Exploring the Mechanism of *Zanthoxylum Bungeanum Maxim* in the Treatment of Osteoarthritis Based on Network Pharmacology, Molecular Docking and Molecular Dynamics Simulation

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Abstract: Zanthoxylum bungeanum Maxim (ZBM) is a traditional Chinese seasoning, and is often used as a Chinese herbal medicine in treating osteoarthritis (OA) and other diseases, but its active ingredients and pharmacological mechanisms are still unclear. We used network pharmacology, molecular docking, and molecular dynamics simulation (MDS) to explore the potential mechanisms of ZBM for the treatment of OA. A total of three potential active ingredients of ZBM, including quercetin, diosmetin, and beta-sitosterol, were screened from the TCMSP. The active ingredient targets were intersected with the OA-related targets obtained from GeneCards, OMIM, PharmGkb, and TTD databases, 43 common ZBM-OA targets were obtained. Hub genes (HIF1A, EGFR, CASP3, IL6, FOS and VEGFA) were obtained in the key target PPI network. GO and KEGG enrichment analysis showed that ZBM is involved in oxidative stress, inflammation, and apoptosis of chondrocytes mainly through regulating AGE-RAGE signaling pathway in diabetic complications, and thus plays a role in the treatment of OA; Molecular docking results showed that the key active compounds in ZBM could bind tightly to key target proteins; the MDS results showed that the active ingredient diosmetin could bind stably to EGFR. This study reveals the potential active ingredients and molecular mechanisms of ZBM for the treatment of OA.

Keywords: Zanthoxylum bungeanum Maxim, osteoarthritis, network pharmacology, molecular docking, molecular dynamics simulation

1. Introduction

Osteoarthritis (OA) is a progressive degenerative disease that usually presents clinically with chronic joint pain and limited mobility [1]. The disease is most prevalent in the elderly population, and the incidence of OA is increasing each year as the world population ages. Its disability rate is among the top 354 most disabling diseases, which seriously affect the daily life of the elderly [2, 3]. Therefore, it is of great practical significance to strengthen the research related to OA to improve people's quality of life. Modern medicine is mainly based on anti-inflammatory and analgesic drugs, intra-articular drug injections, joint repair or replacement, etc., but these treatments are usually slow and expensive, while herbal treatment is effective in the prevention and treatment of osteoarthritis as a multi-component, fewer side effects and moderately priced treatment [4].

According to Chinese medicine, OA belongs to the category of " arthromyodynia", and its pathogenesis is closely related to liver and kidney deficiency, blood stagnation, and paralysis of the meridians, so the disease should be treated by blood-activating and stasis-dissolving, dispersing cold and clearing the channels, tonifying the liver and kidney, and dispelling wind and dampness [5, 6]. *Zanthoxylum bungeanum Maxim* (ZBM) is a plant of the *Rutaceae* family and is a traditional Chinese herbal medicine and flavoring agent, known as "one of the eight great tastes" [7]. The main chemical components are volatile oil, alkaloids, flavonoids, coumarins, lignans, etc., which have pharmacological activities such as anti-inflammatory, antioxidant, analgesic, and antibacterial [8-10]. ZBM has long been used in China for the treatment of "arthromyodynia" and other related diseases, and in Sheng Nong's herbal classic of Materia Medica (Shennong Ben Cao Jing, pinyin in Chinese), it is recorded that ZBM can warm the interior dispel cold, which is used to treat "arthromyodynia" and other related diseases. In

the Compendium of Materia Medica, it is written that ZBM "disperses cold and removes dampness, warms the spleen and stomach, and nourishes the kidney". ZBM was recorded in the Theory of Medicinal Property as " treatment of arthralgia syndrome, lumbar and foot disorders" [7]. Through these ancient texts of Chinese medicine, we can find that the concept of ZBM in treating "arthromyodynia" is consistent with the treatment concept of Chinese medicine of blood-activiating and stasis-dissolving, dispersing cold and clearing the channels, tonifying the liver and kidney, and dispelling wind and dampness. ZBM also plays an important role in the external treatment of osteoarthritis in clinical formulas as a high-frequency component of the formula [11]. However, due to the complex composition of ZBM, through which active ingredients and molecular regulatory mechanisms exert its effect in the treatment of OA, we are still unclear, and in-depth studies are needed to promote the comprehensive utilization of ZBM.

Chinese medicine has the properties of multi-component and multi-target, and it is difficult to fully explain the mechanism of drug action using traditional pharmacological methods. Network pharmacology is a combination of genomics, topology, systems biology, and other multidisciplinary and multiple techniques, which integrate the characteristics of integrity, dynamics and analysis in methodology, and is similar to the synergistic action of traditional Chinese medicine through principal, assistant, complement and guide [12, 13]. Therefore, it has been widely used in the study of the pharmacological mechanism of Chinese traditional compound medicine [14-16]. Molecular docking technology, which uses computer-assisted drug design, is a well-established method to assist in small molecule drug development [17-19]. However, obtaining accurate docking scores is a great challenge due to the complexity of the conformation of candidate small molecules and target proteins [20, 21]. In addition, the conformational transition of target proteins have a significant impact on binding kinetics, which cannot be adequately considered by molecular docking algorithms [22]. Molecular dynamics simulation (MDS) is a molecular simulation method that integrates physical, mathematical, and chemical techniques [23], which can fully consider the impact of conformational changes of proteins on binding kinetics.

Therefore, in this study, the material basis and mechanism of action of ZBM in the treatment of osteoarthritis were investigated using network pharmacology, molecular docking, and MDS to provide a theoretical basis for revealing its potential molecular mechanism in the treatment of OA.

2. Methods

2.1. Screening of active components and acquisition of corresponding targets

All active chemical components of ZBM were obtained based on TCMSP (http://lsp.nwu.edu.cn/tcmsp.php), with oral bioavailability (OB)≥30% and drug-like index (DL)≥0.18 as the screening criteria [24, 25], and the results of the search were integrated and duplicates were removed to obtain the active ingredients of ZBM. Finally, the corresponding targets of action were obtained on TCMSP.

2.2. Acquisition of OA related targets

Using "Osteoarthritis" as the keyword, we searched and screened the disease-related targets in Gene Cards (http://www.genecards/), OMIM (https://omim.org/), PharmGkb (https://www. pharmgkb.org/) and TTD (http://db.idrblab.net/ttd/) databases, integrated the search results, and removed duplicate items to obtain OA disease-related targets.

2.3. Acquisition of ZBM-OA common targets

Intersection of OA-related targets and ZBM targets using R language and drawing the Wayne diagram to finally obtain the ZBM-OA common targets.

2.4. Construction of active compound-target network

The active components of ZBM obtained above were mapped to the ZBM-OA common target using Perl 5.26.3, and then computed using Cytoscape 3.6.1 to obtain the active compound-target regulatory network.

2.5. Construction of protein-protein interaction (PPI) network and screening of key targets

Network analysis of ZBM-OA common targets was performed using the STRING (https://STRING-db.org/) database, with species limited to Homo sapiens and the minimum interaction threshold set to 0.9, and protein interaction relationships were obtained after removing free points. The data of protein interaction relationships were then imported into Cytoscape software, and network node analysis was performed using CytoNCA plug-in, with median values of Betweenness, Closeness, Degree, Eigenvector, LAC, and Network for 2 filters, and finally, core targets were obtained.

2.6. GO and KEGG pathway enrichment analysis of intersection targets

GO enrichment analysis and KEGG enrichment analysis of ZBM-OA common targets were performed in R language based on "ClusterProfiler" and "Pathview" data packages. Both GO and KEGG enrichment analyses were statistically significant at $P<0.05$. The entries obtained from GO enrichment analysis and KEGG enrichment analysis were also visualized with the help of R language.

2.7. Construction of KEGG network

The KEGG data obtained above were mapped using Perl software and computed using Cytoscape to obtain the KEGG relational network.

2.8. Molecular docking

To verify the reliability of the network pharmacological predictions, molecular docking of key compounds to core targets was performed. The chemical structures of key active ingredients were obtained using PubChem (https://pubchem.ncbi.nlm.nih.gov/) database. The obtained chemical structures were energy minimized using Chem3D 14.0.0 software and saved as mol2 format for backup. The 3D structures of the core target proteins were obtained using the PDB (http://www.rcsb.org/) database. Use Auto Dock 1.5.6 software to convert the PDB format of the compound and core target protein to pdbqt format, determine the docking parameters, and save it for backup. Finally, the molecular docking was completed using AutoDock vina software to obtain the binding energy. The visualization of docking results was completed using Pymol software.

2.9. MDS

The Gromacs 5.1.2 software, Amber99sb-ildn force field and SPC water model were used to perform MDS, periodic boundary conditions were set, and the system charge was neutralized using sodium or chloride ions depending on the docking results. The simulation temperature was 300 K. The system was optimized for molecular mechanics in 50000 steps using the steepest descent method before the simulation. The optimized systems were then equilibrated by NVT and NPT with a time step of 2 fs and time duration of 100 ps, respectively, and the system positions were constrained during the equilibration process, and finally the MDS was performed at 300 K for 20 ns. After the simulation, evaluate the root mean square deviation (RMSD) of the complex formed by protein and small molecules, protein radius of gyration, and solvent accessible surface area (SASA). Visualization was performed by qtgrace software.

3. Results

3.1. Screening of active components and prediction of common targets

We obtained a total of 101 components of ZBM from TCMSP. Using OB≥30% and DL≥0.18 as the screening conditions, we obtained 5 active ingredients of ZBM and 84 corresponding targets from TCMSP; using "Osteoarthritis" as the keyword, 1707 OA related targets were retrieved from GeneCards, OMIM, PharmGkb, and TTD databases, as shown in Figure 1. The OA related targets were intersected with ZBM targets, and 43 common targets were obtained (Figure 2), which are the potential targets of ZBM for the treatment of osteoarthritis.
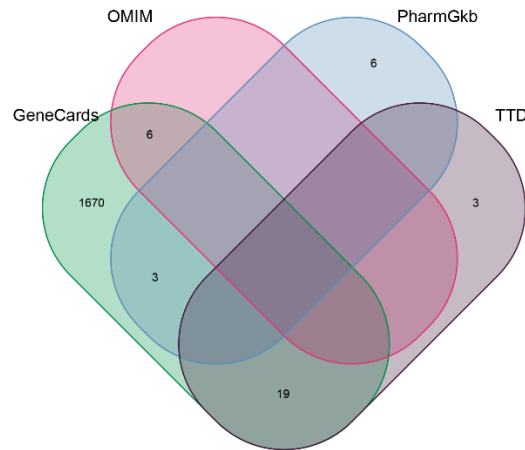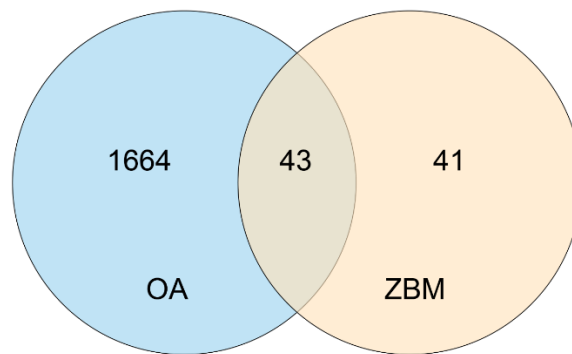
Figure 1: OA related targets.

Figure 2: Venn diagram of ZBM-OA common targets.

3.2. Construction of active compound-target network

Using Cytoscape 3.6.1 software to construct the active compound-target network, the results showed that ZBM acts on 43 targets mainly through 3 active ingredients (Figure 3), which are 43 targets that may affect the occurrence of OA. These three active ingredients are quercetin, beta-sitosterol, and diosmetin, which have important roles in the treatment of osteoarthritis, and the basic information is shown in Table 1. Quercetin has the highest number of connections to the targets, reaching 43.
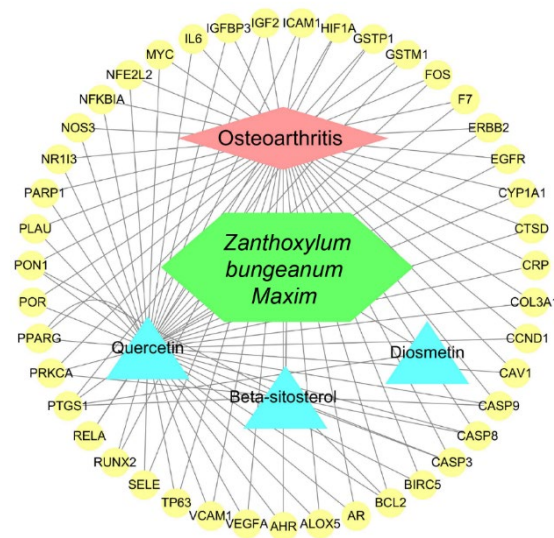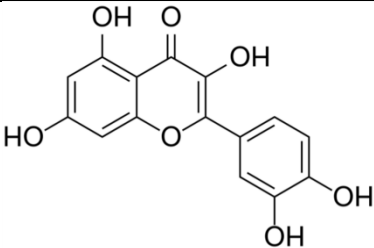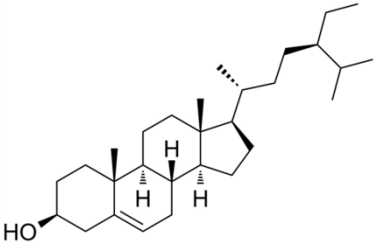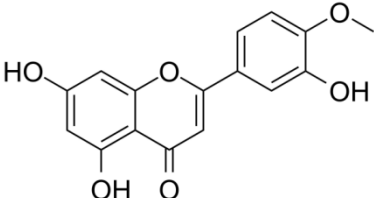

Figure 3: Active compound-target network.

Table 1: Basic information of key compounds.

Chemical composition	Degree value	Composition
Quercetin	43	
Beta-sitosterol	7	
Diosmetin	1	

3.3. Construction of PPI network and screening of key targets

Network analysis of ZBM-OA common targets was performed using the STRING (https://STRING-db.org/) database, species limited to Homo sapiens, and the minimum interaction threshold was set to 0.9, after removing free points. A PPI network map was initially obtained, constructed by 43 nodes representing proteins and 702 edges representing the interactions between the proteins. The data of protein interactions were then imported into Cytoscape 3.6.1 software, network node analysis was performed by CytoNCA plug-in, and the median values of Betweenness, Closeness, Degree, Eigenvector, LAC, and Network were used as screening conditions to obtain the core targets. After the 1st screening, a PPI network graph with 17 nodes and 248 edges is obtained. After the 2nd screening, a PPI network map with 6 nodes and 96 edges was obtained, and the process is shown in Figure 4. These 6 protein targets are HIF1A, EGFR, CASP3, IL6, FOS, and VEGFA, indicating that these proteins play a key role in the whole network, and these may be the key targets for ZBM treatment of OA. This interconnection of nodes also suggests that the active ingredients of ZBM can intervene in the disease process through protein-protein interactions between the targets in addition to direct action on the targets.
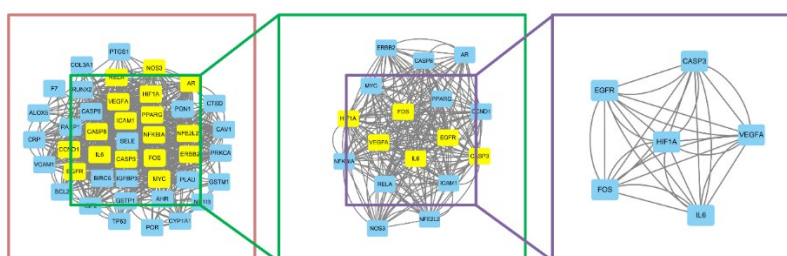

Figure 4: PPI network topology analysis.

3.4. GO enrichment analysis

We performed GO enrichment analysis from three aspects: Molecular function (MF), Biological process (BP), and cellular component (CC). A total of 1613 GO ontology entries were obtained with a P-value < 0.05, including 1461 BP-related entries, 24 CC-related entries, and 128 MF-related entries. The top 10 entries of BP, CC, and MF were ranked by p-value and plotted in bubble diagrams (Figure 5). The entries related to CC are mainly related to membrane raft, membrane microdomain, membrane region,

transcription factor complex, etc. MF The related entries are mainly related to DNA-binding transcription activator activity RNA polymerase I-specific, integrin binding, ubiquitin-protein ligase binding, activating transcription factor binding, etc. These biological effects are closely linked to oxidative stress and inflammatory responses, suggesting that ZBM has the potential to exert anti-osteoarthritic effects by regulating these biological processes.
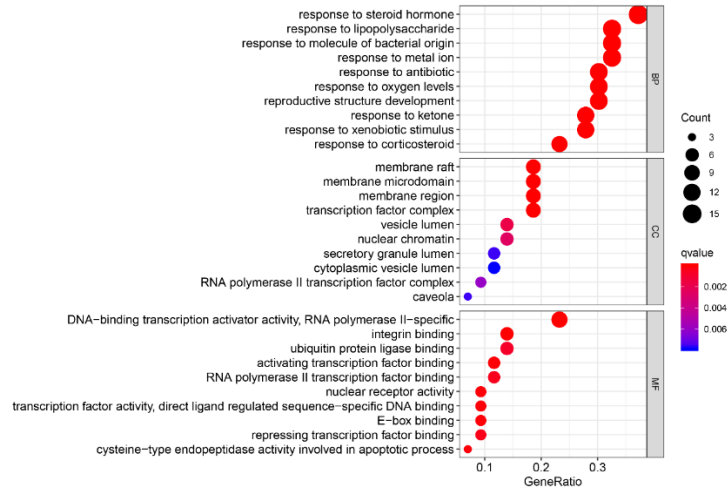

Figure 5: GO enrichment analysis histogram.

3.5. KEGG enrichment analysis and construction of relation network

Table 2: Results of KEGG enrichment analysis.

ID	Term	Gene	Count	P value
hsa04933	AGE-RAGE signaling pathway in diabetic complications	BCL2/CASP3/PRKCA/RELA/VEGFA/CCND1/IL6/ICAM1/SELE/VCAM1/NOS3/COL3A1	12	3.98E-14
hsa04210	Apoptosis	BCL2/CASP9/CASP3/CASP8/RELA/FOS/NFKBIA/BIRC5/PARP1/CTSD	10	9.95E-10
hsa01524	Platinum drug resistance	BCL2/CASP9/CASP3/CASP8/ERBB2/BIRC5/GSTP1/GSTM1	8	2.40E-09
hsa04066	HIF-1 signaling pathway	BCL2/PRKCA/RELA/EGFR/VEGFA/IL6/HIF1A/ERBB2/NOS3	9	2.75E-09
hsa04668	TNF signaling pathway	CASP3/CASP8/RELA/FOS/IL6/NFKBIA/ICAM1/SELE/VCAM1	9	3.51E-09
hsa04151	PI3K-Akt signaling pathway	BCL2/CASP9/PRKCA/RELA/EGFR/VEGFA/CCND1/IL6/ERBB2/MYC/NOS3/IGF2	12	1.09E-07
hsa04926	Relaxin signaling pathway	PRKCA/RELA/EGFR/VEGFA/FOS/NFKBIA/NOS3/COL3A1	8	2.22E-07
hsa05134	Legionellosis	CASP9/CASP3/CASP8/RELA/IL6/NFKBIA	6	3.72E-07
hsa04215	Apoptosis - multiple species	BCL2/CASP9/CASP3/CASP8/BIRC5	5	5.04E-07
hsa04064	NF-kappa B signaling pathway	BCL2/RELA/PLAU/NFKBIA/ICAM1/VCAM1/PARP1	7	7.98E-07
hsa05143	African trypanosomiasis	PRKCA/IL6/ICAM1/SELE/VCAM1	5	1.07E-06
hsa05145	Toxoplasmosis	BCL2/CASP9/CASP3/CASP8/RELA/NFKBIA/ALOX5	7	1.32E-06
hsa04115	p53 signaling pathway	BCL2/CASP9/CASP3/CASP8/CCND1/IGFBP3	6	1.64E-06
hsa01521	EGFR tyrosine kinase inhibitor resistance	BCL2/PRKCA/EGFR/VEGFA/IL6/ERBB2	6	2.62E-06
hsa04657	IL-17 signaling pathway	CASP3/CASP8/RELA/FOS/IL6/NFKBIA	6	7.26E-06
hsa04010	MAPK signaling pathway	CASP3/PRKCA/RELA/EGFR/VEGFA/FOS/ERBB2/MYC/IGF2	9	1.32E-05
hsa04659	Th17 cell differentiation	RELA/FOS/IL6/NFKBIA/HIF1A/AHR	6	1.62E-05
hsa05132	Salmonella infection	BCL2/CASP3/CASP8/RELA/FOS/IL6/NFKBIA/MYC	8	3.07E-05
hsa05130	Pathogenic Escherichia coli infection	CASP9/CASP3/CASP8/RELA/FOS/IL6/NFKBIA	7	5.45E-05

Using a P-value < 0.05 as the screening condition, 71 signaling pathways were obtained from KEGG enrichment analysis, and the top 30 entries were selected for mapping according to the P-value. As shown in Figure 6, the signaling pathways of the core targets mainly involved: AGE-RAGE signaling pathway in diabetic complications, apoptosis, platinum drug resistance, HIF-1 signaling pathway, TNF signaling pathway, PI3K-Akt signaling pathway, relaxin signaling pathway, legionellosis, apoptosis - multiple species, NF-kappa B signaling, etc. This suggests that ZBM can treat OA by regulating multiple complex biological processes. Among them, the AGE-RAGE signaling pathway in diabetic complications is the most significant, and the number of genes acting on this pathway is also high, which indicates that AGE-RAGE signaling pathway in diabetic complications plays an important role in the treatment of OA by ZBM. The target genes involved in the KEGG key entry and their relationship network are shown in Table 2 and Figure 7, from which it can be found that these targets of signaling pathway action mainly contain BCL2/CASP9/CASP3/CASP8/EGFR/IL6/VEGFA/RELA/FOS genes, which are closely related to the occurrence of inflammation.
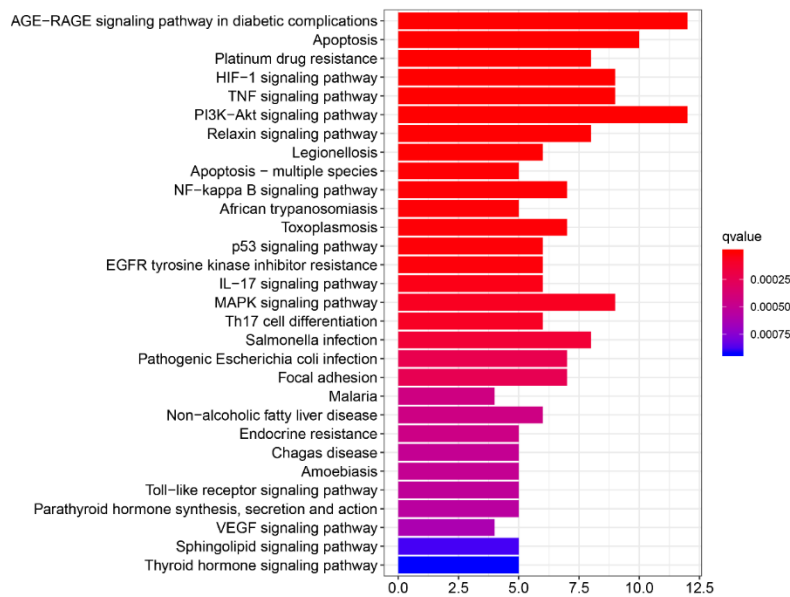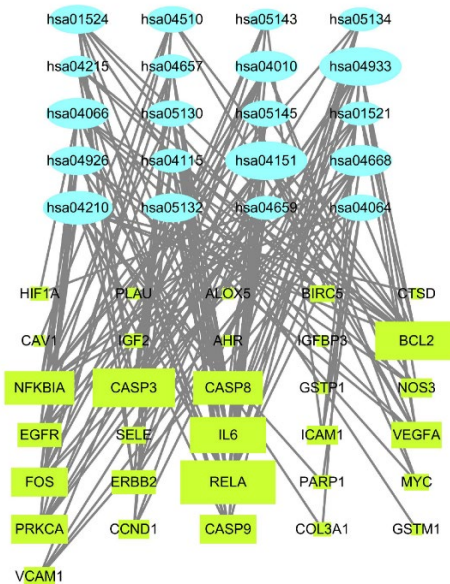

Figure 6: Molecular function enrichment analysis diagram.

Green square nodes represent genes, blue oval circles represent KEGG signaling pathway, if there is a connection between KEGG signaling pathway and genes, it means it belongs to this pathway, the larger the graph, the more connected nodes.

Figure 7: KEGG interaction network diagram.

3.6. Molecular Docking Results

It is generally accepted that the lower the binding energy of a ligand to an acceptor, the higher the likelihood of interaction. We verified the molecular docking of the predicted key compounds and key targets using binding energy ≤ -5.0 kJ/mol as the criterion. The results showed that the binding energies of the key active ingredients of ZBM docked with the key targets were all less than -5.0 kJ/mol, which had good binding activity and proved that the predicted results of this network pharmacological study were reliable (Table 3). Diosmetin and EGFR with the lowest binding energy were selected for molecular docking demonstration (Figure 8). As shown in Figure 8-B, diosmetin formed hydrogen bonding interactions with protein amino acid residues SER719, GLY796, and GLU762; Figure 8-C shows that the hydrophobic pocket shape of small molecule binding in the complex structure is grooved, which helps small molecule binding.
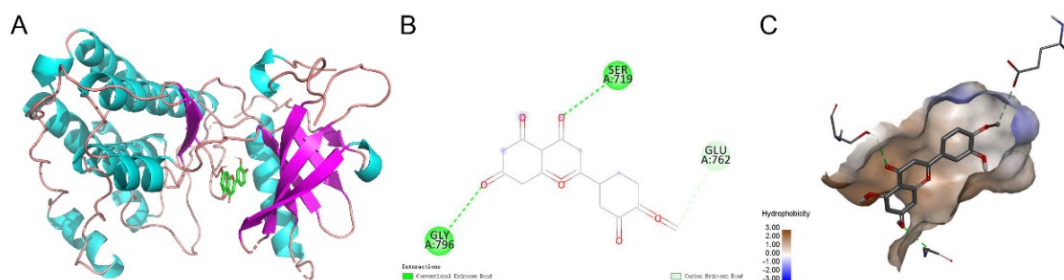

Figure 8: Molecular docking diagram of diosmetin and EGFR.

Table 3: Binding ability of key active ingredients of ZBM docked to key targets.

Chemical composition	Chemical formula	Relative molecular mass	Target spot	Binding energy (kcal/mol)
Quercetin	$C_{15}H_{10}O_7$	302.23	HIF1A	-8.0
			EGFR	-7.8
			CASP3	-7.4
			IL6	-6.6
			FOS	-7.6
			VEGFA	-7.0
Beta-sitosterol	$C_{29}H_{50}O$	414.7	HIF1A	-7.3
			EGFR	-8.0
			CASP3	-7.2
			IL6	-6.9
			FOS	-6.4
			VEGFA	-6.8
Diosmetin	$C_{16}H_{12}O_6$	300.26	HIF1A	-7.3
			EGFR	-8.1
			CASP3	-7.2
			IL6	-6.6
			FOS	-7.2
			VEGFA	-7.0

3.7. MDS

The RMSD fluctuation map of the compound reflects the conformational stability of the protein-small molecule complex. Similarly, we took the lowest binding energy and most stable complex of diosmetin and EGFR for 20 ns MDS, and obtained the RMSD of EGFR protein backbone and ligand diosmetin. As shown in Figure 9, the RMSD of ligand-ligand stabilize after 2 ns, and the fluctuation of RMSD in the first 2 ns is because the small molecule and protein seek lower energy conformation in the dynamic simulation process. In the later 3-20ns, the fluctuation is stable, indicating that the binding between protein and small molecules is relatively stable.
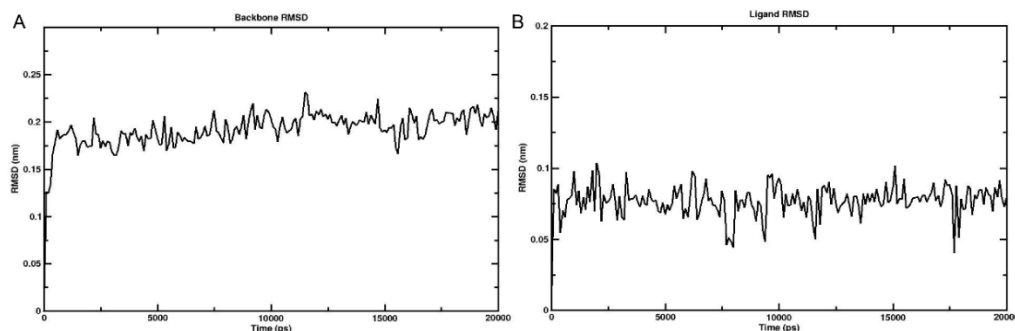
Figure 9: A: the RMSD of backbone-backbone; B: the RMSD of ligand-ligand.

Gyration radius represents the compactness of the protein structure of the complex system. As shown in Figure 10-A, the protein radius of gyration fluctuated less during the MDS of 20 ns, suggesting that the protein has a certain stability of structure after binding to small molecules. SASA indicates the stability of protein conformation. The SASA results showed (Figure 10-B) that the SASA of the complex system gradually becomes smaller and the protein becomes compact as a whole, suggesting that the whole system gradually tends to be stable during the simulation.
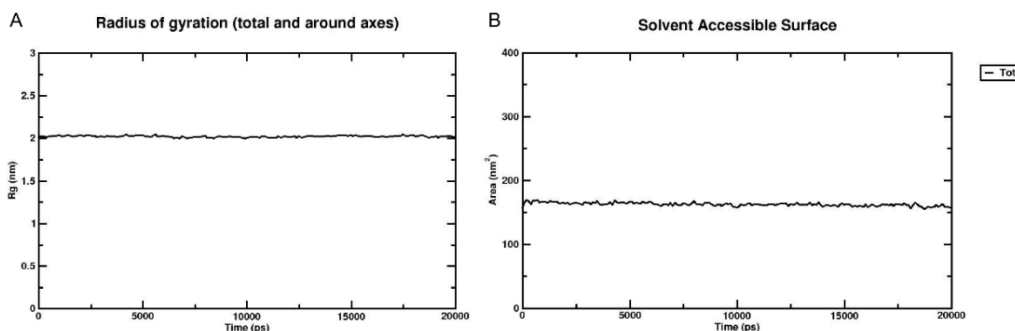

Figure 10: A: radius of gyration; B: solvent accessible surface.

4. Discussion

In traditional Chinese medical science, OA belongs to the category of "arthromyodynia", and ZBM has been used for the treatment of "arthromyodynia" and other related diseases in China for a long time. However, due to the complex active ingredients of ZBM, its pharmacological mechanism for the treatment of OA has not yet been clarified. In this study, we investigated the material basis and mechanism of action of ZBM in the treatment of OA using network pharmacology, molecular docking, and MDS.

The active compound-target regulatory network diagram shows that most of the active ingredients are linked to multiple targets, and different active ingredients can also act on the same target, which indicates the synergistic and multi-target properties of the active ingredients of ZBM. ZBM acts on 43 targets mainly through three active ingredients, which are quercetin, beta-sitosterol and diosmetin, which are important for the treatment of OA. Quercetin and diosmetin belong to flavonoids, which are widely found in vegetables, fruits and herbal medicines. They have various activities such as anti-osteoarthritis, anti-oxidative stress and anti-inflammatory [26-29]. Shuaijie Lv et al. found that quercetin mediates TSC2-RHEB-mTOR pathway to regulate chondrocytes autophagy in knee osteoarthritis and promote chondrocyte synthesis [30]. Mitra Heydari Nasrabadi et al. found the quercetin was useful in the reduction of symptoms of OA and raised the improvement of damaged cartilage [31]. Quercetin also inhibited inflammation and apoptosis by inhibiting IRAK1/NLRP3 signaling, which in turn reduced the progression of osteoarthritis in rats [32]. Hence, it can be a beneficial medical supplement in OA treatment. Diosmetin alleviates IL-1B-induced chondrocyte apoptosis and immune response in neonatal rat osteoarthritis via NF-KB signaling pathway [33]. It also inhibits osteoclast differentiation and reduces subchondral bone loss, which in turn has a therapeutic effect on early osteoarthritis [34]. The above studies suggest that diosmetin could be used as a potential drug for the treatment of OA. These available studies further confirmed the reliability of network pharmacological predictions. Beta-sitosterol is a common phytosterol, which is widely found in plants. Recent studies have found that beta-sitosterol

effectively inhibits the generation of reactive oxygen species (ROS) induced by LPS, partially inhibits the activation of NF-κB [35]. Additionally, beta-sitosterol also regulates bone metabolism balance [36]. But collectively, there are fewer reports on beta-sitosterol anti-OA.

After 2 screens, we obtained a PPI network map with 6 nodes and 96 edges. These 6 protein targets are HIF1A, EGFR, CASP3, IL6, FOS and VEGFA, which play a key role in the whole network and may be the key targets of ZBM for the treatment of OA. The development of OA is mainly caused by chondrocyte apoptosis, inflammatory factor production and associated hydrolytic protein destruction of chondrocytes, and the above 6 proteins are closely associated with these etiologies. HIF1A is well known as one of the major regulators of the hypoxic response and controls hypoxic expression of erythropoietin, as well as the expression of genes with metabolic functions, which is essential in inflammation [37-39]. Sunli Hu et al. showed that HIF1A mediated mitophagy could alleviate OA [40]. Xiaochen Li et al. showed that casticin alleviated MIA-induced knee osteoarthritis by inhibiting of HIF1A/NLRP3 inflammasome activation [41]. Fei Yang et al. showed that miR-411 promotes chondrocyte autophagy by targeting HIF-1alpha, and then alleviate the progression of osteoarthritis [42]. Meng Zhou et al. Showed that the resveratrol may stimulate the HIF1A to promote the matrix accumulation and decrease degradation of human OA chondrocytes [43], indicating that HIF1A may serve as a promising strategy for OA treatment. EGFR is a member of the epidermal growth factor receptor family, and this signaling pathway plays an important regulatory role in biological processes such as cell differentiation and proliferation, and studies have pointed to a close association between EGFR signaling and knee osteoarthritis pathology [44]. In the articular cartilage of OA patients and the articular cartilage of mouse OA models, it is found that there is a certain negative correlation between the activity of the EGFR pathway and the progression of OA [45, 46]. Studies have also shown that, SIRT1 represses the ubiquitination of EGFR by down-regulating PTEN, inhibits extracellular matrix degradation and activates chondrocyte autophagy, thereby performing an OA-alleviating role [47]. EGFR signaling is also necessary to maintain adult cartilage homeostasis and attenuate the progression of osteoarthritis [48]. In summary, EGFR may perform a crucial function in the regulation of OA, providing potential for OA treatment. CASP3 was closely related to chondrocyte apoptosis [49]. Previous studies have shown that CASP3 expression is significantly increased in the chondrocytes of OA patients [50, 51]. It has also been suggested that CASP3 can be used to determine the prognosis and condition of knee osteoarthritis [52]. Therefore, inhibiting the release of inflammatory factors by downregulating CASP3 expression is important for the treatment of osteoarthritis. Interleukin-6 (IL-6) is known as a mediator of inflammation, immune response, and hematopoiesis [40]. IL-6 is detected in synovial fluid and expressed in osteoarthritic cartilage and has an essential role in the pathogenesis of OA [53-55]. Meng Zhou et al. showed that IL-6 was significantly increased in the joint tissues of OA model mice and human OA chondrocytes, suggesting that IL-6 has the function to promote the OA process [43]. Hyewon Park et al. showed that p16INK4a-siRNA nanoparticles reduced IL-6 levels, inhibited inflammation in fibroblast-like synoviocytes, and attenuated cartilage degeneration in osteoarthritis [56]. Fos is closely related to chondrocyte proliferation, differentiation, and inflammation [57]. Motomura et al. showed that specific inhibition of c-Fos/AP-1 and the resulting inhibition of the transactivation of a broad spectrum of downstream MMPs, along with inflammatory cytokines, effectively prevented cartilage destruction and osteophyte formation [58]. Haseeb et al. showed that harpagoside exert a significant chondroprotective effect by inhibiting the IL-1β-induced expression and production of IL-6 in human OA chondrocytes [59]. Vascular endothelial growth factor A (VEGFA) is an angiogenic factor present in adipose tissue. It has been shown that VEGFA is also a pro-inflammatory cytokine [60], and its overexpression may be associated with the development of osteoarthritis [61]. Yuan Liu et al. showed that hUSC-140-Exos increased the secretion of ECM by targeting VEGFA, including collagen II and aggrecan, enhanced cartilage regeneration and subchondral bone remodeling [61]. Yun Bai et al. showed that long non-coding RNA HCAR promotes endochondral bone repair by upregulating VEGF and MMP13 in hypertrophic chondrocyte through sponging miR-15b-5p [62]. Based on the above literature analysis, the reliability of our network pharmacological predictions is further confirmed, these core targets can play an important role in the treatment of OA.

From the results of GO enrichment analysis, it is obvious that the role of ZBM in OA treatment is related to a variety of biological processes. GO enrichment analysis yielded a total of 1613 GO entries, including 1461 BP-related entries, 24 CC-related entries, and 128 MF-related entries. These biological effects are closely related to oxidative stress and inflammatory response, suggesting that ZBM could potentially exert anti-osteoarthritic effects by regulating these biological processes. The results of KEGG enrichment analysis showed that ZBM could treat OA by regulating several signaling pathways, among which the AGE-RAGE signaling pathway in diabetic complications was the most significant and the number of genes acting on this pathway was also high. This suggests that the AGE-RAGE signaling

pathway in diabetic complications plays an important role in the treatment of OA by ZBM. Through literature research, the AGE-RAGE signaling pathway in diabetic complications has been shown to be strongly associated with the occurrence of OA. The AGEs/AGE-RAGE signaling pathway was first proposed by YAMAMOTO et al. in 2000 [63]. The interaction of AGEs with RAGE can cause intracellular oxidative stress and activate the NF-κB signaling pathway by activating the p21ras and MAP kinase pathways [64]. NF-κB increases the expression of various inflammatory factors such as TNF α, VEGF, IL -1α, IL-6, and the inflammatory response of chondrocytes [65-67]. Therefore, activation of the AGEs/AGE-RAGE signaling pathway will aggravate OA.

To further investigate the reliability of the network pharmacology prediction results, molecular docking and MDS analysis of the key active compounds with key target proteins were performed. The results showed that the binding energies of the key active components of ZBM were less than -5.0 kJ/mol, with good binding activity, implying that ZBM could exert its therapeutic effects through these target proteins. We selected diosmetin and EGFR with the lowest binding energy for MDS. The RMSD fluctuation plots of the compound can reflect the conformational stability of the protein-small molecule complexes, with the radius of gyration representing the compactness of protein structure, and SASA indicating the stability of the protein conformation. The results showed that the RMSD of the EGFR protein backbone and ligand diosmetin tended to flatten after 2 ns, indicating that the binding of protein to small molecules was stable. The protein radius of gyration fluctuated less, suggesting that the protein has a certain structural stability after binding to small molecules. The SASA of the complex system gradually became smaller, indicating that the protein as a whole became compact, suggesting that the whole system gradually tended to be stable during the simulation.

5. Conclusion

In summary, this study explored the potential mechanisms of ZBM in the treatment of OA using network pharmacology, molecular docking, and MDS. The results suggest that the active components such as quercetin, diosmetin, and beta-sitosterol in ZBM may participate in the oxidative stress, inflammatory response and apoptosis of chondrocytes by affecting the expression of related targets such as HIF1A, EGFR, CASP3, IL6, FOS, and VEGFA, and regulating the signaling pathway such as AGE-RAGE signaling pathway in diabetic complications, so as to play a role in the treatment of OA. The molecular docking and MDS results also showed that the key active compounds could bind stably to the key target proteins, demonstrating that the predicted results of this network pharmacology study are reliable. This study provides a theoretical basis for uncovering the molecular mechanism of action of ZBM in the treatment of OA.

Conflict of interest

The authors have declared no conflict of interest.

Acknowledgments

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