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Exploring the mechanisms of magnolol in the treatment of periodontitis by integrating network pharmacology and molecular docking

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Key words: Magnolol, Periodontitis, Network pharmacology, Molecular docking

Abstract: Background: Magnolol, a bioactive extract of the Chinese herb *Magnolia officinalis* has a protective effect against periodontitis. This study is aimed to explore the mechanisms involved in the functioning of magnolol against periodontitis and provide a basis for further research. **Methods:** Network pharmacology analysis was performed based on the identification of related targets from public databases. The Protein-protein interaction (PPI) network was constructed to visualize the significance between the targets of magnolol and periodontitis. Subsequently, Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis were performed to predict the functions and the signal regulatory pathways involved in the action of magnolol against periodontitis. The "function-target-pathway" networks were constructed to analyze the core targets and pathways of magnolol against periodontitis. Molecular docking was used to verify the interaction of magnolol and core targets. **Results:** A total of 58 active targets of magnolol and 644 periodontitis-related targets were collected from public databases. A total of 25 targets of magnolol against periodontitis were identified based on the Venn diagram. GO analysis showed that magnolol has a role in the response to oxidative stress, nicotine, and lipopolysaccharide. KEGG enrichment analysis indicated that the mechanism of magnolol against periodontitis was mainly related to the tumor necrosis factor (TNF), phosphoinositide 3-kinase (PI3K/Akt), and mitogen-activated protein kinase (MAPK) signaling pathways. Combined with PPI network and molecular docking results, the core targets of magnolol against periodontitis included AKT1, MAPK8, MAPK14, TNF, and TP53. **Conclusion:** To summarize, the anti-periodontitis mechanisms of magnolol are potentially through regulating the TNF, PI3K/Akt, and MAPK signaling pathways.

Introduction

Periodontitis is a chronic inflammatory disease (Sanz *et al.*, 2020), causing chronic pain, gingival inflammation, and alveolar bone loss (Carvalho *et al.*, 2021). Periodontitis is initiated by disturbances in the balance of the local microbial community (George, 2015). This affects oral health and may aggravate various systemic diseases like type 2 diabetes (Piero *et al.*, 2019). Recent studies showed that periodontitis is the sixth most prevalent disease with a prevalence rate of approximately 50% (GBD 2017 Disease and Injury Incidence and Prevalence Collaborators, 2018). The periodontal treatment mainly focuses on eliminating dental plaque to reduce the number of bacteria, thereby stopping inflammation and its progression. Notably, the use of a local chemotherapeutic agent as an adjunctive to scaling and root planing (SRP) is regarded as the first treatment of option given its limited invasiveness (Taalab *et al.*, 2021). The standard drugs employed for treating periodontitis are antibiotics and oral antiseptics including amoxicillin, metronidazole, and cefradine (Feres *et al.*, 2021). However, the effects of these drugs are limited and also show inevitable drug toxicity. Traditional Chinese medicine (TCM) that emphasizes the holistic treatment has distinctive advantages such as less toxicity in the treatment of periodontitis. Nevertheless, there is lack of the research on the mechanisms of TCM use in treating periodontitis.

Magnolol, the major bioactive polyphenolic component of *Magnolia officinalis*, is known as a TCM in the treatment of gastrointestinal and kidney disorders (Lee *et al.*, 2011). It possesses diverse biological functions, such as anti-cancer, anti-inflammation, and

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anti-oxidation (Chiu *et al.*, 2020; Fukuyama *et al.*, 2020). Multiple studies demonstrated that magnolol plays important roles against tumorigenesis by inhibiting proliferation, migration, and invasion of cancer cells (Shen *et al.*, 2017; Zhu *et al.*, 2019). Magnolol also can mitigate the inflammation and oxidative stress induced by periodontitis. For example, Lu *et al.* (2015) reported that magnolol inhibited *P. gingivalis* lipopolysaccharide-induced inflammation in macrophages (Lu *et al.*, 2015). In another study, Liu *et al.* (2021) found that magnolol was beneficial to improve the impaired wound healing and inflammation caused by combined diabetes mellitus and periodontitis. While these findings suggest that magnolol could be a potential adjuvant for the treatment of periodontitis, the underlying therapeutic mechanisms remain unclear.

Network pharmacology is a powerful research tool based on the theory of pharmacology, biology, and bioinformatics to reveal the mechanisms of disease treatment (Xiong *et al.*, 2020). In recent years, network pharmacology has emerged as a popular method to investigate the potential mechanism of TCM in disease treatments (Chen *et al.*, 2018; Wu *et al.*, 2020). Complex ingredients of TCM present great difficulty in the development of TCM research. Network pharmacology constructs and analyzes the complex "compound-target-disease" network from a systematic and holistic perspective. This approach is different from traditional pharmacology research methods (Shao and Zhang, 2013; Wang *et al.*, 2021). Thus, we used the pharmacology network to investigate the potential mechanism of magnolol against periodontitis.

In this study, network pharmacology was used to screen targets of magnolol in treating periodontitis. Then, the anti-periodontitis mechanisms of magnolol were predicted by GO and KEGG enrichment analysis. Finally, molecular docking technology was used to verify the interaction of magnolol and anti-periodontitis targets. The research flow chart has been presented in Fig. 1.

Materials and Methods

Identification of magnolol-related targets

Magnolol-related targets were obtained from Herb Ingredients' Targets (HIT) (http://hit2.badd-cao.net/) (Yan *et al.*, 2021) and GeneCards (https://www.genecards.org/) (Marilyn *et al.*, 2010) databases. The keyword "magnolol" was applied as a search term, and duplicated targets or those without a Uniprot ID were removed.

Potential targets related to periodontitis

GeneCards, DisGeNET (https://www.disgenet.org/) (Piero *et al.*, 2019), and Therapeutic Target Database (TTD) (http://db.idrblab.net/ttd/) (Chen et al., 2002) databases were used to screen the human periodontitis-related targets. The repeated targets and those with no Uniprot IDs were excluded. VennDiagram (version 1.7.3) (Chen and Boutros, 2011) was used to analyze the overlapping targets of magnolol against periodontitis.

Construction of the protein-protein interaction network

Based on the results of the Venn diagram, the overlapping targets of magnolol and periodontitis were imported into the STRING database to construct the PPI network (Damian *et al.*, 2020). The standard of interaction degree value was set as >0.4. The complex relationships were visualized by ggraph (version 2.0.5) and igraph (version 1.3.1) of the R package, and then cytoHubba was used to select hub genes (Chin *et al.*, 2014).

Functional enrichment analysis

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment were conducted using clusterProfiler (version 4.4.2) (Yu *et al.*, 2012). The principle of KEGG analysis is based on the hypergeometric distribution model that evaluates the significance of the target gene set with the related to special gene and signaling pathway. In addition, a p-value < 0.05 was kept as significant in the GO and KEGG analysis in this study. The cytoHubba was applied to select the core targets based on GO and KEGG results, and the "function-target-pathway" network was conducted using the R language (circlize) (Gu *et al.*, 2014).

Molecular docking

Structures of the core target proteins were downloaded from the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB, http://www.pdb.org/). Pymol and Autodock1.5.6 software were used to modify the protein structure by the removal of original ligands and water molecules, the addition of hydrogen, optimization of amino acids, and calculation of charges (Seeliger and Groot, 2013). The structure file of magnolol in mol2 format was downloaded from the ZINC (https://zinc.docking.org/) database. Open Babel GUI software was used to transform the downloaded protein and magnolol file into the PDBQT version. Finally, molecular docking was operated by Autodock1.5.6, and the results were visualized by Pymol.

Results

Identification of targets of magnolol against periodontitis

According to the HIT and GeneCards databases, a total of 58 targets of magnolol were obtained after removing duplicates and targets without a UniProt ID. GeneCards, DisGeNET, and TTD databases were employed for searching periodontitis-related targets. A total of 664 targets was retrieved by removing duplicates. Then, using VennDiagram (Version 1.7.3), a total of 25 targets of magnolol in the treatment of periodontitis were selected (Table 1 and Fig. 2).

PPI network of targets of magnolol against periodontitis

To investigate the interaction of 25 potential targets for magnolol against periodontitis, the PPI network of these targets was constructed, and a total of 207 interaction relationships were obtained. Then, we put the network into cytoHubaba and identified the top 10 hub targets with high

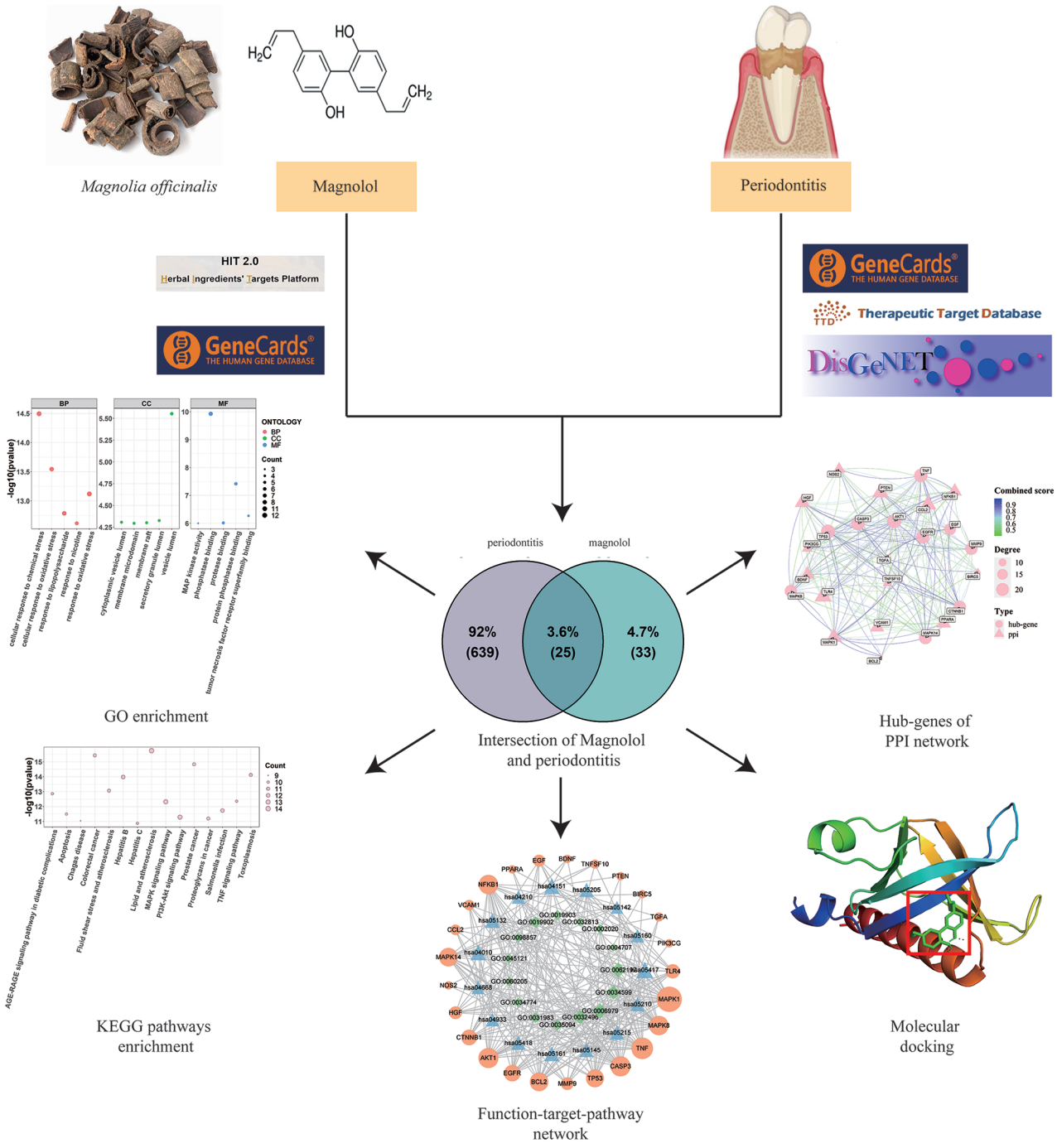

FIGURE 1. Flow chart of the research in this study.

connectivity which are presented as circle points in Fig. 3. These include tumor protein p53 (TP53), AKT serine/threonine kinase 1 (AKT1 1), tumor necrosis factor (TNF), mitogen-activated protein kinase 14 (MAPK14), caspase 3 (CASP3), matrix metallopeptidase 9 (MMP9), epidermal growth factor receptor (EGFR), catenin beta 1 (CTNNB1), epidermal growth factor (EGF), and mitogen-activated protein kinase 8 (MAPK8).

Gene ontology and kyoto encyclopedia of genes and genomes enrichment results

A total of 25 hub genes of magnolol against periodontitis were analyzed by GO and KEGG enrichment. GO function enrichment showed that these 25 targets included 812 biological process (BP) terms, 11 cellular component (CC) terms, and 20 molecular function (MF) terms. The top 15 GO terms have been presented in Fig. 4A and Table 2. These include cellular response to chemical and oxidative stress (BP), vesicle lumen (CC), and phosphatase binding (MF). According to the KEGG pathway enrichment analysis, there were 128 related pathways enriched by targets, and the top 15 signaling pathways have been presented in Fig. 4B and Table 3. The results demonstrated that magnolol is mainly related to the PI3k/Akt, TNF, and MAPK signaling pathways (Fig. 4B).

Combined with core targets, top 15 GO functions, and KEGG pathways of magnolol against periodontitis, the "function-target-pathway" network was constructed

TABLE 1

The 25 targets of magnolol against periodontitis

ID	Gene name	UniProtKB
1	Toll like receptor 4 (TLR4)	O00206
2	Nitric oxide synthase 2 (NOS2)	P35228
3	Brain derived neurotrophic factor (BDNF)	P23560
4	Mitogen-activated protein kinase 14 (MAPK14)	Q16539
5	Mitogen-activated protein kinase 1 (MAPK1)	P28482
6	Mitogen-activated protein kinase 8 (MAPK8)	P45983
7	Tumor necrosis factor (TNF)	P01375
8	C-C motif chemokine ligand 2 (CCL2)	P13500
9	Vascular cell adhesion molecule 1 (VCAM1)	P19320
10	Caspase 3 (CASP3)	P42574
11	Tumor protein p53 (TP53)	P04637
12	Nuclear factor kappa B subunit 1 (NFKB1)	P19838
13	Matrix metallopeptidase 9 (MMP9)	P14780
14	BCL2 apoptosis regulator (BCL2)	P10415
15	Epidermal growth factor receptor (EGFR)	P00533
16	AKT serine/threonine kinase 1 (AKT1)	P31749
17	Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit gamma (PIK3CG)	P48736
18	TNF superfamily member 10 (TNFSF10)	P50591
19	Catenin beta 1 (CTNNB1)	P35222
20	Phosphatase and tensin homolog (PTEN)	P60484
21	Epidermal growth factor (EGF)	P01133
22	Hepatocyte growth factor (HGF)	P14210
23	BIRC5-baculoviral IAP repeat containing 5 (BIRC5)	O15392
24	Peroxisome proliferator activated receptor alpha (PPARA)	Q07869
25	Transforming growth factor alpha (TGFA)	P01135

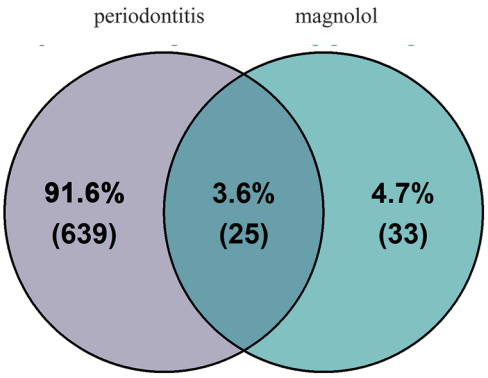

FIGURE 2. Venn diagram of the 25 intersecting targets related to magnolol and periodontitis.

(Fig. 4C). Meanwhile, cytoHubba was used to select the top 10 core genes according to the maximal clique centrality (MCC) score. The top 10 core targets were MAPK1, TNF, AKT1, CASP3, MAPK8, NFKB1, BCL2, MAPK14, TP53, and EGFR. Results showed the top 15 GO functions and KEGG pathways were mainly associated with MAPK1 (25 enriched GO terms and pathways), TNF (21 enriched GO terms and pathways), and CASP3 (20 enriched GO terms and pathways). The top 10 core targets were largely enriched in the cellular response to chemical stress (7 targets), response to oxidative stress (7 targets), and response to lipopolysaccharide (6 targets). Additionally, the core targets were mainly related to the MAPK signaling pathway (9 enrichment targets), TNF signaling pathway (7 enrichment targets), as well as PI3K/Akt signaling pathway (6 enrichment targets) (Fig. 4D).

Molecular docking results

From the intersection of hub targets from the PPI and GO analysis, and KEGG networks, a total of 7 targets (TP53, AKT1, TNF, MAPK14, CASP3, EGFR, MAPK8) were selected and further used for molecular docking with magnolol (Fig. 5A). In order to verify the accuracy of magnolol and core target by molecular docking, the standard of binding affinity was set as <−5.0 kcal/mol, which suggested the ligand has a good binding activity to the receptor protein. As shown in Table 4 and Figs. 5B–5F, magnolol showed good binding with 5 core targets, namely MAPK14 (binding affinity = −6.54 kcal/mol, Fig. 5B), TP53 (binding affinity = −6.15 kcal/mol, Fig. 5C), TNF (binding affinity = −6 kcal/mol, Fig. 5D), MAPK8 (binding affinity =

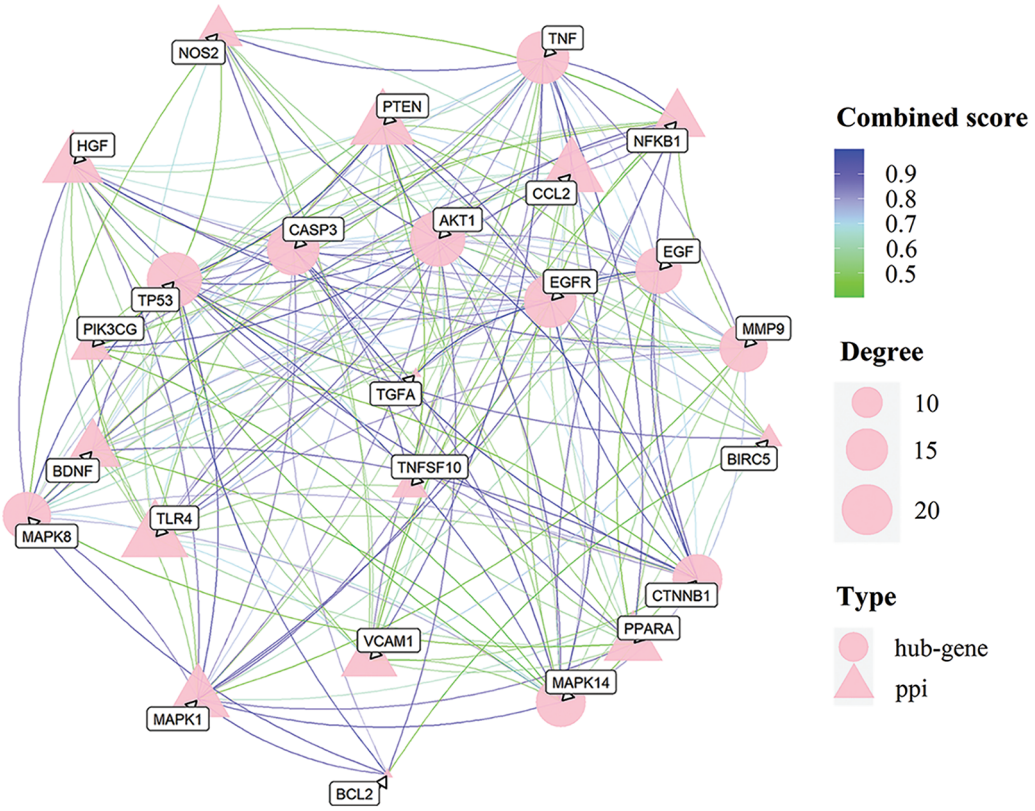

FIGURE 3. Protein-protein interaction (PPI) network of the 25 hub targets of magnolol against periodontitis.

−5.7 kcal/mol, Fig. 5E), and AKT (binding affinity = −5.67 kcal/mol, Fig. 5F).

Discussion

Periodontitis is a noninfectious chronic disease initiated by dysbiosis of the local microbial community (Lin *et al.*, 2022). Periodontitis was shown to lead to the direct activation of osteoclast activity, and eventually cause the loss of alveolar bone due to the overactivation of the host immune response (Lamont *et al.*, 2018). In short term, the traditional treatment methods including SRP (scaling and root planning) and oral antiseptics are effective in reducing the impact of periodontitis and improving the clinical symptoms (Elashiry *et al.*, 2021). However, the effects of these therapies are limited and non-selective. The treatment of periodontitis is aimed to reduce the risk of infection and inflammation. In recent years, TCM the compounds used have been reported to have significant therapeutic effects on health disorders including periodontitis (Yuan *et al.*, 2021; Scab *et al.*, 2019). Magnolol, a binaphthalene polyphenolic compound derived from the stem bark and root of *Magnolia officinalis* is widely used in the treatment of inflammation, anxiety, etc. (Yang *et al.*, 2008; Weeks, 2009; Cicalău *et al.*, 2021). Previous studies showed that magnolol could alleviate the clinical symptoms associated with periodontitis (Ho *et al.*, 2001; Lu *et al.*, 2013). However, the mechanistic aspects of how magnolol functions in the treatment of periodontitis are still unclear. Through network pharmacology, we obtained 25 potential targets for magnolol against periodontitis. Network pharmacology, a new research tool, is largely used to reveal the complex mechanisms of TCM in treatment (Bi *et al.*, 2021; Zhang *et al.*, 2021). In the current study, we used network pharmacology to investigate the possible mechanisms of magnolol in the treatment of periodontitis.

Significantly, GO function analysis demonstrated that the 25 targets were mainly enriched in BPs, such as responses to oxidative stress, nicotine, and lipopolysaccharide. Oxidative stress has been demonstrated to be a crucial contributing factor in the development of periodontitis through the overaccumulation of reactive oxygen species (Sczepanik *et al.*, 2020). For example, Ying *et al.* (2020) found that low-intensity pulsed ultrasound could eliminate oxidative stress through the PI3K-Akt/Nrf2 pathway to regulate alveolar bone homeostasis (Ying *et al.*, 2020). Despite magnolol having shown anti-oxidative stress potential in several disorders such as acute alcoholic liver damage (Liu *et al.*, 2019) and multiple sclerosis (Bibi *et al.*, 2022), its effect on oxidative stress in periodontitis remains largely unclear. Interestingly, a previous investigation indicated that magnolol could inhibit oxidative stress to ameliorate the symptoms of diabetic periodontitis, which may account for the findings of the present study (Liu *et al.*, 2021). Smoking is a risk factor for periodontitis as it disrupts the repair process during subsequent periodontal treatment (Garcia *et al.*, 2018). A previous study indicated that nicotine consumption enhanced the inflammatory response and the development of periradicular lesions in rats (Pinto *et al.*, 2020). Lipopolysaccharide, which is a bacterial virulence factor, is one of the main causes of the aggravation of edema and vasodilation in inflammatory periodontal tissues (Kim *et al.*, 2021). A previous study reported that the activity of circulating lipopolysaccharide

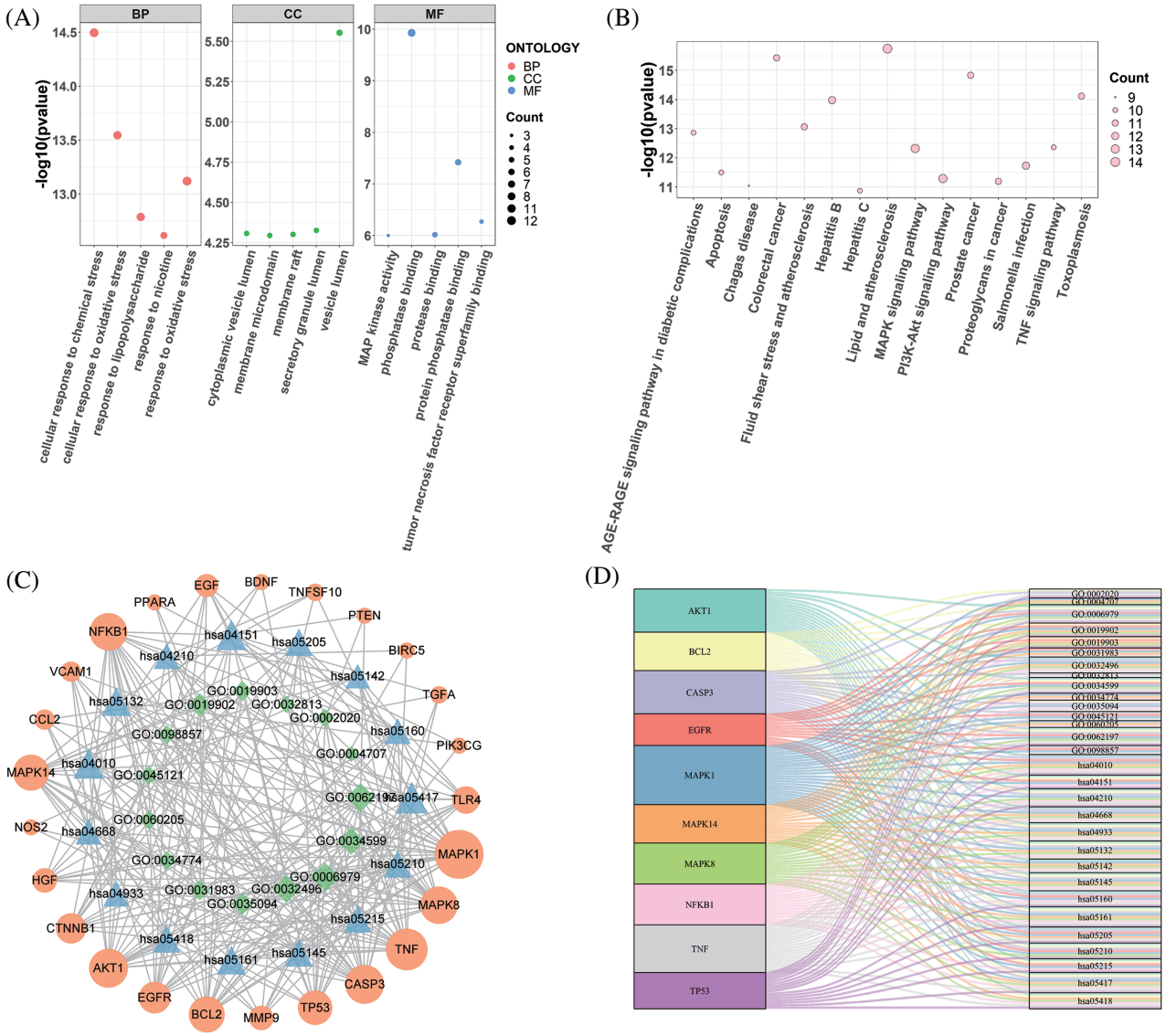

FIGURE 4. Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis of 25 targets of magnolol against periodontitis. (A) The top 15 significantly enriched GO terms, including 5 biological progress (BP) terms, 5 cellular component (CC) terms, and 5 molecular function (MF) terms; (B) The top 15 KEGG pathways enrichment terms; (C) The "function-target-pathway" network based on the 25 core targets with top 15 GO terms and KEGG pathways; (D) The network of 10 key targets related to top 15 GO terms and KEGG enrichment pathways.

TABLE 2

The Top 15 enriched terms of Gene Ontology (GO) enrichment

Gene ontology	ID	Description	*p*-value
BP	GO:0062197	Cellular response to chemical stress	3.20E-15
BP	GO:0034599	Cellular response to oxidative stress	2.85E-14
BP	GO:0006979	Response to oxidative stress	7.60E-14
BP	GO:0032496	Response to lipopolysaccharide	1.63E-13
BP	GO:0035094	Response to nicotine	2.42E-13
CC	GO:0031983	Vesicle lumen	2.80E-06
CC	GO:0034774	Secretory granule lumen	4.71E-05
CC	GO:0060205	Cytoplasmic vesicle lumen	4.92E-05
CC	GO:0045121	Membrane raft	5.00E-05

(Continued)

Table 2 (continued)

Gene ontology	ID	Description	*p*-value
CC	GO:0098857	Membrane microdomain	5.07E-05
MF	GO:0019902	Phosphatase binding	1.17E-10
MF	GO:0019903	Protein phosphatase binding	3.81E-08
MF	GO:0032813	Tumor necrosis factor receptor superfamily binding	5.38E-07
MF	GO:0002020	Protease binding	9.64E-07
MF	GO:0004707	MAP kinase activity	9.96E-07

TABLE 3

The Top 15 enriched pathways of Kyoto Encyclopedia of Genes (KEGG) analysis

ID	Description	*p*-value
hsa05417	Lipid and atherosclerosis	1.79E-16
hsa05210	Colorectal cancer	3.70E-16
hsa05215	Prostate cancer	1.48E-15
hsa05145	Toxoplasmosis	7.61E-15
hsa05161	Hepatitis B	1.05E-14
hsa05418	Fluid shear stress and atherosclerosis	8.68E-14
hsa04933	AGE-RAGE signaling pathway in diabetic complications	1.37E-13
hsa04668	TNF signaling pathway	4.40E-13
hsa04010	MAPK signaling pathway	4.76E-13
hsa05132	Salmonella infection	1.86E-12
hsa04210	Apoptosis	3.17E-12
hsa04151	PI3K-Akt signaling pathway	5.12E-12
hsa05205	Proteoglycans in cancer	6.40E-12
hsa05142	Chagas disease	9.12E-12
hsa05160	Hepatitis C	1.35E-11

was increased in periodontitis patients, suggesting the essential role of targeting lipopolysaccharide in the treatment of periodontitis (Pussinen *et al.*, 2022). Collectively, our findings demonstrated that the potential targets of magnolol against periodontitis were mainly implicated in the regulation of oxidative stress, nicotine, and lipopolysaccharide.

The KEGG pathway enrichment analysis showed that magnolol exerted its anti-periodontitis effect through multiple signaling pathways, including the TNF pathway, MAPK pathway, and the phosphatidylinositol-3-kinase (PI3K)-Akt signaling pathway. The TNF signaling pathway shows significant effects on the response to inflammation, regulation of the immune system, and induction of cell proliferation among many human pro-cancer processes (Lousa *et al.*, 2022). The MAPK signaling pathway is involved in diverse inflammatory responses including regulating the expression levels of inflammatory factors, activating the immune system, and degrading matrix components (Kim *et al.*, 2018). It was previously documented that the activation of the MAPK pathway in periodontitis aggravated inflammation and alveolar bone loss (Wang *et al.*, 2020). Another study revealed that inhibition of the MAPK/c-fos/NFATC1 pathway could alleviate ligation-induced periodontitis (Xu *et al.*, 2020). Notably, a previous study suggested that magnolol might suppress periodontitis by regulating the p38 MAPK signaling pathway (Lu *et al.*, 2015). PI3K is a serine/threonine and lipid kinase, and Akt is the main downstream target of PI3K. PI3K/Akt signaling participates in the regulation of cell autophagy. Liu *et al.* (2018) reported that the lipopolysaccharide-induced inhibition of the PI3k/Akt/mTOR signaling pathway could promote the autophagy of human gingival fibroblasts (Liu *et al.*, 2018). Taken together, KEGG enrichment analysis revealed that TNF, MAPK, and PI3K/Akt signaling pathways might be involved in the functional mechanisms of magnolol against periodontitis. In addition, we reported 10 hub targets from the PPI network, namely TP53, AKT1, TNF, MAPK14, CASP3, MMP9, EGFR, CTNNB1, EGF, and MAPK8. Further, 7 core targets (TP53, AKT1, TNF, MAPK14, CASP3, EGFR, and MAPK8) were obtained from the "function-target-pathway" network for molecular docking. The results showed that the binding energies of magnolol with AKT1, MAPK8, MAPK14, TNF, and TP53 were less than −5.0 kcal/mol. This suggests a good binding capability between these molecules. AKT1 is a serine/threonine kinase, which has critical roles in the regulation of osteoblast differentiation, suggesting the importance of AKT1 in the treatment of periodontitis (Eiichi *et al.*, 2014). Several natural compounds, such as panduratin A, have been demonstrated to suppress osteoclastogenesis to prevent periodontitis progression by inhibiting MAPK signaling (Kim *et al.*, 2018). A previous study found elevated MAPK8 in the human gingival fibroblasts under the pathological conditions of periodontitis (Herath *et al.*, 2013). Moreover, another study indicated that suppression of the MAPK14 signaling could ameliorate the symptoms of periodontitis (Wang *et al.*, 2021). Despite these findings, research into the role of MAPK8 and MAPK14 in the pathological process of periodontitis remains scarce. Unlike previous research, our study revealed the significance of both MAPK8 and MAPK14 in the anti-periodontitis mechanisms of magnolol functioning. TNF, an essential cytokine, which is related to the innate response to periodontal pathogens (Aleksandrowicz *et al.*, 2021). Notably, a previous study revealed that the expression of TNF was remarkably increased in different inflammatory

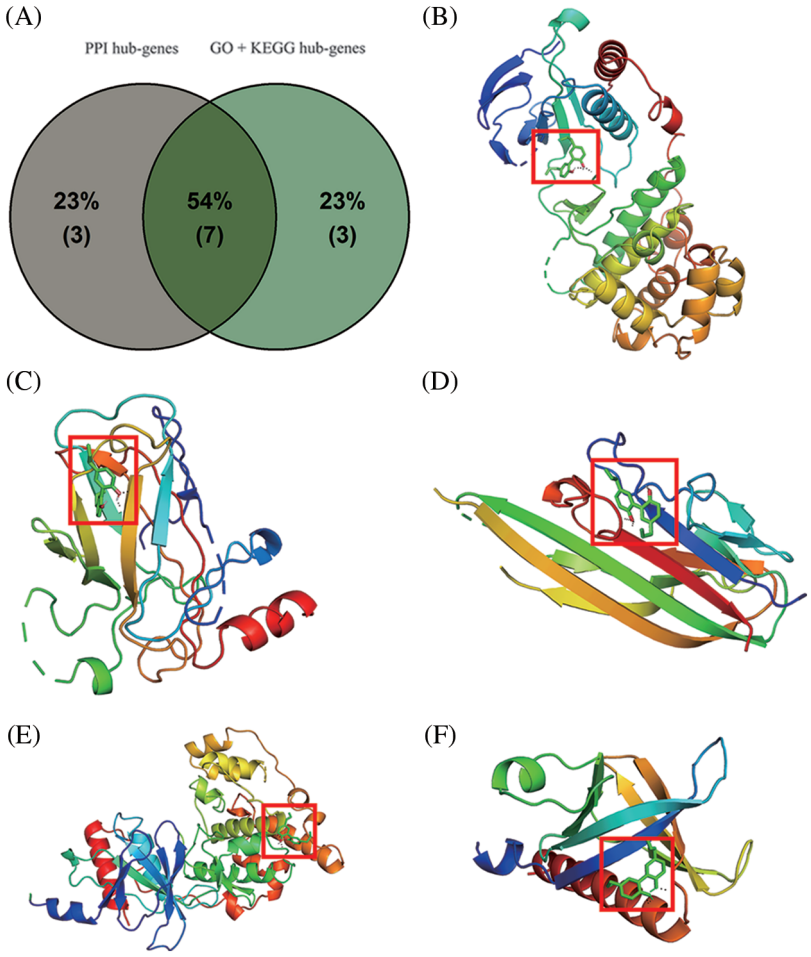

FIGURE 5. Molecular docking of core targets with magnolol. (A) Venn diagram of the hub-genes from Protein-Protein Interaction (PPI) and Gene ontology and Kyoto Encyclopedia of Genes and Genomes (GO + KEGG network); (B) Magnolol bound with mitogen-activated protein kinase 14 (MAPK14), binding affinity = −6.54 kcal/mol; (C) Magnolol bound with tumor protein p53 (TP53), binding affinity = −6.15 kcal/mol; (D) Magnolol bound with tumor necrosis factor (TNF), binding affinity = −6 kcal/mol; (E) Magnolol bound with mitogen-activated protein kinase 8 (MAPK8), binding affinity = −5.7 kcal/mol; (F) Magnolol bound with AKT1, binding affinity = −5.67 kcal/mol. Red boxes represent binding sites.

TABLE 4

Molecular docking of magnolol and the 5 core targets

Compound	Target	Free binding energy (kcal/mol)
Magnolol	MAPK14	−6.54
	TP53	−6.15
	TNF	−6
	MAPK8	−5.7
	AKT1	−5.67

disorders including periodontitis (Li *et al.*, 2020). TP53, a transcription factor, has direct effects on the processes of proliferation and differentiation in dental stem cells (Chen *et al.*, 2021). The increased activity of TP53 could induce an immune and inflammatory response which promotes the occurrence of diseases including periodontitis (McCubrey *et al.*, 2022). Collectively, magnolol could act on AKT1, MAPK8, MAPK14, TNF, and TP53 to exert the therapeutic effect on periodontitis.

Our study has some limitations. We only verified the interaction between the core targets and magnolol through molecular docking however, the effect of protein-molecule interactions counts on the binding site of the proteins. Besides, the crosstalk between the key targets and pathways revealed by network pharmacology analysis should be further elucidated. Additional *in vitro* and *in vivo* experiments are required to verify the pharmacological effect of magnolol against periodontitis and its mechanisms, which will be conducted in our subsequent investigations.

In conclusion, our findings demonstrated the multi-target and multi-pathway mechanisms of magnolol functions in the treatment of periodontitis. AKT1, MAPK8, MAPK14, TNF, and TP53 were revealed as core targets of magnolol against periodontitis. The underlying mechanisms of magnolol against periodontitis might be associated with the regulation of AKT, MAPK, and TNF signaling pathways. Our study provides the theoretical basis for the further investigations on the mechanisms of magnolol in treating periodontitis.

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Author Contributions: The authors confirm their contribution to the paper as follows: study conception and design: DC and CL; data collection: DC; analysis and interpretation of results: DC and CL; draft manuscript preparation: DC. All authors reviewed the results and approved the final version of the manuscript.

Availability of Data and Materials: The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Ethics Approval: Not applicable.

Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

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