, it has been discovered that the enhancement of the AGE-RACG signaling pathway, the end product of glycation, is an essential mechanism for the aggravation of periodontal disease in diabetic patients [8]. HCMV is a beta herpes virus, which can infect various epithelial cells, T cells, and monocyte-macrophages. HCMV is often detected in the active sites of periodontal lesions in aggressive periodontitis, severe chronic periodontitis, palmoplantar keratosis periodontal destruction syndrome, and necrotizing ulcerative gingivitis.

[15]. Domestic studies have shown that the detection rate of HCMV in patients with aggressive periodontitis is 43.8%, and the detection rate of HMV in patients with chronic

periodontitis is 47.5%, which is significantly higher than 12.9% in healthy controls, $P<0.01$ [16].

The study revealed the mechanism of Drynariae for the treatment of periodontitis, mainly through anti-infection, inhibition of inflammation, immune regulation, endocrine function regulation to play a synergistic effect, providing a theoretical basis for the rational clinical application.

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