Predicting the levels of orcinol glucoside during the treatment of osteoporosis by network pharmacology and molecular docking

xia liu
Chongqing Traditional Chinese Medicine Hospital

Mingchun Huang
Chongqing Traditional Chinese Medicine Hospital

Chen Yang
Chengdu University of Traditional Chinese Medicine

Qin Wang
Chongqing Traditional Chinese Medicine Hospital

Mei Zhang (✉ zhangmei63@cdutcm.edu.cn)
Chongqing Traditional Chinese Medicine Hospital

Research

Keywords: orcinol glucoside, osteoporosis, network pharmacology, molecular docking, molecular mechanisms

DOI: https://doi.org/10.21203/rs.3.rs-102341/v1

License: ☑️ ☑️ This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License
Abstract

Introduction: As a traditional Chinese medicine (TCM), *Curculigo orchioides* Gaertn. (Xianmao) has been widely used to treat bone-related diseases. However, the active components of this TCM, and the specific mechanisms by which it exerts effect, have yet to be elucidated. To identify potential targets for orcinol glucoside (OG), an active constituent of *C. orchioides*, during the treatment of osteoporosis (OP) by adopting a network pharmacology approach.

Methods: First, we mined the Similarity ensemble approach (SEA), SwissTargetPrediction, DisGeNET, and Genecards databases were mined for data related to the prediction of OG- and OP-related targets. Next, we identified the common targets for OG and OP, and then used STRING software to create a protein-protein interaction (PPI) network. Then, we used topological analysis to identify which of the common targets were most significant. Then, we used the common significant targets and g:profiler to perform gene ontology (GO) term and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis. Finally, we used molecular docking to predict the targets of OG that were most relevant to the treatment of OP and investigated the potential pharmacological mechanisms that might be involved.

Results: In total, 130 potential targets of OG, and 4582 targets relevant to OP, were subjected to network analysis. There were 73 common targets; these identified the principal pathways linked to OP. In addition, topological analysis identified 14 key targets. Most of the predicted targets played crucial roles in the PI3K-AKT signaling pathway. Molecular docking identified ten core targets (*VEGFA*, *IL6*, *EGFR*, *MAPK1*, *HRAS*, *CCND1*, *FGF2*, *IL2*, *MCL1* and *CDK4*), thus indicating that OG may promote osteoblast proliferation and differentiation by accelerating progression of the cell cycle.

Conclusions: This research provides a theoretical base for identifying the specific potential mechanisms of OG in treatment of OP.

Introduction

Osteoporosis (OP) is a common metabolic bone disease that is characterized by low bone mineral density and microarchitectural changes in the bone, thus resulting in debilitating fragility and fractures [1]. OP is a global health issue that shows a close association with the aging population. Current estimates show that the population of patients with OP in China will increase by 60 million to over 120 million by the year 2050 [2]. The pathogenesis of OP is very complicated, including physical variables, nutritional status, endocrine factors, genetic factors, and a range of immune factors [3]. Numerous clinical therapies have been applied to treat OP, including calcium and vitamin D, bisphosphonates, anti-RANKL, selective estrogen receptor modulators, anabolic agents, and sclerostin inhibitors [4]. However, these drugs can only partially ease bone loss; their lasting clinical use is limited by high costs and low tolerability [5].

Traditional Chinese medicine (TCMs), such as Epimedium (Yinyanghuo), *Curculigo orchioides* Gaertn. (Xianmao), have been used for many years to treat OP [6]. The effects of water extract from epimedium
treatment on osteoporosis can be mitigated through a mechanism associated with several neuropeptides that regulate the brain/spinal cord/bone axis [7]. *C. archioides* ethanol extract can inhibit bone absorption in rats underoing oophorectomy, increase serum phosphorus and calcium levels, and have a certain protective effect on osteoporosis; however, this product does not affect bone formation [8]. Phytochemical studies previously demonstrated that *C. archioides* contains and abundance of phenols and phenolic glycosides, triterpenes and triterpenoid glycosides, lignans, lignan glycosides, and many other types of compounds [9]. OG is one of the major bioactive phenolic glycosides of *C. archioides* and is reported to have a wide range of pharmacological actions in mouse models, including antiosteoporosis [10], anxiolytic [11] and antidepressive [12] effects. The specific mechanisms underlying the therapeutic effects of OG in patients with OP, however, has yet to be elucidated. The role of OG in the treatment of OP can be better understood by detailed studies on molecular targets and related signal pathways.

With the rapid progression of bioinformatics technology, the development of network pharmacology has proven to be an innovative method for investigating the effects of TCM [13–14]; this new discipline involves network analysis, molecular docking, experimental methods, and integrates multiple information sources [15]. Therefore, network-based methods are expected to significantly enhance our understanding of drug effect by considering multiple sources of information [16].

In this research, we systematically explored the potential targets and molecular mechanisms of OG in the treatment of OP. First, we identified the molecular targets of OG and then predicted targets of disease using different types of bioinformatics platforms. Second, we selected the most significant common targets for OG and OP used STRING software to create a protein-protein interaction (PPI) network; Cytoscape was used to visualize the results of PPI. Third, we carried out enrichment analysis of common targets according to gene ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. Finally, the therapeutic effect of OG, and several key targets identified by topological analysis, was confirmed by molecular docking experiments.

**Methods**

**The prediction of targets for OG**

PubChem (https://pubchem.ncbi.nlm.nih.gov/) is the world’s largest free chemical database and offers information related to compound structures and biological activities. First, we used OG to search the PubChem database to acquire canonical SMILES strings. These strings were then sent to SwissTargetPrediction (http://new.swisstargetprediction.ch/?) and Similarity ensemble approach (SEA) (http://sea.bkslab.org/) to identify targets. In this part of the analysis, the species was set to “human” and the prediction results were collated and classified.

**Screening of targets related to OP**
Disease-related gene screening was carried out by screening two free public databases: the DisGeNET database v6.0 (https://www.disgenet.org/) [17] and Genecards database (https://www.genecards.org/) [18] with the keyword “osteoporosis”. We also used UniProtKB (https://www.uniprot.org/) [19] to acquire the names of standard targets with “homo sapiens” as the selected organism.

After collating the targets and removing any duplicates, the final list of predicted targets were estimated to represent common targets. Next, the common targets of OG that showed relevance with regards to its effects on OP were identified. We were particularly interested in targets that may play an active role in the proliferation and differentiation of osteoblasts by accelerating cell cycle progression.

**Construction of a visualization network**

The identified targets were then inputted into the STRING database v11.0 (https://string-db.org/) [20] for PPI analysis, including their physical and functional associations. During this analysis, we set the score > 0.4 medium confidence, and the results were exported in tab-separated value (TSV) format. PPI results were subsequently imported into Cytoscape 3.7.2 [21] to visualize the results.

Next, we used Network Analyzer to calculate the network topological parameters by treating the network as undirected [22]. We also used the CytoNCA plugin [23], and the “without weight” method to determine the three centralities: degree centrality (DC), betweenness centrality (BC), and closeness centrality (CC).

In this study, the nodes with a DC and BC score that were greater than the median by two-fold were taken as key targets in the network. We assumed that these were the key targets for anti-osteoporosis. In this experiment, the median DC was 8, and the median BC was 15.14135.

**GO and KEGG pathway enrichment analysis**

GO and KEGG pathway enrichment analyses was carried out for OG targets in the treatment of OP. To facilitate this analysis, we used a web server for functional enrichment analysis known as g:Profiler (https://biit.cs.ut.ee/gprofer/gost) [24]. This analysis was performed and visualized by OmicShare tools, a free online platform for data analysis (https://www.omicshare.com/tools/).

**Molecular docking**

Three-dimensional (3D) structures of the ligand was obtained from PubChem. The crystal structures of key targets were then downloaded from the RCSB Protein Data Bank (https://www.wwpdb.org/) and modified using PyMOL [25], including solvents, ligands, and original ligands. The key target proteins were as follows: VEGFA (PDB ID: 3QTK), IL6 (PDB ID: 409H), EGFR (PDB ID: 3IKA), MAPK1 (PDB ID: 4FV4), HRAS (PDB ID: 6ZL3), CCND1 (PDB ID: 6P8E), FGF2 (PDB ID: 5X1O), IL2 (PDB ID: 4NEM), MCL1 (PDB ID: 4WMR), and CDK4 (PDB ID: 2W99). Next, Autodock Vina (Olson Research Group, Scripps Research Institute) was used to analyze the binding characteristics of the ligand for each protein [26]. First, we used Autodock Tools to save the proteins and ligand in PDBQT formats [27]; the grid box parameters are shown in Table 1. Exhaustiveness was set to the value of 60 for all docking analysis. Other settings were
all set as default values. After docking, we selected the lowest binding affinity score for each protein for further analysis. Ligplus [28] and PyMOL were used to visualize the interactions between OG and the key targets as two-dimensional (2D) and three-dimensional (3D) graphs.

### Table 1 Grid box parameters used for molecular docking

| Targets | PDB ID | Grid Center | Npts | Spacing |
|---------|--------|-------------|------|---------|
| VEGFA   | 3QTK   | 24.624 -1.635 -7.692 60 60 60 1.000 |
| IL6     | 4O9H   | -34.017 23.971 11.472 60 60 60 1.000 |
| EGFR    | 3IKA   | -10.730 27.973 36.465 60 60 60 1.000 |
| MAPK1   | 4FV4   | -16.691 3.162 15.987 60 60 60 1.000 |
| HRAS    | 6ZL3   | 29.700 -22.911 14.229 60 60 60 1.000 |
| CCND1   | 6P8E   | 31.466 12.636 44.714 60 60 60 1.000 |
| FGF2    | 5X1O   | 31.752 2.507 261.200 60 60 60 1.000 |
| IL2     | 4NEM   | -14.928 8.061 18.532 60 60 60 1.000 |
| MCL1    | 4WMR   | -3.495 -12.165 7.518 60 60 60 1.000 |
| CDK4    | 2W99   | 29.545 19.537 14.264 60 60 60 1.000 |

### Results

#### Screening of potential targets

After the elimination of duplicates, SEA and SwissTargetPrediction databases identified a total of 130 predicted targets for OG (Table S1). DisGeNET and Genecards databases further identified a total of 4582 targets associated with OP (Table S2). The intersection between these two lists of targets identified 73 common targets of OG that were associated with OP.

#### OG-OP target network

Based on the 73 common targets, we created a PPI network by importing the genes of the common targets into the STRING database. Then, Cytoscape 3.7.2 software was used to visualize the PPI network, which consisted of 73 nodes and 461 edges (Fig. 1). According to strict criteria (twice the median of DC and BC), 14 critical nodes ("key targets") were further identified, as shown in Table 2. These 14 key targets may represent valuable targets for OG in the treatment of OP.
Table 2
Topological parameters of the targets

| Gene   | DC   | BC    | CC  |
|--------|------|-------|-----|
| GAPDH  | 49   | 1058.475 | 0.75 |
| VEGFA  | 45   | 640.9635 | 0.72 |
| IL6    | 44   | 763.3865 | 0.72 |
| EGFR   | 41   | 440.131 | 0.692308 |
| MAPK1  | 33   | 279.9036 | 0.631579 |
| HRAS   | 32   | 154.2462 | 0.631579 |
| MMP9   | 30   | 305.9342 | 0.615385 |
| CCND1  | 30   | 90.02024 | 0.615385 |
| ESR1   | 29   | 272.7521 | 0.605042 |
| FGF2   | 27   | 124.6053 | 0.6 |
| IL2    | 25   | 118.7386 | 0.595041 |
| MCL1   | 22   | 51.10877 | 0.571429 |
| CDK4   | 20   | 35.86626 | 0.5625 |
| F2     | 16   | 93.15169 | 0.541353 |

GO terms and KEGG pathway enrichment analyses

Next, the 73 common targets were imported to the g: Profiler for GO and KEGG analysis. GO terms and KEGG pathways with a \( p \)-value < 0.05 were considered to be notably enriched. The top 20 elements were then visualized using OmicShare tools (Fig. 2).

As shown in Fig. 2, the top five items of biological processes (each with a \( p \) value < 0.05) included organonitrogen compound metabolic process, cell population proliferation, protein metabolic process, regulation of response to stress, and response to chemicals. The top five items for the cellular components category included cyclin-dependent protein kinase holoenzyme complex, serine/threonine protein kinase complex, protein kinase complex, extracellular region, and extracellular space. The top five items for the molecular functions category included catalytic activity, catalytic activity, acting on a protein, protein kinase activity, metalloendopeptidase activity, and serine-type endopeptidase activity.

KEGG analysis further showed that 54 of the 73 common targets (74.0%) were notably enriched in 51 pathways (Table S3). Among the enriched pathways, we identified abnormalities in terms of the PI3K-AKT signaling pathway in OP. The predicted targets that refer to the PI3K-AKT signaling pathway are indicated in red in Fig. 3.
Molecular Docking

Ten select key targets, VEGFA, IL6, EGFR, MAPK1, HRAS, CCND1, FGF2, IL2, MCL1 and CDK4, were obtained because they were not only the key targets of PPI network, but also played a major role in the PI3K-AKT signaling pathway. These genes were subjected to OG for molecular docking. As summarized in Table 3, the binding energies were computed to evaluate the binding affinities of the ten targets with OG. A binding affinity <-7 showed strong binding activity [26].

| Targets  | PDB ID | Binding Energy (ΔG)/kcal·mol⁻¹ |
|----------|--------|-------------------------------|
| VEGFA    | 3QTK   | -7.3                          |
| IL6      | 4O9H   | -6.7                          |
| EGFR     | 3IKA   | -7.0                          |
| MAPK1    | 4FV4   | -7.2                          |
| HRAS     | 6ZL3   | -7.4                          |
| CCND1    | 6P8E   | -7.0                          |
| FGF2     | 5×1O   | -7.9                          |
| IL2      | 4NEM   | -6.1                          |
| MCL1     | 4WMR   | -7.2                          |
| CDK4     | 2W99   | -7.8                          |

The mode of action for OG with VEGFA is shown in Fig. 4A; the hydroxyl group of OG is able to form two hydrogen bonds with the amino acid Asn55 (2.82 Å) and Asp56 (3.00 Å) within the active pocket. In addition, Cys53, Cys54, Phe29, Arg98, Lys101, Ile39, and Glu57, can all form hydrophobic interactions; these help to stabilize the entire region that is used for interaction. In addition, the results in Fig. 4B show that OG binds to IL6 by forming several hydrophobic interactions with the surrounding residues: Lys46, Arg104, Leu39, Leu101, and Ala112.

As shown in Fig. 4C, we observed hydrophobic interactions between OG and seven residues in 3IKA (Leu718, Ala743, Leu844, Leu792, Gly796, Val726, and Met790). Two hydrogen bonds, Met793 (3.10 Å) and Lys745 (2.92 Å), further enhanced the interaction between OG and the 3IKA protein. Figure 4D shows that OG binds to MAPK1 by forming several hydrophobic interaction with several surrounding residues: Cys164, Ala50, Leu154, Val37, Ile29, and Leu105. Analysis also showed that OG forms three H-bonds with Met106 (3.05 Å), Gln103 (3.17 Å) and Lys52 (3.34 Å).

As shown in Fig. 4E, we observed hydrophobic interactions between OG and seven residues in 6ZL3 (Leu718, Ala743, Leu844, Leu792, Gly796, Val726, and Met790). Two hydrogen bonds, Met793 (3.10 Å) and Lys745 (2.92 Å), further enhanced the interaction between OG and the 3IKA protein. Figure 4D shows that OG binds to MAPK1 by forming several hydrophobic interaction with several surrounding residues: Cys164, Ala50, Leu154, Val37, Ile29, and Leu105. Analysis also showed that OG forms three H-bonds with Met106 (3.05 Å), Gln103 (3.17 Å) and Lys52 (3.34 Å).

The interplay between OG and HRAS is shown in Fig. 4E; three H-bonds were observed with Lys117 (2.80 Å), Ala146 (3.02 Å), and Lys147 (3.02 Å), in the binding pocket of HRAS. In addition, hydrophobic
interactions with five residues in HRAS (Phe28, Gly15, Ala18, Asn116, and Asp30), along with pi-pi stacking with Phe28, further facilitated the binding of OG to HRAS.

As shown in Fig. 4F, OG interacted with CCND1 by Asn221, Pro220, Arg218, Leu217, Ile177, and Gly214. Furthermore, OG can form H-bonds with Asn174 (3.22 Å), His181 (3.00 Å), and Asn222 (3.16 Å). Figure 4G shows that OG binds to a pocket in FGF2 which consists of Pro36, His50, Ile51, Val40, Lys66, Tyr73, and Ala84. Furthermore, OG can form two H-Bonds with Leu74 (2.94 Å), and Gly67 (3.14 Å).

The data shown in Fig. 4H further shows that OG can form hydrophobic interactions with four residues in IL2 (Pro34, Ile28, Ile24, and Gln74) and one H-Bond (Asn30 (3.33 Å)).

Figure 4I show the binding ability between OG and MCL1 (Fig. 4I) which involves hydrophobic interactions with Phe254, Val253, Thr266, Leu267, Gly271, Ile294, Leu290, Met250, and Val249. In addition, one H-Bond with Arg263 (3.30 Å), along with pi-pi stacking with Phe270, further facilitated the binding of OG to MCL1.

The docking results between OG and CDK4 are shown in Fig. 4J. OG binds to a pocket in CDK4 which consists of Ile12, Glu144, Val20, Asn145, Phe93, Ala157, Leu147, Val72, and Ala33. Four H-Bonds (Asp158 (3.11 Å), Lys35 (3.02 Å), Val96 (3.17 Å), and His95 (3.28 Å)) further enhance the interaction between OG and the CDK4 protein.

**Discussion**

With the increasing incidence of OP and the poor therapeutic efficacy of clinical drugs, there is an urgent need to develop new treatment strategies. Therefore, the search of complementary and alternative medicine has become our top priority. As a famous kidney-tonifying traditional medicine, *C. orchioides* has been widely used against OP. The major active constituent of OG is *C. orchioides*; previous research has shown that *C. orchioides* exerts significant effects against OP [10]. Here, we used network pharmacology and molecular docking approaches to investigate existing literature and confirm potential mechanisms underlying the role of OG.

We hypothesized that OG can be used as a therapeutic for the treatment of OP and that OG promotes the proliferation and differentiation of osteoblasts by accelerating cell cycle progression. We then carried out a series of investigations to test this hypothesis. Another active molecule, Curculigoside, also isolated from *C. orchioides* has been reported to have a similar therapeutic mechanism and exhibits anti-OP effects by inducing the proliferation and differentiation of osteoblasts [29] and by reducing the inflammatory response [30].

Enrichment analysis of KEGG pathways showed that OG targets were enriched in the PIK3-AKT signaling pathway. Previous research also showed that the activation of the PI3K-AKT signaling pathway exhibits a strong correlation with the occurrence and development of OP [31]. During the pathogenesis of OP, the PIK3-AKT pathway contributes to the progression of disease through cell survival, proliferation,
differentiation, and apoptosis [32]. Therefore, targeting the PI3K/Akt signaling pathway may be a potential treatment for osteoporosis.

In this study, we first identified 73 common targets for drugs and diseases that might also represent targets for OG during the treatment of OP. Based on topological analysis, we further identified 14 key targets, including GAPDH, VEGFA, IL6, EGFR, MAPK1, HRAS, MMP9, CCND1, ESR1, FGF2, IL2, MCL1, CDK4, and F2. Among the top 20 enriched KEGG pathways, the PI3K-AKT signaling pathway was shown to be particularly important as abnormalities were clearly evident in OP. It was evident that the key target genes were not exactly the same as those in the PI3K-AKT signaling pathway. Proteins that were confirmed as key targets and involving the PI3K-AKT signaling pathway were thus selected for molecular docking.

In total, ten targets were selected for the molecular docking studies: VEGFA, IL6, EGFR, MAPK1, HRAS, CCND1, FGF2, IL2, MCL1 and CDK4. Cell proliferation and differentiation can be adjusted via alterations of the cell cycle phase, thus causing indirect effects on bone formation [33]. Our molecular docking studies suggest that OG can affect the osteoblast cell cycle and has strong affinity for CCND1 (-7.0 kcal·mol⁻¹), and CDK4 (-7.8 kcal·mol⁻¹); these are key players in cell cycle progression. Consistent with this hypothesis, pharmacological studies have consistently shown that related compounds can downregulate CCND1 or CDK4 to repress osteogenic proliferation and differentiation [34–35]. Studies have also shown that VEGFA, EGFR, MAPK1, and FGF2 play a critical role in the proliferation and differentiation of osteoblast cells [36–39]. In this study, we found that OG may activate VEGFA, EGFR, and MAPK1, as their binding affinities for interaction were all <7.0. Furthermore, IL6 and IL2 are involved not only in inflammatory responses but also in the regulation of bone mineral density, osteoclast differentiation and activation [40–41]. However, our molecular docking results for IL6 and IL2 showed binding affinities of -6.7 and −6.1 kcal·mol⁻¹; these are higher than −7.0 kcal·mol⁻¹ and therefore indicate that OG exerts only weak inhibition on inflammatory responses.

**Conclusion**

By combining network pharmacology and molecular docking, we showed that OG is a promising treatment for OP and acts via several key targets (VEGFA, IL6, EGFR, MAPK1, HRAS, CCND1, FGF2, IL2, MCL1 and CDK4). Via these targets, OG may promote the proliferation of osteoblast by altering cell cycle progression. However, further experimental testing is now required to investigate the specific mechanisms underlying the action of OG on OP.

**Abbreviations**

OG: orcinol glucoside; OP: osteoporosis; GO: gene ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; BC: betweenness centrality; CC: closeness centrality; DC: degree centrality; Npts: number of points in x-, y-, z-dimension; VEGFA: Vascular endothelial growth factor A; IL6: Interleukin-6; EGFR: Epidermal growth factor receptor; MAPK1: Mitogen-activated protein kinase 1; HRAS: GTPase HRas;
CCND1: G1/S-specific cyclin-D1; FGF2: Fibroblast growth factor 2; IL2: Interleukin-2; MCL1: Induced myeloid leukemia cell differentiation protein Mcl-1; CDK4: Cyclin-dependent kinase 4.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The data and materials used or analyzed during this current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

The authors are grateful to National Natural Science Foundation of China (Grant number: 81774202), Chongqing Municipal Natural Science Foundation (Grant number: cstc2018jcyjAX0388) and the Chongqing Municipal Performance Incentive Foundation (Grant number: cstc2018jxj1130032) for financial support.

Authors’ contributions

MZ designed the study. XL, MH, CY and QW carried out the experimental work., MZ, XL and MH analyzed the experimental data and wrote the paper. All authors reviewed the manuscript and approved its submission.

Acknowledgements

Not applicable.

References

1. Black DM, Rosen CJ. Postmenopausal Osteoporosis. N Engl J Med. 2016; 374(3): 254-262.
2. Zeng Q, Li N, Wang Q, et al. The Prevalence of Osteoporosis in China, a Nationwide, Multicenter DXA Survey. J Bone Miner Res. 2019; 34(10): 1789-1797.
3. Zhao HY, Zhao N, Zheng P, et al. Prevention and Treatment of Osteoporosis Using Chinese Medicinal Plants: Special Emphasis on Mechanisms of Immune Modulation. J Immunol Res. 2018;2018(4): 1-11.

4. Goode SC, Wright TF, Lynch C. Osteoporosis Screening and Treatment: A Collaborative Approach. J Nurse Pract. 2020; 16(1): 60-63.

5. Khan M, Cheung AM, Khan AA. Drug-Related Adverse Events of Osteoporosis Therapy. Endocrinol Metab Clin North Am. 2017; 46(1): 181-192.

6. Li CR, Li Q, Liu RJ, et al. Medicinal herbs in the prevention and treatment of osteoporosis. Am J Chinese Med. 2014; 42(1): 1–22.

7. Liu HR, Xiong YQ, Wang HX, et al. Effects of water extract from epimedium on neuropeptide signaling in an ovariectomized osteoporosis rat model, J Ethnopharmacol. 2018; 221(15): 126-136.

8. Cao DP, Zheng YN, Qin LP, et al. Curculigo orchioides, a traditional Chinese medicinal plant, prevents bone loss in ovariectomized rats. Maturitas. 2008, 59(4): 373-380.

9. Nie Y, Dong X, He Y., et al. Medicinal plants of genus Curculigo: Traditional uses and a phytochemical and ethnopharmacological review. J Ethnopharmacol. 2013; 147(3): 547-563.

10. Zhou XY, Liu ZZ, Huang B, et al. Orcinol glucoside facilitates the shift of MSC fate to osteoblast and prevents adipogenesis via Wnt/β-catenin signaling pathway. Drug Des Devel Ther. 2019; 13: 2703-2713.

11. Wang XH, Li GY, Li P, et al. Anxiolytic effects of orcinol glucoside and orcinol monohydrate in mice. Pharm Biol. 2015; 53(6): 876-881.

12. Ge JF, Gao WC, Cheng WM, Lu WL, et al. Orcinol glucoside produces antidepressant effects by blocking the behavioural and neuronal deficits caused by chronic stress. Eur Neuropsychopharmacol. 2014; 24(1): 172-180.

13. Xu HH, Li SM, Xu R, et al. Prediction of the underlying mechanism of Bushenhuoxue formula acting on knee osteoarthritis via network pharmacology-based analyses combined with experimental validation. J Ethnopharmacol. 2020; 263: 113217.

14. Xu XY, Niu LL, Liu Y, et al. Study on the mechanism of Gegen Qinlian Decoction for treating type II diabetes mellitus by integrating network pharmacology and pharmacological evaluation. J Ethnopharmacol. 2020; 262: 113129.

15. Park M, Park SY, Lee HJ, et al. A Systems-Level Analysis of Mechanisms of Platycodon grandiflorum Based on A Network Pharmacological Approach. Molecules. 2018; 23(11): 2841.

16. Boezio B, Audouze K, Ducrot P, et al. Network-based Approaches in Pharmacology. Mol Inform. 2017; 36(10): doi: 10.1002/minf.201700048. Epub 2017 Jul 10.

17. Piñero J, Bravo À, Rosinach NQ, et al. DisGeNET: A comprehensive platform integrating information on human disease-associated genes and variants. Nucleic Acids Res. 2016; 45(D1): D833-D839.

18. Stelzer G, Rosen N, Plaschkes I, et al. The GeneCards Suite: From Gene Data Mining to Disease Genome Sequence Analyses. 2016; 54: 1.30.31-1.30.33.
19. Consortium U. UniProt: a worldwide hub of protein knowledge. Nucleic Acids Res. 2018; 47(D1): D506-D515.

20. Szklarczyk D, Gable AL, Lyon D, et al. STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. Nucleic Acids Res. 2018; 47(D1): D607-D613.

21. Shannon P, Markiel A, Ozier O, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res. 2003; 13(11): 2498-2504.

22. Assenov Y, Ramírez F, Schelhorn SE, et al. Computing topological parameters of biological networks. 2008; 24(2):282-284.

23. Tang Y, Li M, Wang J, et al. CytoNCA: a cytoscape plugin for centrality analysis and evaluation of protein interaction networks. Bioinformatics. 2015; 127: 67-72.

24. Raudvere U, Kolberg L, Kuzmin I, et al. g:Profiler: a web server for functional enrichment analysis and conversions of gene lists (2019 update). Nucleic Acids Res. 2019; 47(W1): W191-W198.

25. DeLano WL. The PyMOL Molecular Graphics Palo Alto, CA: DeLano Scientific. 2002.

26. Trott O, Olson AJ. AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comp Chem. 2010; 31(2): 455-461.

27. Morris GM, Huey R, Lindstrom W, et al. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. J Comput Chem. 2009; 30(16): 2785-2791.

28. Laskowski RA, Swindells MB. LigPlot+: multiple ligand-protein interaction diagrams for drug discovery. J Chem Inf Model. 2011; 51(10): 2778-86.

29. Shen QP, Zeng DL, Zhou Y, et al. Curculigoside promotes osteogenic differentiation of bone marrow stromal cells from ovariectomized rats. J Pharm Pharmacol. 2013; 65(7): 1005-1013.

30. Zhu FB, Wang JY, Zhang Y, et al. Curculigoside regulates proliferation, differentiation, and pro-inflammatory cytokines levels in dexamethasone-induced rat calvarial osteoblasts. Int J Clin Exp Med. 2015; 8(8): 12337-12346.

31. Han LH, Mao XZ, Wang K, et al. Phosphorylated peptides from Antarctic krill (Euphausia superba) ameliorated osteoporosis by activation of osteogenesis-related MAPKs and PI3K/AKT/GSK-3β pathways in dexamethasone-treated mice. J Funct Foods. 2018; 47: 447-456.

32. Zhu LY, Xie YY, Wen BT, et al. Porcine bone collagen peptides promote osteoblast proliferation and differentiation by activating the PI3K/Akt signaling pathway. J Funct Foods. 2020; 64: 103697.

33. Chen X, Deng Y, Zhou Z, et al. 17β-estradiol combined with testosterone promotes chicken osteoblast proliferation and differentiation by accelerating the cell cycle and inhibiting apoptosis in vitro. Vet Res Commun. 2010; 34(2): 143–152.

34. Wang LJ, Cai HQ. Let-7b downgrades CCND1 to repress osteogenic proliferation and differentiation of MC3T3-E1 cells: An implication in osteoporosis. Kaohsiung J Med Sci. 2020; doi: 10.1002/kjm2.12236.
35. Zhang J, Yu XH, Yan YG, et al. PI3K/Akt signaling in osteosarcoma. Clin Chim Acta. 2015; 444, 182-192.

36. Duan XC, Murata Y, Liu YQ, et al. Vegfa regulates perichondrial vascularity and osteoblast differentiation in bone development. Development. 2015; 142(11): 1984-1991.

37. Chandra A, Lan S, Zhu J, et al. Epidermal growth factor receptor (EGFR) signaling promotes proliferation and survival in osteoprogenitors by increasing early growth response 2 (EGR2) expression. J Biol Chem. 2013; 288(28): 20488-20498.

38. Zhao P, Xiao L, Peng J, et al. Exosomes derived from bone marrow mesenchymal stem cells improve osteoporosis through promoting osteoblast proliferation via MAPK pathway. Eur Rev Med Pharmacol Sci. 2018; 22(12): 3962-3970.

39. Coffin JD, Homer-Bouthiette C, Hurley MM. Fibroblast Growth Factor 2 and Its Receptors in Bone Biology and Disease. J Endocr Soc. 2018; 2(7): 657-671.

40. Gür A, Denli A, Nas K, et al. Possible pathogenetic role of new cytokines in postmenopausal osteoporosis and changes during. Rheumatol Int. 2002; 22(5): 194-198.

41. Ma XY, Guo ZX, Gao WS, et al. LncRNA-NEF is downregulated in postmenopausal osteoporosis and is related to course of treatment and recurrence. J Int Med Res. 2019; 47(7): 3299-3306.

Figures
**Figure 1**

The orcinol glucoside-osteoporosis target network. The color and size of each circle reflects the node degree for the common targets.
Figure 2

GO terms and KEGG pathway enrichment analysis of 73 common targets (p-value < 0.05). (A) The top 20 biological processes. (B) The top 20 cellular components. (C) The top 20 molecular functions. (D) The top 20 KEGG pathways. The color scales indicate different thresholds for p-values while the sizes of the dots represent the number of genes corresponding to each term.
Figure 3

Orcinol glucoside is expressed as a target in the PI3K-AKT signaling pathway. The red rectangles indicate the identified targets.
Figure 4

Molecular models of orcinol glucoside binding to its predicted protein targets: (A) VEGFA, (B) IL6, (C) EGFR, (D) MAPK1, (E) HRAS, (F) CCND1, (G) FGF2, (H) IL2, (I) MCL1, and (J) CDK4 (shown as 3D diagrams and 2D diagrams)

Supplementary Files
This is a list of supplementary files associated with this preprint. Click to download.

- supportinginformation.xlsx