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Network pharmacological and molecular docking verification of the mechanism of Osteoking in preventing deep vein thrombosis of lower limb

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Abstract. – OBJECTIVE: The aim of this study was to predict the mechanism of Osteoking in preventing deep vein thrombosis (DVT) of the lower limb by network pharmacology and molecular docking.

MATERIALS AND METHODS: The relevant active components and targets of Osteoking were collected through the TCMSP database, and the relevant disease targets of DVT were collected through the GeneCards, OMIM, and DisGeNET databases. The intersecting gene targets of Osteoking and DVT were obtained using Venny 2.1.0 software. PPI network construction and core target selection using Cytoscape 3.9.0 software. The Metascape database was used for GO and KEGG enrichment analysis of relevant targets. Finally, the molecular docking of the main active components and key targets was carried out.

RESULTS: There are 361 potential targets and 71 core targets of Osteoking in preventing deep vein thrombosis of the lower limb. Signal pathways are involved in various diseases such as cancer, diabetic complications, atherosclerosis, and more. Some of the most common pathways include AGE-RAGE signaling pathway and Calcium signaling pathway. Molecular docking results showed that the main active components of Osteoking had relatively stable binding activities with the key targets.

CONCLUSIONS: Osteoking can play a role through multiple targets and multiple signal pathways to prevent the formation of deep venous thrombosis of the lower limb after fracture.

Key Words:
Osteoking, deep venous thrombosis, Network pharmacology, Molecular docking.

Introduction

Deep vein thrombosis (DVT) is a common complication in orthopedics, which is more common in patients with fracture and lower limb paralysis and can lead to lower limb swelling, pain, and other complications, even pulmonary embolism in severe cases, which is life-threatening[1]. Studies[2,3] have shown that the incidence of DVT in fracture patients is as high as 50-60%, while the mortality rate is 6.25-4.17%. Besides fracture, gender, age, obesity, hyperglycemia, hypertension, and limb paralysis are all high-risk factors for DVT[4,5]. Patients with fractures have venous stasis caused by immobilization of both lower limbs, while the fracture itself and operation can lead to vascular endothelial injury and hypercoagulability, increasing the risk of deep venous thrombosis of lower limbs[6].

Osteoking, also known as henggu bone healing agent, is produced in Yunnan Province, China, and it is mainly made of traditional Chinese medicines such as Panax notoginseng, Radix Astragali, and Ginseng[7], with a long history[8]. Studies[9-11] have shown that Osteoking can delay the progress of osteoporosis and osteoarthritis through TGF-β and other signal pathways, and it has a remarkable curative effect. With the deepening of the research on Osteoking, its function has been continuously explored. In 2005, Zhao et al[12] analyzed 62 patients with intertrochanteric fracture through a randomized controlled study and found that Osteoking had a satisfactory effect in preventing postoperative DVT. However, it is a pity that no other research has been found on the prevention of deep venous thrombosis of lower limbs with Osteoking, and its mechanism of action has not yet been clarified. Therefore, the purpose of this paper is to predict the mechanism of Osteoking in preventing deep vein thrombosis of lower limbs by network pharmacology and to verify the reliability of molecular docking method, so as to provide a reference for clinical and scientific research.

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Materials and Methods

Screening of Active Components and Targets of Osteoking

The main components of Osteoking (Tangerine Peel, Safflower, *Radix Astragali, Notoginseng, Ginseng,* Eucommia Bark, and Datura Flower) were searched using the TCMSP database (https://old.tcmsp-e.com/tcmsp.php). The active components of the drug were screened out with an oral bioavailability (OB) of ≥30% and a drug-like property (DL) of ≥0.18. The SMILES numbers of the active components were obtained from PubChem database (https://pubchem.ncbi.nlm.nih.gov), and the targets corresponding to the active components were retrieved from SwissTargetPrediction database (http://www.swisstargeting.ch).

Screening of Targets For DVT

The databases of Gene Cards (https://www.genecards.org), OMIM (https://www.omim.org), and Disgene (https://www.disgenet.org) were used to search for the related targets of DVT, with the search term "deep vein thrombosis of lower limb". Collate the obtained targets and delete the duplicates.

Screening of Potential Targets

The intersection targets of drug targets and disease targets were obtained by Venny 2.1.0 software (Madrid, Spain), and the Venn diagram was produced. The intersection targets were the potential targets.

Construction of Protein-Protein Interaction (PPI) Network

A protein-protein interaction (PPI) network model was constructed using the String database (https://cn.string-db.org). The species were set as "Homo sapiens," and the minimum interaction score was 0.9 for screening. The obtained data were imported into Cytoscape 3.9.0 software(California, USA), and the built-in software (Centiscape 2.2) was used to calculate the network topology parameters and screen out the core targets according to closeness (0.001-0.002), betweenness (391.479-10,239.242) and degree (43.468-213).

Construction of Drug-Active Ingredient-Target Network Diagram

The composition data were imported into Cytoscape 3.9.0 software to produce a drug-active ingredient-target network diagram, and topology analysis was performed to screen the main active ingredients of the drug according to the degree value.

KEGG and GO Enrichment Analysis

The Metascape database (https://metascape.org) was used for KEGG and GO enrichment analysis of potential targets. Among them, GO analysis included biological process (BP), cell components (CC), and molecular function (MF) analysis, with $p<0.01$. The data of the first 20 analyzed items were imported into the bioinformatics database (http://bioinformatics.com.cn) to produce a bubble chart.

Molecular Docking

The 2D structural diagrams of the first three components of the main active components (sorted according to degree value) were obtained using the TCMSP database and PubChem database and imported into Chem3D software (Massachusetts, USA) to minimize energy and prepare small-molecule ligands. The protein structures of the first three targets (sequenced according to degree value) of the core targets were obtained using the PDB database (https://www.rcsb.org), and protein receptors were prepared by removing water molecules and ligands using Pymol software (Pennsylvania, USA). Finally, the molecular docking of ligand and receptor was carried out, and the docking binding energy was <-4.0 kcal/mol, which indicated that the target protein and small molecular ligand could freely bind[13].

Statistical Analysis

Metascape database was used for GO and KEGG pathway enrichment analysis. The GO and KEGG enriched terms were collected for biological process (BP), cell component (CC), and molecular function (MF), at a cutoff of $p<0.01$.

Results

Screening of Potential Targets for Drugs and Diseases

A total of 1,230 targets and 114 active components of Osteoking were retrieved through the TCMSP database. A total of 1,954 disease targets for deep vein thrombosis of the lower limb were retrieved from the GeneCards, OMIM, and DisGeNET databases. A total of 361 potential targets were obtained by intersection with Venny diagram (Figure 1).

Construction of Protein-Protein Interaction (PPI) Network

The 361 potential targets were imported into the String database to build the model, and the relevant data were imported into Cytoscape 3.9.0 software

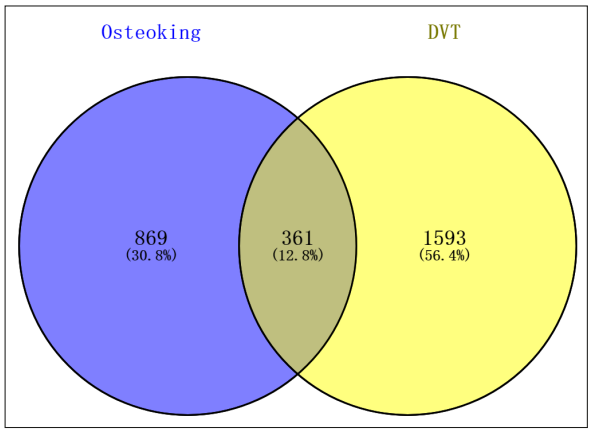

Figure 1. Venny diagram of drug-disease potential targets.

to calculate the network topology parameters. By setting the parameters of closeness concentration, between concentration and degree, we finally obtained 71 core targets, of which *AKT1, ALB, TNF, IL-6,* and *VEGFA* genes were the main targets (Figure 2).

Construction of Drug-Active Ingredient-Target Network Diagram

The composition data were imported into Cytoscape 3.9.0 software to produce the drug-active ingredient-target network diagram (Figure 3). Topological analysis was performed to screen the main active components of the drug according to the degree value (Table I).

KEGG and GO Enrichment Analysis

The Metascape database was used for enrichment analysis, and the first 20 items of data were taken to make bubble charts. The KEGG enrichment analysis showed that Osteoking mainly acts on DVT

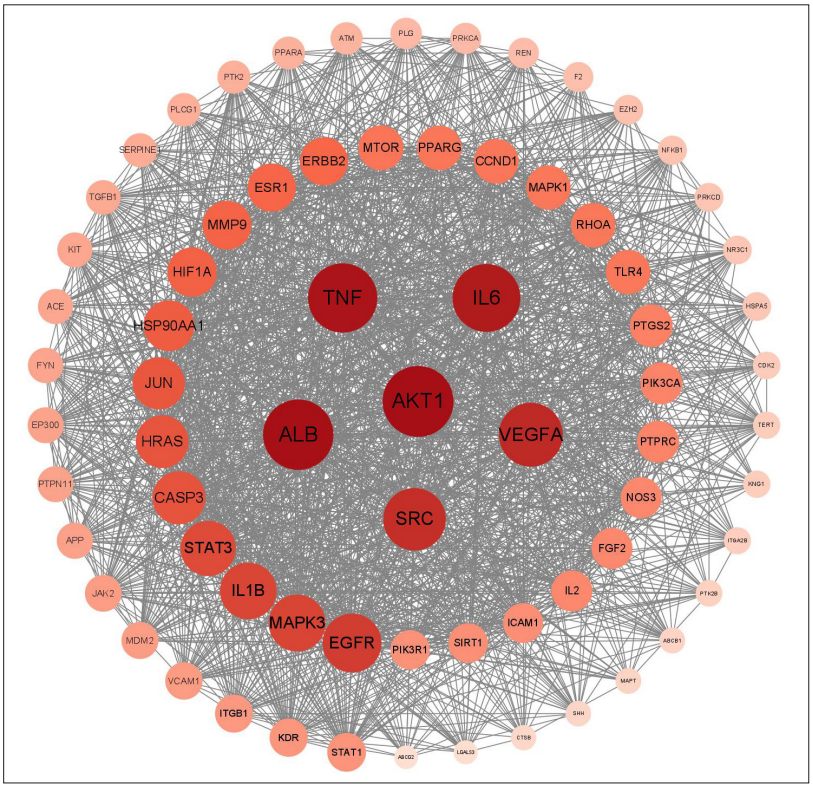

Figure 2. Drug-disease core target map.

Table I. Top 5 main active ingredients.

Molecule Name	Mol ID	Degree	BC	CC
Kaempferol	MOL000422	85	384	0.001686
Quercetin	MOL000098	75	335	0.001675
Ginsenoside rh2	MOL005344	40	683	0.002451
Diop	MOL002879	26	312	0.002392
Mairin	MOL000211	24	639	0.002336

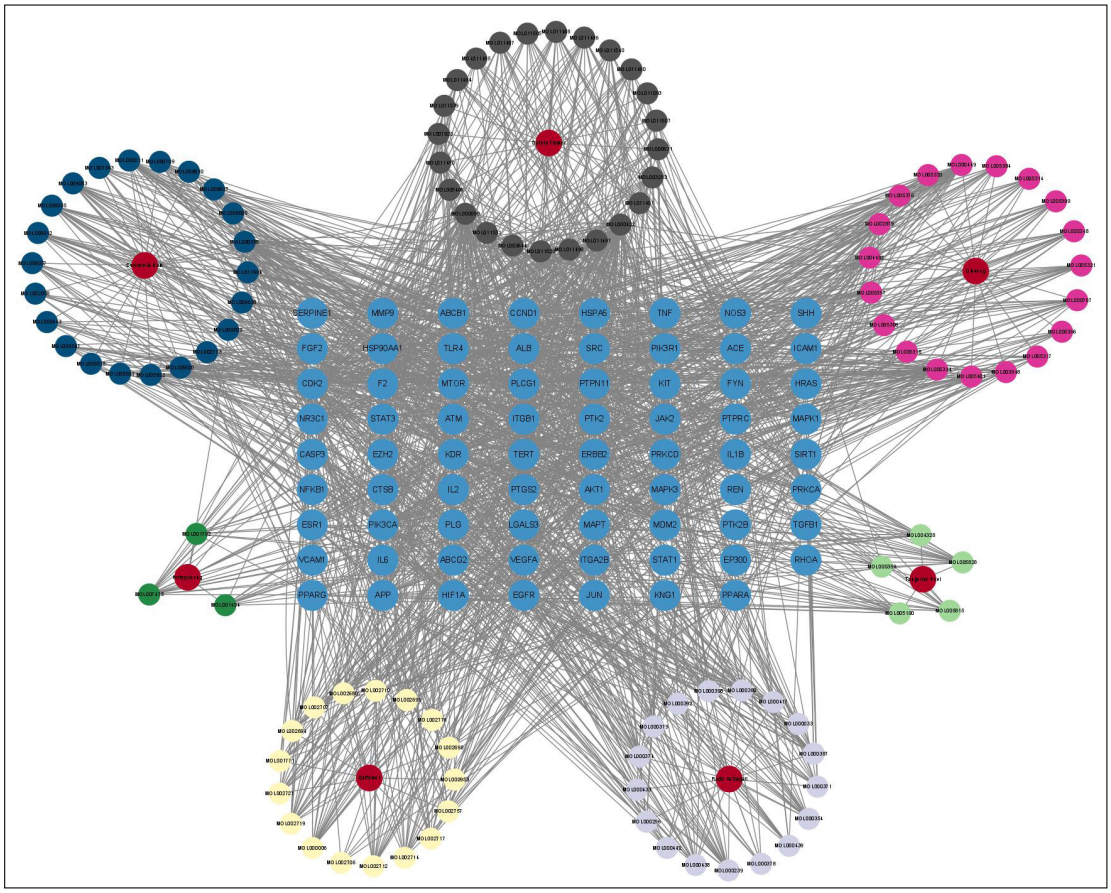

Figure 3. Drug-active ingredient-target network diagram.

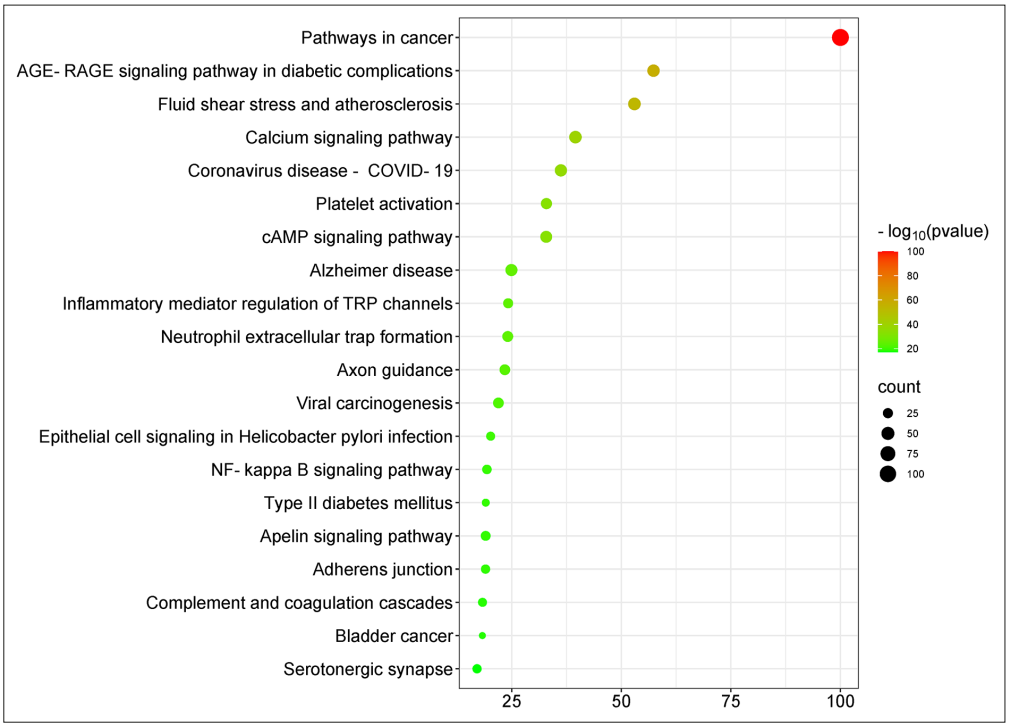

Figure 4. KEGG pathway enrichment analysis diagram.

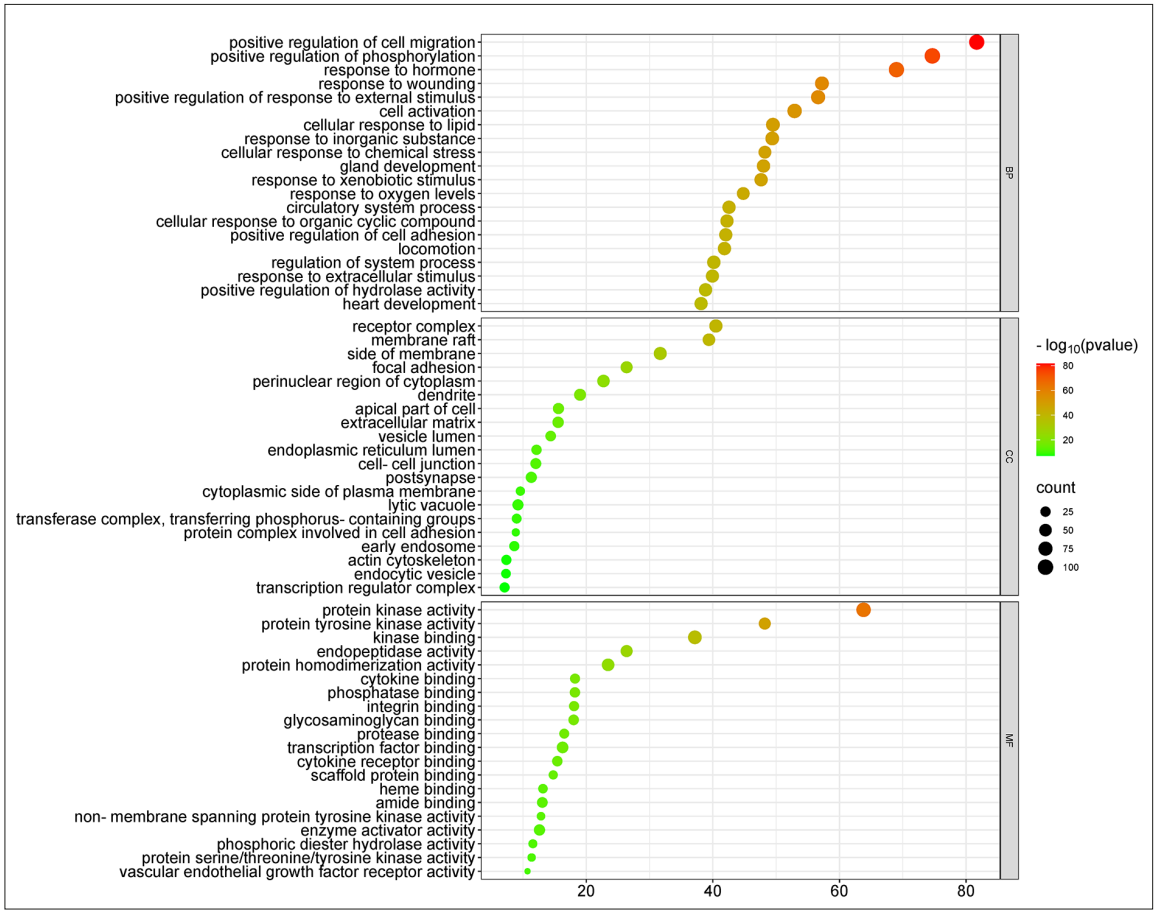

Figure 5. GO enrichment analysis diagram.

through Pathways in cancer, AGE-RAGE signaling pathway in diabetic complications, Fluid shear stress and atherosclerosis, calcium signaling pathway, etc., (Figure 4). The results of GO biological process analysis showed that its biological processes were mainly involved in positive regulation of cell migration, positive regulation of phosphorylation, response to hormones, etc. The cellular components mainly involve receptor complex, membrane raft, membrane side, and so on. Molecular functions mainly include protein kinase activity, tyrosine kinase activity, and kinase binding (Figure 5).

Molecular Docking

The first three main active components (kaempferol, quercetin, and ginsenoside rh2) were molecular docked with the top three core targets (*AKT1, ALB, TNF*). The results showed that the binding energies of the three active components to *AKT1, ALB, TNF* were all <-4.0 kcal/mol, and they could dock

Table II. Molecular docking information table.

Targets	Molecule Name	Mol ID	Binding energy (Kcal/mol)
AKT1	Kaempferol	MOL000422	-4.67
	Quercetin	MOL000098	-4.84
	Ginsenoside rh2	MOL005344	-6.29
ALB	Kaempferol	MOL000422	-6.13
	Quercetin	MOL000098	-6.27
	Ginsenoside rh2	MOL005344	-8.12
TNF	Kaempferol	MOL000422	-4.67
	Quercetin	MOL000098	-5.28
	Ginsenoside rh2	MOL005344	-6.68

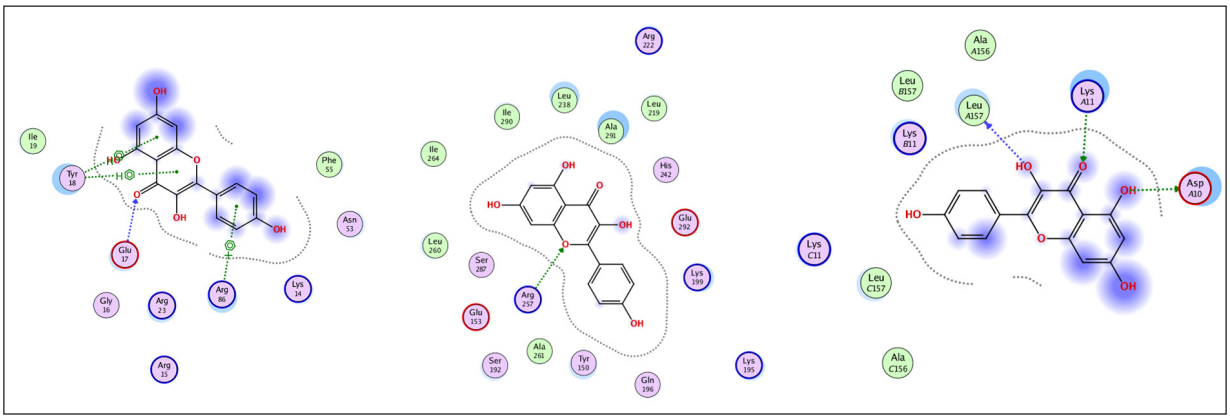

Figure 6. Molecular docking diagram of kaempferol with *AKT1, ALB, TNF.*

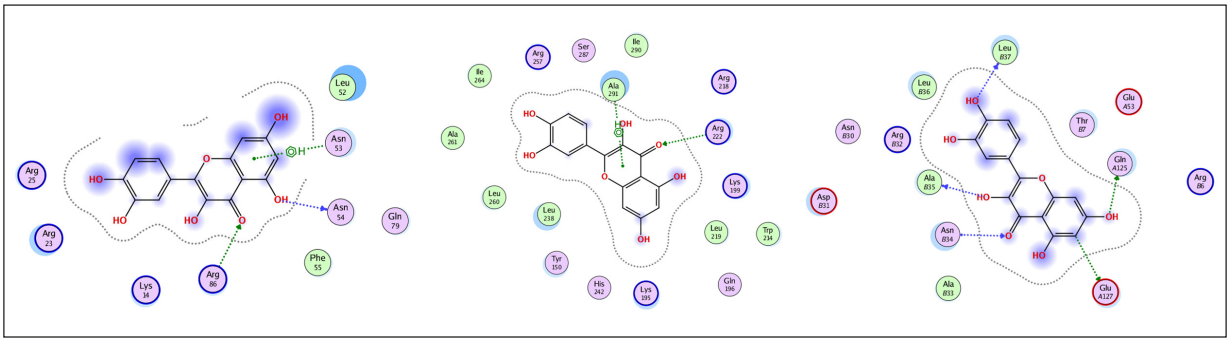

Figure 7. Molecular docking diagram of quercetin with *AKT1, ALB, TNF.*

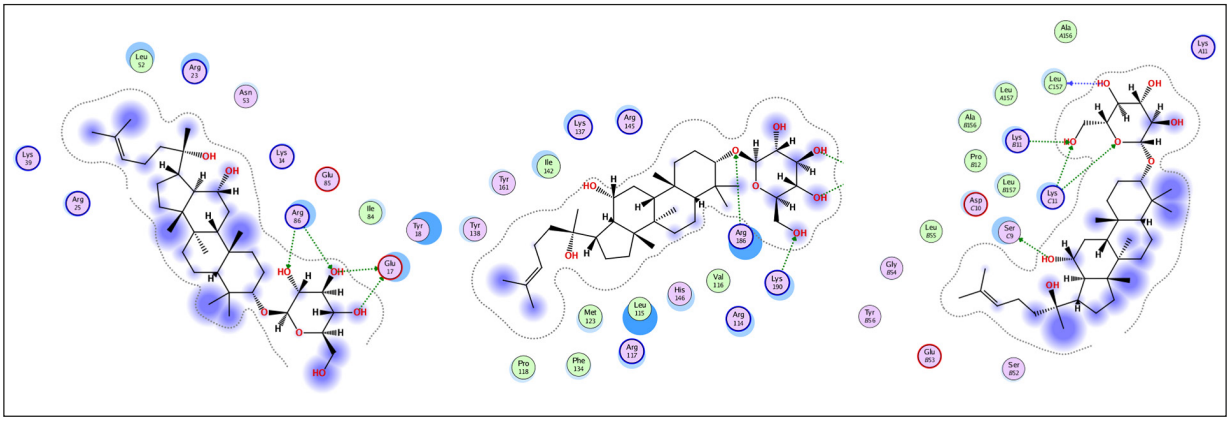

Figure 8. Molecular docking diagram of ginsenoside rh2 and *AKT1, ALB, TNF.*

spontaneously. Among them, the binding energies of kaempferol to *ALB*, quercetin to *ALB, TNF*, and ginsenoside rh2 to *AKT1, ALB*, and *TNF* were all <-5 kcal/mol, indicating a good docking binding activity[14]. Among them, the docking activities of ginsenoside rh2 and *ALB* were the most significant (Table II). Docking visualization is shown in Figures 6-8.

Discussion

The results show 71 core targets of Osteoking and deep vein thrombosis of lower limbs, mainly *AKT1, ALB, TNF, IL-6, VEGFA*, and other gene targets. ALB is a biomarker of malnutrition, and Xue et al[15] found that ALB is an independent risk

factor for deep vein thrombosis of lower limbs through logistic multivariate regression analysis. Patients with fractures can lead to acute loss of ALB. Pain and prolonged bed rest can cause appetite loss and nutritional consumption[16]. A variety of reasons jointly cause the continuous decline of ALB levels in patients and increase the risk of deep vein thrombosis of lower limbs. Interleukin (IL) and tumor necrosis factor (TNF) are considered to be the key mediators in the aggravation of the inflammatory process and have strong pro-inflammatory activity. Among them, IL-6 and TNF-α are the most critical, and they are involved in various inflammatory activities. TNF-α is closely related to many components of the human immune system[17]. When TNF-α is stimulated, the level of substance P increases, which induces the expression of IL-1β, IL-6, and IL-8[18]. AKT, as a key signal node in the inflammatory reaction, can cause phosphorylation of its downstream signal molecule Mammalian target of rapamycin (mTOR), thus promoting giant cell polarization and secreting inflammatory factors such as IL-1, IL-6, and TNF-α[19,20]. These inflammatory factors play a key role in the procoagulant process, especially inducing the expression of tissue factor (TF), which can activate the coagulation cascade and form thrombus[21]. VEGFA can combine with VEGFR-1 and VEGFR-2 to promote angiogenesis[22]. It has the function of maintaining normal vascular endothelial cells, and blocking VEGF pathway can lead to endothelial dysfunction. Inhibition of vascular endothelial cell apoptosis or endothelial cell regeneration will destroy the integrity of endothelial cells, exposure of procoagulant phospholipids under blood vessels and aggregation of various cytokines can promote thrombosis.

The results of KEGG and GO enrichment analysis showed that the active components of the Osteoking may exert molecular functions such as protein kinase activity, protein tyrosine kinase activity, and kinase binding in the receptor complex, membrane raft, side of membrane through the positive regulation of cell migration, positive regulation of phosphorylation, response to hormone and other biological processes. Furthermore, it regulates pathways in cancer, AGE-RAGE signaling pathway in diabetic complications, fluid shear stress and atherosclerosis, the calcium signaling pathway, and other pathways. Research[23] shows that the risk of VTE in cancer patients is 9 times higher than that in non-cancer patients, and the incidence of thrombosis in cancer patients is increasing year by year[24]. Tumor cells can activate the coagulation pathway through various mechanisms, and at the same time, they are exposed to pro-inflammatory stimuli in the cancer microenvironment, causing endothelial cell damage, inducing inflammatory reactions, and increasing the risk of DVT[25,26]. The atherosclerosis pathway and calcium signal pathway involved in Osteoking mainly participate in the classical coagulation pathway. Atherosclerosis damages vascular endothelium and promotes deep vein thrombosis. The calcium pathway can be activated by other coagulation factors to participate in the endogenous coagulation pathway and exogenous coagulation pathway and promote coagulation. KEGG analysis suggests that Osteoking can also be used in other pathways to jointly prevent the formation of deep venous thrombosis of lower limbs.

In order to verify the reliability of the predicted target and signal pathway, three components with the highest activity and three core targets with the highest degree value were selected for molecular docking. The results showed that kaempferol, quercetin, and ginsenoside rh2 could be well docked with *AKT1, ALB,* and *TNF* targets, among which the docking activity of ginsenoside rh2 and *ALB* was the most significant, and the docking binding energy was -8.12 kcal/mol, which further verified the reliability of the results.

Limitations

However, there are some limitations in this study. Through the method of network pharmacology, we explored the mechanism of Osteoking in preventing deep vein thrombosis of lower limbs. Although the results are verified by molecular docking, experiments are still needed to increase the reliability of this study. Therefore, in the next step, we will carry out experiments on this study to provide a new treatment for the prevention of deep vein thrombosis after fracture surgery in the clinic.

Conclusions

Osteoking has many active components, among which Kaempferol, Quercetin, and Ginsenoside rh2 are the most prominent. They can be used as several key targets of DVT, and the active components have good binding activity with the key targets. It can play a role through multiple targets and multiple signal pathways to prevent the formation of deep venous thrombosis of lower limbs after fracture.

Informed Consent
Not applicable.

Ethics Approval
Not applicable.

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Conflict of Interest
The authors declare that they have no conflict of interest.

Authors' Contributions
All authors made a significant contribution to the work reported and agreed to be accountable for all aspects of the work. X.-L. Luo proposed this research, collected data and prepared the initial draft of the manuscript. J. Liang and D.-K. Gao collect data and analyze it. C.-R. Fang summarize the data and draw graphs. Y.-T. Chen and Q. Na evaluate the manuscript and give suggestions for revision.

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Availability of Data and Materials
Publicly available datasets were analyzed in this study. All data can be obtained from the related websites marked in this paper.

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