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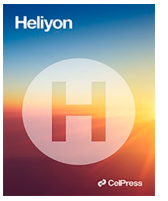

Research article

Preliminary investigation on the mechanism of anti-periodontitis effect of *Scutellariae Radix* based on bioinformatics analysis and *in vitro* verification

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ABSTRACT

Objective: To investigate the material basis, targets and molecular mechanism of *Scutellariae Radix* against periodontitis to provide theoretical basis for clinical applications.
Materials and methods: The active compounds and targets of *Scutellariae Radix* were obtained from Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) database, and the periodontitis-related targets were collected by integrating Online Mendelian Inheritance in Man (OMIM), Therapeutic Target Database (TTD), Genecards and Gene Expression Omnibus (GEO) database together. The potential targets of *Scutellariae Radix* against periodontitis were obtained from the intersection of two target sets. Metascape database was used for Gene Ontology (GO) term enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis. Discovery Studio software was used for molecular docking between key targets and compounds to evaluate their binding affinity. Western blot was used to check the expression of PTGS2 and MMP9 to verify the regulatory effects of baicalein, the main active compound of *Scutellariae Radix*, on human periodontal ligament stem cells (hPDLSCs) cultured under inflammatory environment which induced by lipopolysaccharide (LPS).
Results: 15 active compounds of *Scutellariae Radix* and 53 common targets for periodontitis treatment were identified. Among these targets, the 10 core targets were AKT1, IL-6, TNF, VEGFA, TP53, PTGS2, CASP3, JUN, MMP9 and HIF1A. GO and KEGG analysis mainly focused on response to LPS and pathways in cancer. Molecular docking showed that the main active compounds had good binding affinity with key targets. Cell experiments confirmed that baicalein can interfere the expression of pro-inflammatory factors PTGS2 and MMP9 proteins and exert anti-inflammatory effects.

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Conclusion: Current study preliminarily analyzed the mechanism of *Scutellariae Radix* against periodontitis, which provide a new idea for the utilization of *Scutellariae Radix* and the development of novel medicine for the clinical treatment of periodontitis.

1. Introduction

Currently, modern medicine research believed that the development of periodontitis was caused by bacterial invasion and host immunological imbalance, which often starts with plaque as the initiating factor [1]. The plaque colonizes the supragingival or subgingival areas and invades into periodontal tissue, leading to the formation of periodontal pockets and progressive resorption of alveolar bone. It was believed that the development of periodontitis is result by bacterial invasion and host immune imbalance, which often starts with plaque as the initiating factor, then plaque is planted on the gingival or subgingival parts of teeth, invading periodontal tissue, leading to the formation of periodontal pockets and progressive resorption of alveolar bone. It is a chronic inflammatory disease whose clinical symptoms mostly manifested as loose teeth, gum bleeding, and it is also the main cause of tooth loss [2]. According to *the Global Burden of Diseases, Injuries, and Risk Factors Study 2017* (GBD 2017) report, the prevalence of severe periodontitis is approximately 11 %, affecting approximately 743 million people, the prevalence of mild periodontitis is even higher, affecting at least half of the global population [3]. In China, the situation of periodontitis is not optimistic. According to the results of *the* 4th *National Oral Health Survey in the Mainland of China*, the periodontal health rate of Chinese adults was only 9.1 %, the detection rate of calculus in 35▪44 years old residents was 96.7 %, the detection rate of gingival bleeding was 87.4 %, and the prevalence of severe periodontitis (stage III or IV) was as high as 10.6 % [4,5]. The prevalence of periodontitis remains at a high level, which shows it is no time to delay to prevent and control periodontitis.

Although mechanical therapy supplemented by antibiotics were used for periodontal diseases treatment normally, there have some disadvantages which induce bacterial resistance and have potential toxic side effects during the treatments such as nausea, abdominal pain, dental and oral cavity discolorations, drug-induced lupus, autoimmune hepatitis and diarrhoea [6,7]. In contrast, traditional Chinese medicine (TCM) plays an important role during the treatment or prevention of different kinds of disease [8], because they have high efficacy, long history usage, minimum side effects, and the most important is TCM can attenuate the patient's clinical symptoms, as well as rebalance the healthy status of human body based on the holistic treatment in TCM theory. Currently, lot of Chinse herb medicine were used for the treatment of periodontal diseases and already obtained satisfactory effects, but the detailed molecular mechanisms are still not clear [9,10]. *Scutellariae Radix*, a traditional herb medicine, is a perennial herb of the genus *Scutellaria* in the family Labiatae, and its root is mainly used as herb medicine in many prescriptions. The application of *Scutellariae Radix* was first recorded in *Shen Nong's Materia Medica* which has been written in Eastern Han Dynasty. According to TCM theory, it was used for cleaning away heat, detoxifying toxicosis and stopping bleeding for a long history [11]. Modern pharmacological analysis explored that *Scutellariae Radix* has multiple bioactivities, such as anti-inflammatory, antibacterial, antiviral and antioxidant effects, and also has the potential to treat periodontitis [12,13].

Because herb medicine have very complex compounds, so they always have the characteristics of multi-target and multi-pathway efficacy. Although the clinical efficacy of *Scutellariae Radix* has been recognized in recent years, there are few reports on the pharmacodynamic material basis and molecular mechanism of *Scutellariae Radix* on the treating of periodontitis, and there are still lack systematic research with related animal studies and cellular studies to investigate its molecular mechanisms, which limits the further development and utilization of *Scutellariae Radix*.

In addition, network pharmacology, a new generation of drug research model, based on systems biology [14], polypharmacology [15] and computer technology, can construct a network relationship model of "disease-target-drug" from the perspective of multi-drugs, multi-compounds and multi-targets. It breaks the previous drug research and development concept of "single compound-single target-single disease", and also breaks the previous pharmacodynamic substance research model of "chemical composition separation-molecular structure identification-biological activity evaluation", which greatly improves the efficiency of drug discovery. It is also beneficial to observe the effects of active compounds on the disease -target network from an overall level, screen out the key pharmacodynamic compounds and related targets, and improve the experimental efficiency for the subsequent experimental verification of key targets. At present, there is no research on *Scutellariae Radix* treating periodontitis by using network pharmacological analysis. Therefore, we use the network pharmacology method to explain the relationship between *Scutellariae Radix*, and periodontitis related targets from a holistic and systematic perspective, so as to reveal the molecular mechanism that how *Scutellariae Radix* works for the periodontitis treatments. Molecular docking is a commonly used method for Computer-aided drug design (CADD), which can test the binding affinity between drug molecules and target proteins [16]. In our study, we investigated the interaction between key active compounds of *Scutellariae Radix* and key protein receptors of periodontitis through molecular docking to explored the molecular binding mechanism of drug compound-target at the molecular level. Meanwhile, we cultured hPDLSCs in *vitro* and constructed a cell inflammation model to investigate the regulation effects of baicalein on the protein expression of targets with better binding affinity in molecular docking, in order to more rigorously verify the results of bioinformatics screening, and provide some theoretical basis for clinical usage and development of new drugs for periodontitis therapy in future.

2. Materials and methods

2.1. Screening of active compounds and corresponding targets

The data of active compounds of *Scutellariae Radix* were searched in TCMSP (Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform, https://tcmsp-e.com/) [17], and the corresponding targets were also obtained from TCMSP database. The absorption, distribution, metabolism and excretion (ADME) properties of compounds have a significant impact on their activity. A compound is more likely to exhibit its activity if it is well absorbed by the body and reaches an effective concentration in the target tissue or organ. In this study, the screening of the active compounds was mainly based on two parameters, OB and DL, which are the two key parameters for ADME. Considering that *Scutellariae Radix* can be administered both orally and topically, we screened with the generally accepted screening criteria of OB $\geq$ 30 % and DL $\geq$ 0.18 recommended by the database in this study. These two parameters were also used to screen the compounds in the published articles on the treatment of periodontitis with traditional Chinese medicine [18,19]. The protein database UniProt (https://www.uniprot.org/)was used to standardize the target information of human genes and obtain the corresponding target genes [20].

2.2. Identification of periodontitis-related targets

In order to ensure the comprehensiveness and accuracy of periodontitis-related target collection, we mainly used OMIM (http://www.omim.org) [21], TTD (http://db.idrblab.net/ttd/) [22], GeneCards (https://www.genecards.org) [23] and GEO (http://www.ncbi.nlm.nih.gov/geo/) [24] databases. They are widely used and recognized databases in the fields of biology and genetics, which can guarantee the breadth and depth of the information provided. Among them, OMIM is a comprehensive database of human genes and genetic disorders, focusing on the association between disease phenotypes and their causative genes. TTD is a database that provides information on therapeutic targets and related diseases. It proposes a set of drug target determination strategies based on the association of drug, target, and disease, which can strictly confirm targets related to periodontitis. GeneCards is a comprehensive database that aggregates information about human genes from multiple sources and can quickly retrieve all genetic information related to specific diseases such as periodontitis. The GEO database stores a large number of gene expression data sets that researchers can use to identify differentially expressed genes in specific disease states. In the OMIM and TTD databases, we included all the genes obtained from the input of "periodontitis". In the GenenCards database, we conducted a secondary screening of the initial genes to obtain genes that are more closely related to periodontitis. The genes were ranked based on the Relevance score from the highest to the lowest, and those greater than the median were chosen. The gene array data was downloaded from GEO database (Series: GSE 79705), which contained the gene sequencing information of 8 periodontitis patients and 4 healthy controls. R software (4.1.0) was used to analyze the original gene array data, and Limma program package was used to analyze the differential genes. The filter criteria of significantly different genes were set as $P < 0.05$, Fold change (FC) > 2. The original volcano map of the gene array was drawn using the Plot program package. Finally, the targets of periodontitis obtained from the three databases and the differentially expressed genes obtained from GSE79705 were combined to acquire the identified targets of periodontitis.

2.3. Network construction

The active compounds and target genes information of *Scutellariae Radix* was imported into Cytoscape 3.9.1 software to construct a "*Scutellariae Radix*-active compound-target" network. The drug target genes were intersected with the relevant targets of periodontitis, and the active compounds of the drug were reverse searched by the intersected targets, and then using Cytoscape 3.9.1 software to build a "*Scutellariae Radix*-compound-target-periodontitis" network. In addition, the "Analyze network" function is used to analyze the topology of network. In constructed network, nodes represent *Scutellariae Radix*, periodontitis, active compounds and target genes, which are connected by edges; the degree value represents the number of edges that connect a node to other nodes, and the greater value, more nodes are connected, indicating that the node is more essential and plays a pivotal role in the whole network, which may be a key compound or a hub gene.

2.4. Protein-protein interaction network construction

The target genes related to periodontitis and the active compounds of *Scutellariae Radix* were both inputted to Venny 2.1 online tool (http://bioinfogp.cnb.csic.es/tools/venny/index.html) to plot the Venn diagram and obtain shared targets. The intersection genes were imported into the String database (http://string-db.org) [25] with species limited to *Homo sapiens* and confidence >0.4 to construct a protein-protein interaction (PPI) network. Cytoscape 3.9.1 software was used to visualize PPI network. The plug-in cytoHubba was used to calculate the Degree value of the network nodes, and top 10 key targets with the highest Degree value were selected for subsequent molecular docking verification.

2.5. GO and KEGG pathways enrichment analysis

GO and KEGG pathways enrichment analysis were performed on Metascape database (http://metascape.org/) [26], with $P < 0.05$ as statistically significant. The analysis results were processed on the bioinformatics platform (http://www.bioinformatics.com.cn/) for visualization. Finally, the "Gene -pathway" network was constructed in Cytoscape 3.9.1 software.

Table 1
General information of active compounds in Scutellariae Radix.

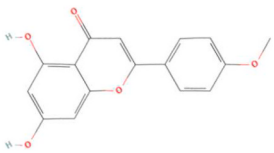
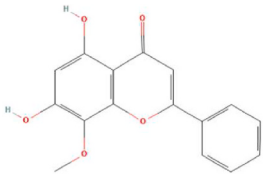
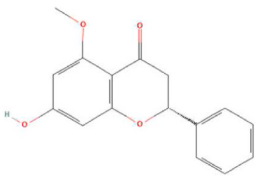
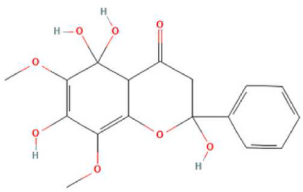
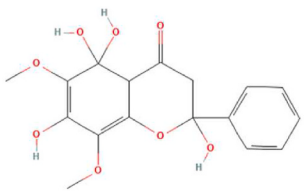
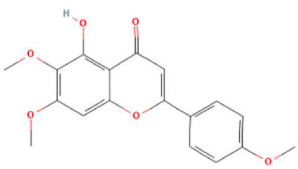
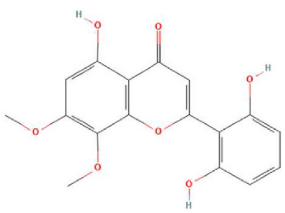
TCMSP ID	active compounds	OB (%)	DL	Structure
MOL001689	acacetin	34.97	0.24	
MOL000173	wogonin	30.68	0.23	
MOL000228	(2R)-7-hydroxy-5-methoxy-2-phenylchroman-4-one	55.23	0.2	
MOL002714	baicalein	33.52	0.21	
MOL002909	5,7,2,5-tetrahydroxy-8,6-dimethoxyflavone	33.82	0.45	
MOL002915	Salvigenin	49.07	0.33	
MOL002917	5,2',6'-Trihydroxy-7,8-dimethoxyflavone	45.05	0.33	

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Table 1 (*continued*)

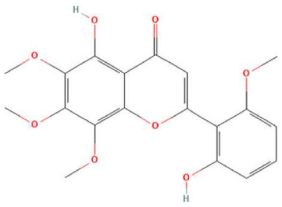
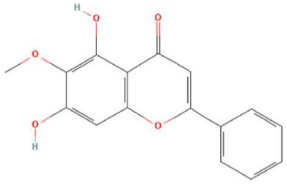
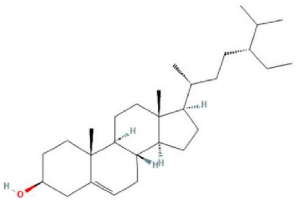
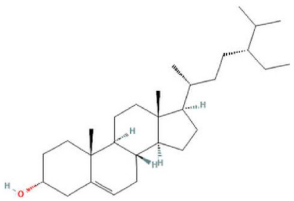
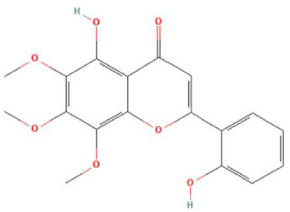
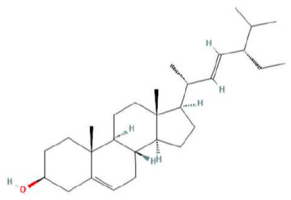
TCMSP ID	active compounds	OB (%)	DL	Structure
MOL002927	Skullcapflavone II	69.51	0.44	
TCMSP ID	active compounds	OB (%)	DL	Structure
MOL002928	oroxylin A	41.37	0.23	
MOL000358	beta-sitosterol	36.91	0.75	
MOL000359	sitosterol	36.91	0.75	
MOL000552	5,2'-Dihydroxy-6,7,8-trimethoxyflavone	31.71	0.35	
MOL000449	Stigmasterol	43.83	0.76	

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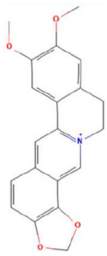
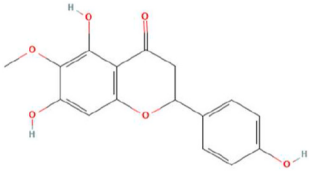
Table 1 (*continued*)

MOL002897	epiberberine	43.09	0.78	
MOL012245	5,7,4'-trihydroxy-6-methoxyflavanone	36.63	0.27	

2.6. Molecular docking

Discovery Studio (DS4.0) was used to conduct molecular docking between the top 10 key targets in PPI network and core compounds from the "*Scutellariae Radix*-compound-target-periodontitis" network, so as to explore whether the active compounds of *Scutellariae Radix* could combine with the key targets and play an important role in treating periodontitis.

2D structures of the core compounds were downloaded from PubChem database (https://pubchem.ncbi.nlm.nih.gov/) [27], and the structures were optimized by Chem3D software. 3D structures of key target proteins were downloaded from the PDB database (https://www.rcsb.org/) [28]. The original ligand was extracted and redocked into the active pocket of the receptor protein, and the root-mean-square deviation (RMSD) of the original ligands in the crystal structure were calculated. It is generally believed that when RMSD$\leq$ 2 Å indicates that the molecular docking model can better reproduce the original ligand and protein binding mode, and that the docking results are highly reliable [29]. Then, the core active compounds (ligands) of *Scutellariae Radix* and key target proteins were imported into DS4.0 for molecular docking, and the docking scoring value (-CDOCKER interaction energy) was calculated. The higher the -CDOCKER interaction energies, the greater the binding affinity between the protein and the ligand [30]. In the process of molecular docking, we use the ligand expansion method to define the docking box, that is, to expand a certain range outward from the center of the original ligand position (considering the active site generated by the original ligand and the size of the ligand to be docked), and the receptor residues in this range constitute the relevant active site. The parameter setting of CDOCKER docking is set to 0.5 for Pose Cluster Radius under Top Hits, and the rest settings are default.

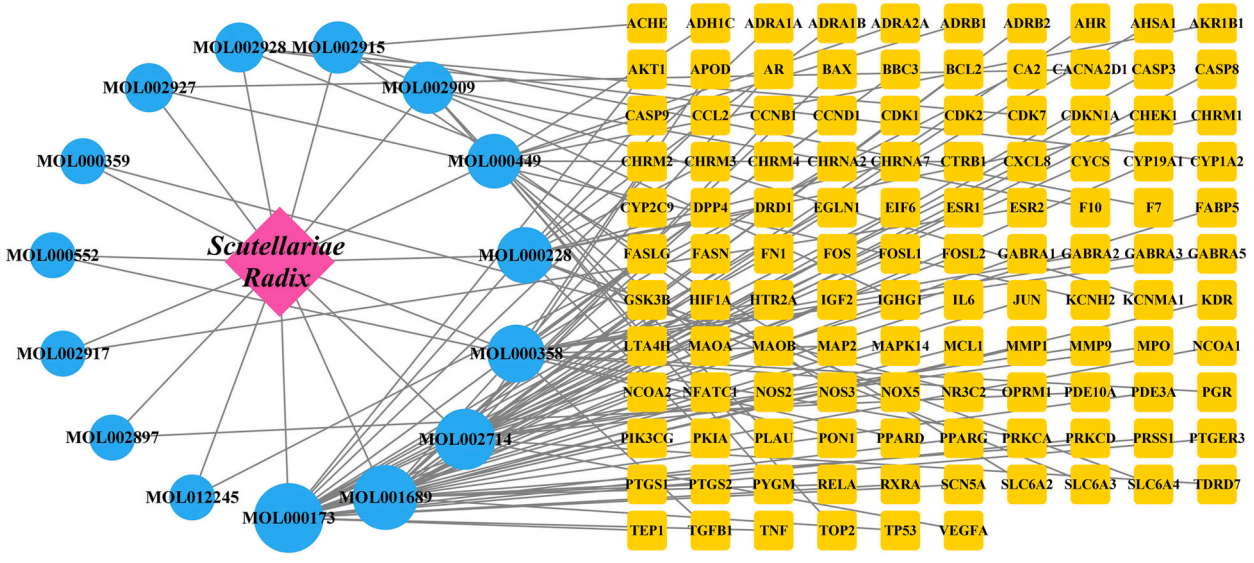


Fig. 1. Active compound-target network of *Scutellariae Radix*. The diamond node represents *Scutellariae Radix*, the circle nodes represent active compounds, and the rectangular nodes represent targets.

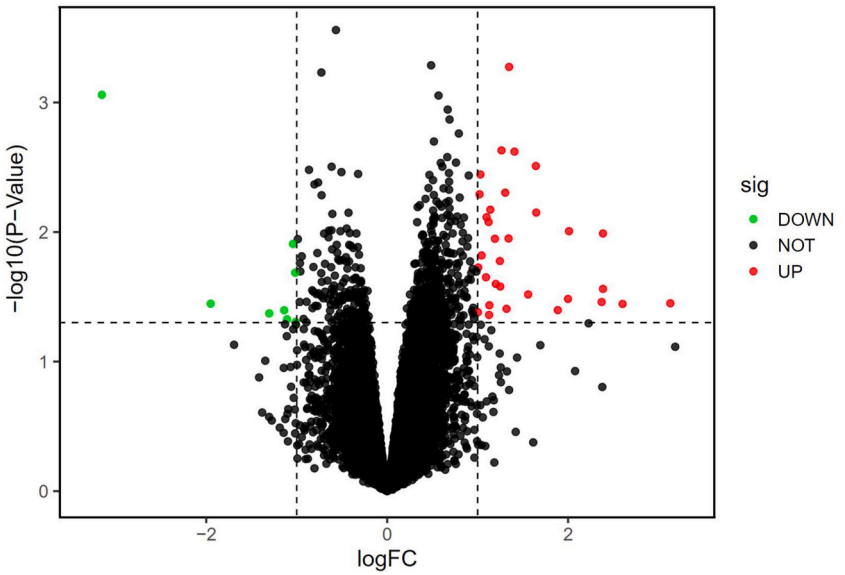


Fig. 2. Volcano map of differentially expressed genes. Red dots represent upregulated genes, and green dots represent downregulated genes.

2.7. Experimental validation

Baicalein was chosen for subsequent experimental validation, because as a flavonoid compound, it possesses a variety of pharmacological effects and has significant research value. Besides, it is one of the most abundant compounds in *Scutellariae Radix*. Moreover, compared with other active compounds, baicalein has higher content, lower price and is easy to extract and obtain, which provides great convenience for clinical application.

2.7.1. Cell culture and identification

The hPDLSCs were harvested from the removed wisdom teeth in clinical approved by the Biomedical Ethics Committee of Henan University (HUSOM2020-059), and identified with flow cytometry. The hPDLSCs were grown in Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEM/F-12) media with 10 % fetal bovine serum (FBS) and 1 % penicillin/streptomycin double antibody at 37 °C with 5 % CO_2. The cells were passaged during the logarithmic growth phase, with roughly 80 % cell fusion. The cell density of hPDLSCs at P4 was adjusted to 1×10^6 cells/ml and divided them into five 2 mL EP tubes with 100 μL each. The blank control group did not add antibody, and the 4 single staining groups were added with 5 μL PE-labeled CD34, APC-labeled CD45, FITC-labeled CD90, and PE-Cyanine7-labeled CD105 antibodies, respectively. After mixing well, incubate at 4 °C for 30 min away from light, then wash twice with DPBS, and finally add 200 μL of DPBS suspension for machine detection.

2.7.2. Cell viability assay

hPDLSCs were seeded in 96-well plates with a cell density of 5×10^3 cells/well. After 24 h of cell apposition, the medium was changed into different concentrations of baicalin (0.01 μM, 0.1 μM, 1 μM, 10 μM, 100 μM). The control group was 0.1 % DMSO, and the blank group was the medium without cells. After 24 h of incubation, the old medium was discarded and the cells were washed twice with medium to remove drug interference, then new medium containing 10 % CCK-8 was added and incubated for 2 h, thereafter, the absorbance at 450 nm was measured with an enzyme marker.

2.7.3. Western blot analysis

The hPDLSCs were inoculated in 6-well plates at 1×10^6 cells/ml and divided into control group, LPS group, baicalin group and LPS + baicalin group, with 3 replicate wells in each group. After 24 h of cell culture, LPS and LPS + baicalin group added 85 μg/mL LPS for 24 h to induce inflammation, subsequently, baicalin and LPS + baicalin group added baicalein for another 24 h. The total proteins of each group were extracted with RIPA lysate, centrifuged at 12,000 g for 10 min at 4 °C. The proteins were separated by 10 % SDS-PAGE gel electrophoresis, then transferred to PVDF membrane, blocked with 5 % skimmed milk powder solution at room temperature for 2 h, washed with TBST, and incubated with primary antibody (PTGS2 at 1:1000, MMP9 at 1:500 and GAPDH at 1:5000) at 4 °C overnight. The next day, the membrane was washed three times with TBST for 10 min each time, and incubated with the corresponding secondary antibody for 1 h. Finally, the ECL luminescent liquid was added to expose, and the gray value of each protein band was analyzed by Image J software, with GAPDH as the internal reference.

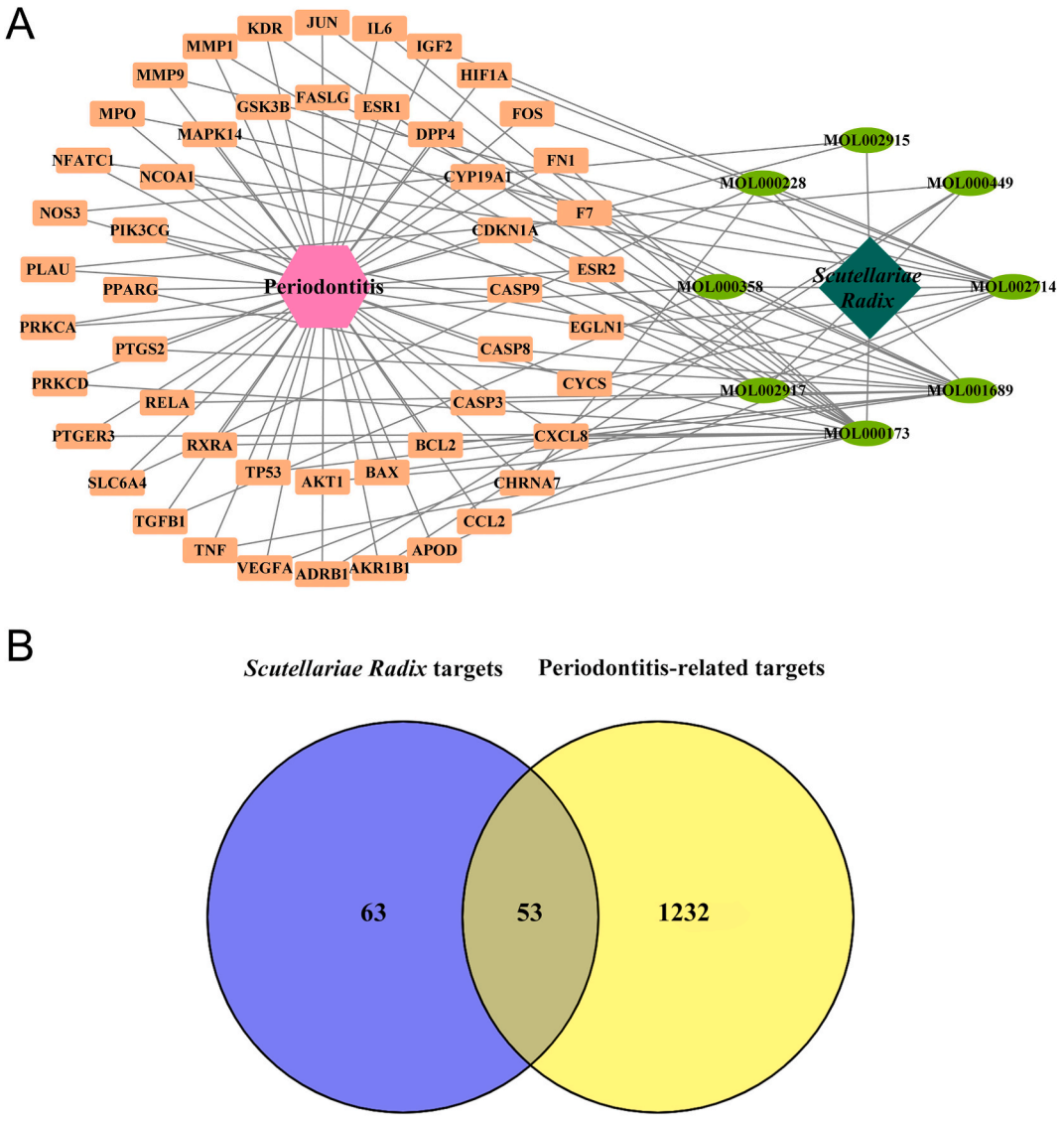


Fig. 3. "*Scutellariae Radix*-compound-target- periodontitis" network and Venn diagram. (A) "*Scutellariae Radix*-compound-target- periodontitis" network. The diamond node represents *Scutellariae Radix*, the elliptical nodes represent active compounds, the regular hexagonal nodes represent periodontitis, and the rectangular nodes represent targets. (B) Venn diagram showing the intersection of *Scutellariae Radix* and periodontitis-related targets.

2.8. Statistical analysis

All experiments were repeated three times and data were expressed as mean ± standard deviation (*S.D.*). Graphpad Prism 8.0 software was used for statistical analysis. One-way ANOVA was used to test for significant differences between three or more groups. Differences were statistically significant when $P < 0.05$.

3. Results

3.1. Active compounds and common targets

The TCMSP database search revealed that *Scutellariae Radix* contains 143 compounds, and 15 active compounds were obtained after screening. The basic information of the active compounds of *Scutellariae Radix* is shown in Table 1, and the target genes were obtained by normalizing the target information with UniProt. In Fig. 1, the pink node represents *Scutellariae Radix*, the blue nodes represent 15 *Scutellariae Radix* active compounds, the yellow rectangles represent genes, and the size of the active compounds node represents the number of targets connected to it.

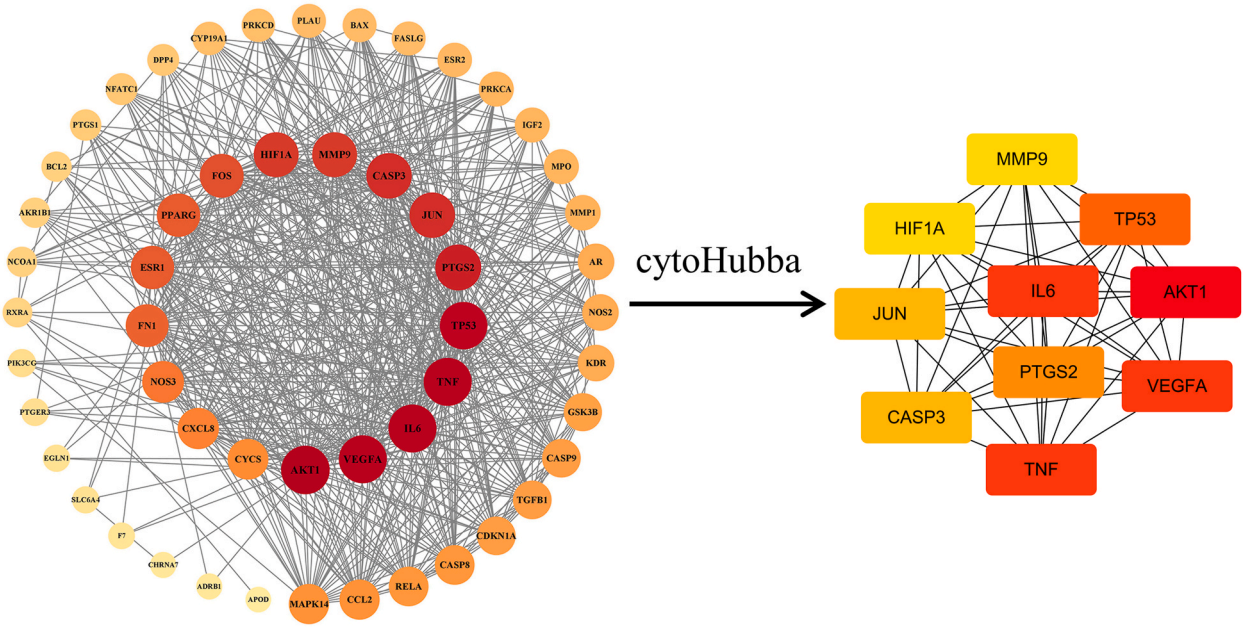


Fig. 4. Protein-protein interaction network map. The left side is the interaction network diagram of 53 shared targets, and the right side is the top 10 key targets with the highest degree value extracted by the cytoHubba plug-in.

3.2. Screening of periodontitis-related targets

A total of 1252 disease targets related to periodontitis were identified by OMIM, TTD and GeneCards databases after deduplication. Among them, 19 targets and 1 target were retrieved from OMIM and TTD databases, respectively. A total of 2435 targets were initially retrieved from the GeneCards database, and the Relevance score of 1285 targets were greater than the median for subsequent analysis. In addition, we also obtained 40 differentially expressed genes by secondary analysis of gene array in GEO database, and the results were visualized by using a volcano plot (Fig. 2). These differentially expressed genes may be closely related to the occurrence and development of periodontitis. After deleting the duplicates, a total of 1285 candidate genes were included for the next step analysis (Sup. 1).

3.3. Potential targets of Scutellariae Radix in treating periodontitis

The "*Scutellariae Radix*-compound-target-periodontitis" network was created by using Cytoscape 3.9.1 software (Fig. 3A), which fully reflected how *Scutellariae Radix* interfered with periodontitis through multi-compound and multi-target methods. According to the topological analysis results, the main active compounds of *Scutellariae Radix* include wogonin (MOL000173), acacetin (MOL001689), baicalein (MOL002714), Stigmasterol (MOL000449), Salvigenin (MOL002915), (2R)-7-hydroxy-5-methoxy-2-phenylchroman-4-one (MOL000228), beta-sitosterol (MOL000358), 5,2',6'-Trihydroxy-7,8-dimethoxyflavone (MOL002917), indicating that these active components are the most important active compounds of *Scutellariae Radix* in the treatment of periodontitis. Veen diagram (Fig. 3B) shows drug targets, disease targets, and their common targets.

3.4. PPI network analysis and key targets

A total of 606 protein interaction relationships involving 53 targets were obtained through the STRING database. Cytoscape software was used to extract the top 10 targets according to degree value, as shown in Fig. 4, the top 10 targets were AKT1, IL-6, TNF, VEGFA, TP53, PTGS2, CASP3, JUN, MMP9 and HIF1A with degree from high to low. The topological significance is positively associated with the darkness of the colour, darker red targets denoting more meaningful spots.

3.5. GO and KEGG pathway enrichment analyses

GO function enrichment analysis showed that there were 504 GO terms significantly enriched. It includes 377 biological processes (BP), mainly involving response to hormone, tube morphogenesis, response to lipopolysaccharide and positive regulation of cell death; 39 cellular components (CC), mainly involving membrane raft, transcription regulator complex and cytoplasmic vesicle lumen; 88 molecular functions (MF), mainly involving RNA polymerase II-specific DNA-binding transcription factor binding and protein domain specific binding (Fig. 5A). KEGG pathway analysis showed 142 pathways related to *Scutellariae Radix* in the treatment of periodontitis,

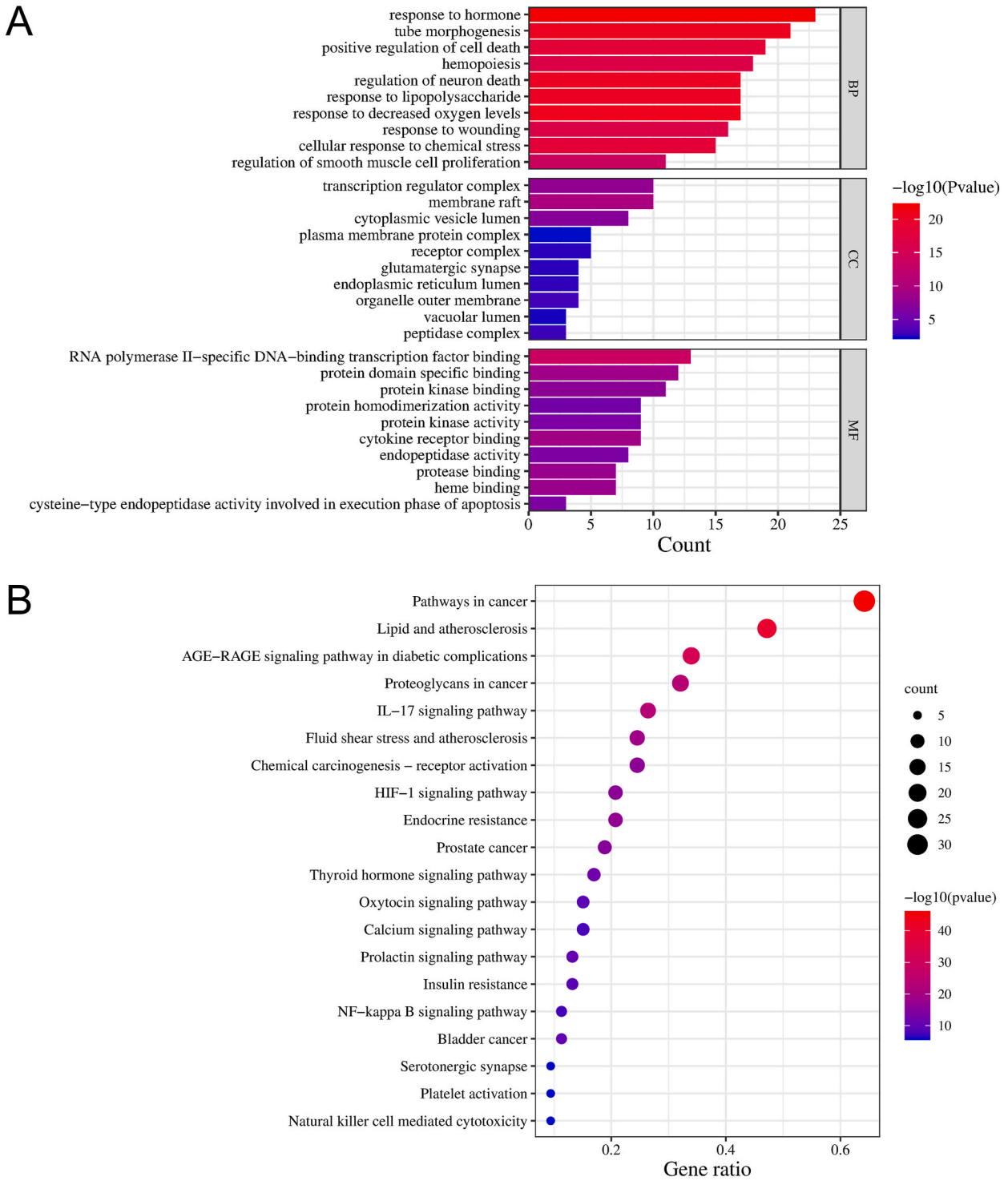


Fig. 5. GO and KEGG enrichment analysis of *Scutellariae Radix* in the treatment of periodontitis. (A) The top 10 terms of BP, CC and MF in GO enrichment analysis. The color of terms turned from blue to red. The redder the bar was, the smaller the *P* value was. (B) The top 20 pathways of KEGG enrichment analysis. The color of bubbles turned from blue to red. The redder the bubble was, the smaller the *P* value was.

including pathways in cancer, AGE-RAGE signalling pathway in diabetic complications, IL-17 signalling pathway and HIF-1 signalling pathway (Fig. 5B). These results indicate that *Scutellariae Radix* can treat periodontitis by regulating the coordination of multiple biological processes and pathways. As shown in Fig. 6, Cytoscape 3.9.1 software was used to create a "Gene-pathway" network for the

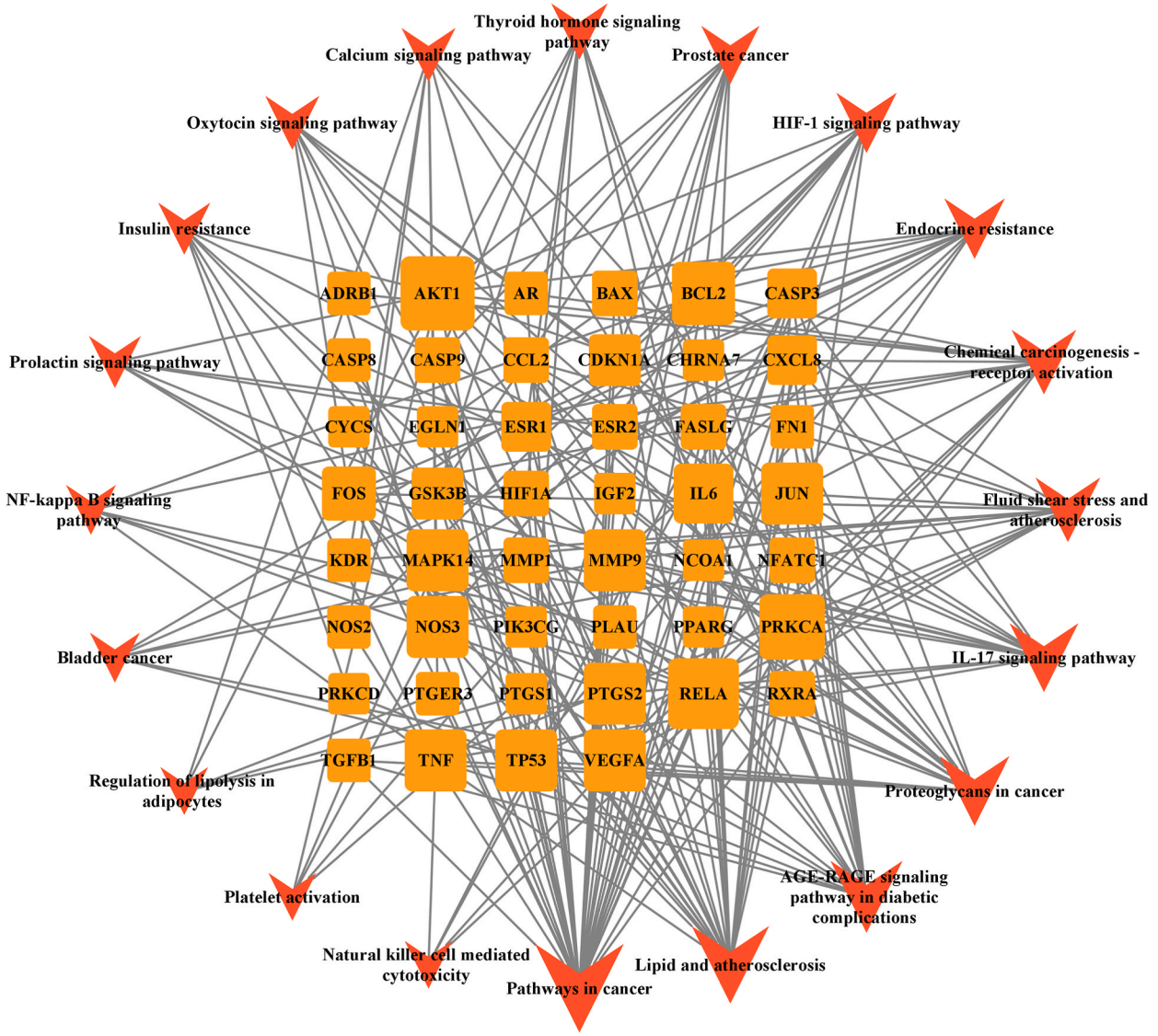


Fig. 6. Gene-pathway network of the *Scutellariae Radix* against periodontitis. The orange nodes represent targets, and the red nodes represent the top 20 pathways. The size of a node is proportional to its importance in the network.

Table 2
Molecular information of key target proteins.

Target	PDB ID	Ligand	Resolution/Å	RMSD/Å	Three dimensional coordinates of the active site
AKT1	4EKL	ORF	2.00	0.6442	x = 28.217614 y = 5.260013 z = 11.402263
IL-6	1ALU	TLA	1.90	1.7070	x = −7.569861 y = −13.013527 z = −0.146933
TNF	2AZ5	307	2.10	0.3594	x = −7.959711 y = 68.026486 z = 20.210744
TP53	5O1F	9GQ	1.38	0.3741	x = 124.150372 y = 101.357155 z = −45.149223
PTGS2	5IKV	FLF	2.51	0.3821	x = 166.024448 y = 220.463759 z = 217.605931
CASP3	1RHM	NA4	2.50	1.8395	x = −75.703496 y = 30.912291 z = 60.752341
JUN	5FV8	GOL	1.99	1.8183	x = 30.851905 y = 3.516270 z = −1.917952
MMP9	2OW1	7 MR	2.20	0.3027	x = 25.943000 y = 7.902000 z = 50.558000
HIF1A	3HQU	UN9	2.30	0.7703	x = −37.015666 y = 25.871624 z = −4.128520

top 20 significant KEGG pathways. The network consisted of 66 nodes and 232 edges, with 20 KEGG pathways and 42 genes. The significance of genes and pathways is proportional to their size. Topological analysis revealed that ATK1, which has the highest degree, is engaged in numerous signalling pathways and is regarded as the core gene. Several other genes, such as RELA, TNF, JUN, MMP9 and

Table 3
Molecular docking of key target proteins and active compounds.

Name	Molecular docking score for key targets (-CIE)			
	wogonin	acacetin	baicalein	primary ligand
AKT1	36.66	36.27	34.21	59.17
IL-6	26.22	27.01	25.39	21.17
TNF	27.23	25.78	27.03	46.98
TP53	36.74	35.66	32.85	46.83
PTGS2	39.84	41.68	38.75	46.04
CASP3	29.29	29.97	23.02	58.01
JUN	25.39	27.67	25.42	13.16
MMP9	39.98	41.91	39.65	65.21
HIF1A	33.88	35.13	39.46	47.69

VEGFA, also have a greater degree value, indicating that the pharmacological process of *Scutellariae Radix* in treating periodontitis with multiple targets and different pathways.

3.6. Molecular docking results

AKT1, IL-6, TNF, VEGFA, TP53, PTGS2, CASP3, JUN, MMP9 and HIF1A were selected as key targets. Wogonin, acacetin and baicalein, were used as small molecules for molecular docking. We calculated RMSD value to verify the accuracy of the results in network analysis. The results showed that the RMSD value of AKT1, IL-6, TNF, TP53, PTGS2, CASP3, JUN, MMP9 and HIF1A molecular docking models were 0.6442 Å, 1.7070 Å, 0.3594 Å, 0.3741 Å, 0.3821 Å, 1.8395 Å, 1.8183 Å, 0.3027 Å and 0.7703 Å, respectively, all of which were less than 2 Å (Table 2). It was concluded that the binding mode of original ligand and protein could be better reproduced and the docking results were highly reliable. VEGFA was not analyzed in depth because there has no appropriate crystal structure in PDB database. The specific information of the target proteins was shown in Table 3.

The nine target proteins mentioned above were docked with three main active compounds by the CDOCKER protocol available on the DS4.0 software, and the docking results are shown in Table 3. The value represents the score of -CDOCKER interaction energy (-CIE), and the higher value means stronger docking affinity between the active compounds and the target proteins. All -CIE scores in Table 3 are greater than zero, indicating that drug compounds can spontaneously bind to target proteins. Among them, the binding affinity of the three active compounds with IL-6 and JUN proteins are greater than that of the primary ligand of the protein, and they also have good binding affinity with other proteins. Fig. 7(A - I) represents the molecular binding mode of AKT1 - wogonin, IL-6 – acacetin, TNF – wogonin, TP53 – wogonin, PTGS2 – acacetin, CASP3 – acacetin, JUN – acacetin, MMP9 – acacetin, HIF1A – baicalein, respectively.The active compounds form hydrogen bonds, van der Waals forces and π-π interactions with amino acid residues of key targets and bind stably in our docking models.

3.7. Experimental validation

3.7.1. Cell culture and identification

Cell morphology observed under the microscope and shown in Fig. 8A, the cells are mostly fusiform or spindle-shaped. Flow cytometry was used to detect the expression of cell surface markers. As shown in Fig. 8B, cells negatively expressed hematopoietic-derived surface markers CD34 (1.52 %) and CD45 (0.03 %), and positively expressed mesenchymal-derived surface markers CD90 (99.99 %) and CD105 (99.87 %), which proved that the cultured hPDLSCs were mesenchymal-derived stem cells and could be used for subsequent experiments.

3.7.2. Effect of baicalein on the cell viability of hPDLSCs

As shown in Fig. 9A, baicalein at 0.01 μM, 0.1 μM and 1 μM concentrations promoted the cell viability significantly when compared with the control group ($P < 0.05$); while baicalein at 10 μM concentration could inhibit the cell viability, but the difference has no significant ($P > 0.05$), but the high concentration of baicalin (100 μM) can significantly inhibit the cell viability ($P < 0.05$). In conclusion, the low concentration of baicalin could promote the cell viability of hPDLSCs with a concentration depend manner, but the high concentration can decrease the cell viability which means inhibit the cell growth. According to the statistical results, baicalein at a concentration of 0.1 μM had a better promotion effect on the cell viability of hPDLSCs, so this concentration was chosen for the following experiments.

3.7.3. Baicalein inhibited the protein expression of PTGS2 and MMP9 in LPS-induced hPDLSCs

Based on our above studies of network pharmacology and molecular docking, we screened and predicted that baicalein had high binding affinity with PTGS2 and MMP9, which remind us they are may be the main targets of baicalein against periodontitis. For further verification, we detected the expression of PTGS2 and MMP9 proteins by using Western blot, and the results verified our conclusion. As shown in Fig. 9B (sup. 2) and 9C, PTGS2, also known as COX2, was not expressed in both control and baicalein groups. The PTGS2 and MMP9 expression in LPS-induced hPDLSCs was significantly higher compared with the control group, while the addition of baicalein can inhibit the expression of PTGS2 and MMP9 significantly ($P < 0.05$).

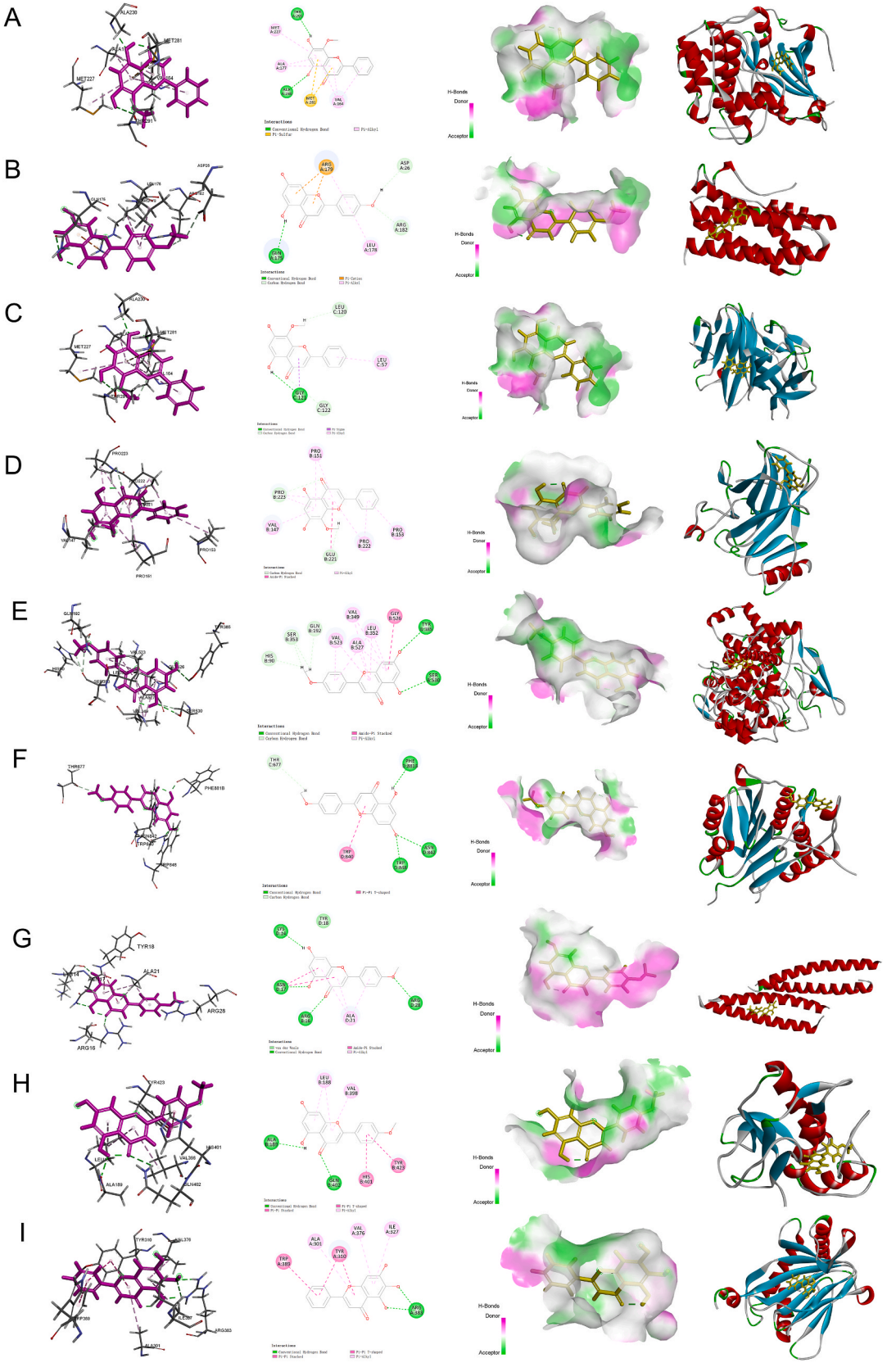


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Fig. 7. The represented results for the proposed action mode of molecular docking. (A) ATK1 (PDB ID: 4EKL) - wogonin; (B) IL-6 (PDB ID: 1ALU) - acacetin; (C) TNF (PDB ID: 2AZ5) - wogonin; (D) TP53 (PDB ID: 5O1F) - wogonin; (E) PTGS2 (PDB ID: 5IKV) - acacetin; (F) CASP3 (PDB ID: 1RHM) - acacetin; (G) JUN (PDB ID:5FV8) - acacetin; (H) MMP9 (PDB ID: 2OV1) - acacetin; (I) HIF1A (PDB ID: 3HQU)- baicalein.

4. Discussion

Periodontitis is an infectious disease which caused by plaque microorganisms, but its development is also regulated by some other factors, such as host immune inflammatory response and external environmental factors. In recent years, with the further understanding of periodontitis, many scholars have found that viruses are also involved in the occurrence and development of periodontitis [31,32]. Studies have found that traditional Chinese medicine has a wide range of physiological activities such as killing microorganisms, promoting tissue regeneration, enhancing immunity and reducing inflammation, but with minimum side effects. Therefore, Chinese herb medicine is very useful and may become an important choice for the clinical treatment of periodontal disease.

The main active compounds of *Scutellariae Radix* include flavonoids, phytosterols and alkaloids. Baicalein and wogonin are two kinds of flavonoids with high contents in *Scutellariae Radix*, they can promote the proliferation of human periodontal ligament cells and matrix mineralization, inhibit the differentiation of osteoclasts, and then lead to continuous apoptosis of mature osteoclasts [33,34]. In addition, baicalein can inhibit the production of LPS -induced inflammatory factors by regulating NF-κB pathway and prevent the formation of new blood vessels, cut off the supply of energy to inflammatory cells to achieve anti-inflammatory effects [35]. Besides, flavonoids from *Scutellariae Radix* also have some other physiological activities such as killing microorganisms and bootstring immunization [36,37]. Stigmasterol and beta-sitosterol, as typical phytosterols, also have good bioactivities such as anti-inflammatory, anti-oxidation, antibacterial, and regulation of the immune system [38]. The beta-sitosterol exist in lots of herbs, also in *Scutellariae Radix,* which can regulate the balance of bone metabolism. Zeng et al. found that beta-sitosterol in the leaves of *Eucommia ulmoides* can increase the ratio of OPG/ODF (Osteoprotegerin/Osteoclast Differentiation Factor) in osteoblast, which not only directly promotes osteogenesis, but also collaboratively achieves osteoprotective effects by effectively inhibiting osteoclast activities [39]. The mutual promotion and co-infection of herpesvirus and periodontal pathogens are important causes of periodontal disease. Studies have shown that alkaloids have strong antiviral effects [40,41] and epiberberine in *Scutellariae Radix* also belongs to alkaloids, which means *Scutellariae Radix* may control the development of periodontitis through its antiviral effect. The possible mechanism is that alkaloids enter cells, block virus adsorption and invasion, and inhibit the expression of viral genome to achieve antiviral effects [42].

According to PPI network analysis, the top 10 targets related to the treatment of periodontitis were AKT1, IL-6, TNF, VEGFA, TP53, PTGS2, CASP3, JUN, MMP9 and HIF1A. AKT, a serine/threonine protein kinase, can be activated by PI3K and recruited to the plasma membrane. AKT related to cell metabolism, survival migration, and gene expression when acute inflammation occurs, and AKT1 can cause edema and leukocyte extravasation by regulating vascular permeability [43]. IL-6 is a multifunctional inflammatory cytokine that plays a significant role in the inflammatory mediator network and linked to the progress of periodontal disease. In the inflammatory process of periodontitis, IL-6 promotes the release of IL-1, MMP and other cytokines in inflammatory area, aggravate the damage of periodontal tissue and inhibit the regeneration of periodontal ligament [44–46]. TNF-α can promote bone resorption by increasing the number of osteoclast precursor cells, induce osteoclast differentiation of macrophages in bone marrow and osteoclast maturation [47]; TNF-α was proved that can activate the NF-κB signalling pathway and promote the release of inflammatory factors such as IL-6, IL-8, therefore affecting the process of inflammation [48,49]. VEGFA is the most important growth factor during vascular development, which promotes the differentiation of bone progenitor cells and also promotes the secretion of bone-related cytokines [50], VEGFA is essential for neovascularization and vascular permeability, and it was considered to play an important role in the pathology of periodontitis in recent years [51]. PTGS2, also known as COX2, can promote the conversion of arachidonic acid (AA) into prostaglandin E2 (PGE2), which exerts biological effects by binding to the G protein-coupled prostaglandin E2 receptor 4, promotes the expression of cyclic adenosine monophosphate (cAMP), and then increases the release of inflammatory factors IL-6 and TNF [52,53]. CASP3 is a key protease which can be activated during the process of cell apoptosis [54]. The tumour suppressor gene TP53 and proto-oncogene JUN work in opposite effects to control DNA repair, apoptosis, and cell proliferation [55]. MMP9 is one of the important matrix metalloproteinases, which can degrade the extracellular matrix of periodontal tissue and plays an important role in the development of periodontitis [56]; MMP9 which works like PTGS2, is an inflammation-related factor with increased expression in gingival tissue of patients with periodontitis [57,58]. Chronic inflammation can cause microcirculation disorder and hypoperfusion in periodontal tissue, leading to the formation of hypoxic microenvironment in periodontal tissue. In addition, HIF-1α, the most important hypoxia response regulator, plays an important role in regulating alveolar bone resorption in periodontal tissue [59].

The pathways of *Scutellariae Radix* in the treatment of periodontitis include pathways in cancer, lipid and atherosclerosis, AGE-RAGE signalling pathway in diabetic complications and IL-17 signalling pathway. The most frequently related pathway among them is pathways in cancer. There is a clear correlation between chronic inflammation and cancer, with malignant cells expressing a cellular phenotype similar to that of inflammatory cells [60], and long-term persistent inflammation can alter cancer progression through some inflammatory molecules or pathways [61] Atherosclerosis is a process of passive deposition of lipids in the vessel wall, and it has been shown that periodontitis can directly by bacteria modify the structure of lipids or indirectly by triggering inflammatory pathways to affect lipid synthesis, modification, and the formation of atheroma [62]. AGEs increase the expression of IL-6 and ICAM-1 through RAGE, MAPK and NF-κB pathways, which may aggravate the pathogenesis of periodontal disease [63]. IL-17 is a cytokine secreted by T helper 17 (Th17) cells, which can induce the secretion of a variety of pro-inflammatory factors and play a synergistic role with these cytokines to amplify the inflammatory response. It was found that IL-17A and Th17 cells are key factors in the destruction of alveolar bone which induced by periodontal inflammation [64].

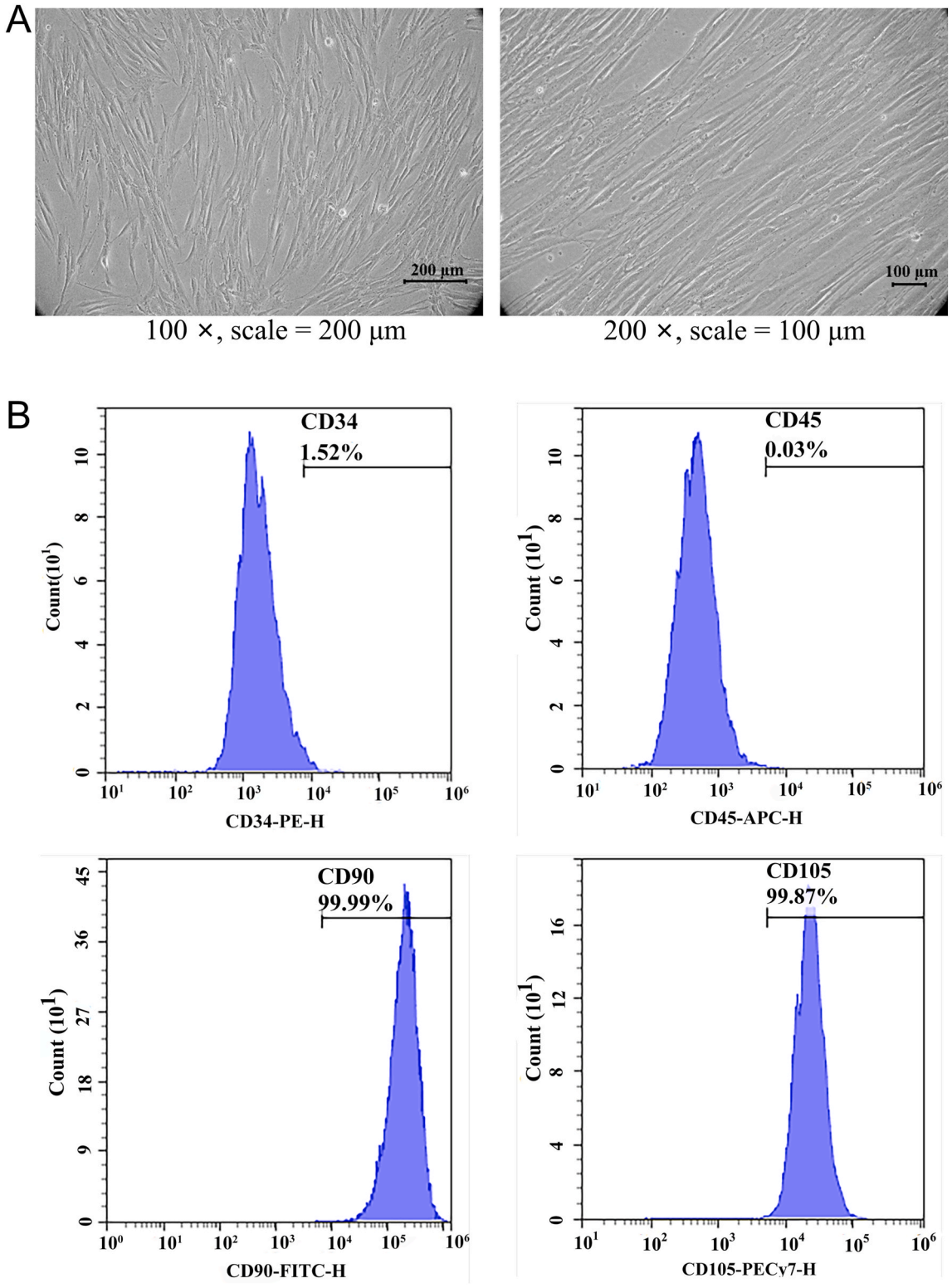

Fig. 8. hPDLSCs characterization. (A) The morphology of hPDLSCs observed at 100× and 200 × magnification. (B) Flow cytometry assay to assess the CD markers in the surface of hPDLSCs.

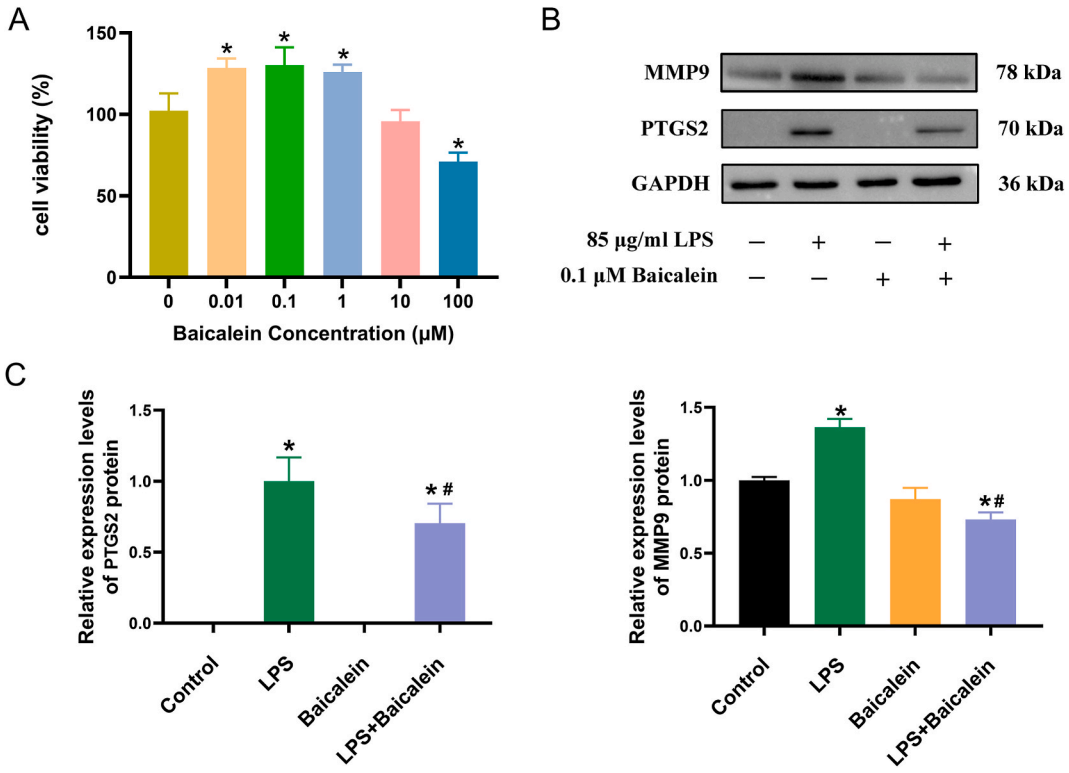

Fig. 9. Results of cell viability and Western blotting assays. (A) The effect of baicalein on the cell viability of hPDLSCs. *$P < 0.05$, compared with the control group. (B) Proteins expression bands. (C) Quantitative analysis of the gray value of PTGS2 and MMP9 proteins * $P < 0.05$ vs. control group, #$P < 0.05$ vs. LPS group.

Molecular docking confirmed that three main compounds of *Scutellariae Radix* could spontaneously bind to the key active regions of AKT1, IL-6, TNF, TP53, PTGS2, CASP3, JUN, MMP9 and HIF1A proteins through hydrogen bonding, electrostatic interaction, hydrophobic interaction and van der Waals force. The whole protein-ligand interaction system was stable, which preliminarily verified the analysis results of network pharmacology. In addition, cellular experiments also demonstrated that baicalein, the most predominant compound of *Scutellariae Radix*, could reduce the expression of PTGS2 and MMP9, inflammation-related factors which are induced by LPS *in vitro*, indicating that baicalein could indeed exert anti-inflammatory effects by binding to target proteins and regulating theirs expression. Notably, PTGS2 (COX2) is an inducible enzyme that is usually absent or expressed at very low level in healthy tissues and organs, but can be highly induced because of the inflammation response, so it is usually detected after inflammatory stimulation [65,66]. In current experiment, the expression of PTGS2 cannot be detected both in control and baicalein treated group, which demonstrated that baicalein does not cause any inflammation reaction by itself and has a good safety profile.

However, our study may still have some limitations. Firstly, *Scutellariae Radix* can be used both orally and topically, but currently, there is no good screening method to identify potential compounds for topical use. In order to increase the likelihood that the screened compounds are effective, we used the generally accepted screening criteria recommended by the database, but this may limit our interpretation of the results. In addition, when collecting disease-related targets, although we have searched four commonly used databases to ensure the comprehensiveness of the target collection as much as possible, we also acknowledge that this does not exhaust all the results, and there may still be some targets missing, limiting the interpretation of the results. Furthermore, while our analysis in the pharmacology section revealed that multiple active compounds of *Scutellariae Radix* may exert a synergistic effect through multiple targets and various signaling pathways, in the cell experiment part, we only verified the flavonoid compound with the highest content in *Scutellariae Radix*, but did not verify the synergistic effect of multiple active compounds and additional pathways. In this regard, we will conduct more in-depth research in the future.

5. Conclusions

In summary, we are first systematically investigated the pharmacodynamic material basis, key targets and network regulation mechanism of *Scutellariae Radix* against periodontitis through network pharmacology, molecular docking and experimental validation, revealed the main mechanism of *Scutellariae Radix* against periodontitis from a holistic and systematic perspective, and elucidated how baicalein, its main active substance, perform its inhibitory effects by checking the expression of PTGS2 and MMP9 after induced by LPS *in vitro*, all these providing scientific basis for subsequent studies and optimization for the deep exploration of *Scutellariae Radix* and

design of novel drugs for the treatment of periodontitis in future.

Data availability statement

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

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CRediT authorship contribution statement

Jixian Feng: Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Yan Li:** Writing – original draft, Validation, Project administration, Investigation, Formal analysis, Data curation. **Juan Liu:** Visualization, Methodology, Formal analysis, Conceptualization. **Ningli Li:** Writing – review & editing, Visualization, Methodology. **Bin Sun:** Writing – review & editing, Software, Resources. **Shizhen Zhao:** Writing – review & editing, Software, Resources. **Yuankun Zhai:** Writing – review & editing, Supervision, Resources, Methodology, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e35744.

Abbreviations

AKT1	AKT serine/threonine kinase 1
BP	biological processes
CASP3	Caspase 3
CC	cellular components
DL	drug-likeness
GO	Gene Ontology
hPDLSCs	human periodontal ligament stem cells
HIF1A	Hypoxia inducible factor 1 subunit alpha
IL-6	Interleukin 6
JUN	Jun proto-oncogene
KEGG	Kyoto Encyclopedia of Genes and Genomes
LPS	lipopolysaccharide
MF	molecular functions
MMP9	Matrix metallopeptidase 9
OB	oral availability
PPI	protein-protein interaction
PTGS2	Prostaglandin-endoperoxide synthase 2
TNF	Tumor necrosis factor
TP53,	Tumor protein p53
VEGFA	Vascular endothelial growth factor A

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