

Received: 2017.03.10
Accepted: 2017.05.17
Published: 2017.10.27

A Network Pharmacology Approach to Determine the Active Components and Potential Targets of *Curculigo Orchioides* in the Treatment of Osteoporosis

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

ABCDEF G 1,2
ACG 1
BCD 1
BD 1
BD 3
BD 3
AEG 1
Nani Wang
Guizhi Zhao
Yang Zhang
Xuping Wang
Lisha Zhao
Pingcui Xu
Dan Shou

1 Department of Medicine, Zhejiang Academy of Traditional Chinese Medicine, Hangzhou, Zhejiang, P.R. China
2 School of Pharmacy, Second Military Medical University, Shanghai, P.R. China
3 School of Pharmacy, Zhejiang University of Traditional Chinese Medicine, Hangzhou, Zhejiang, P.R. China

Corresponding Authors: Guizhi Zhao, e-mail: 35509790@qq.com, Dan Shou, e-mail: shoudanok@163.com

Source of support: The project was sponsored by National Natural Science Foundation of China (No. 81603252), China Postdoctoral Science Foundation (No. 2016M602987), Zhejiang Provincial Natural Science Foundation of China (No. LQ17H280002), Zhejiang Provincial TCM Foundation (No. 2016ZQ003) and Zhejiang Provincial Medicine Plan (No. 2016RCB003)

Background: Osteoporosis is a complex bone disorder with a genetic predisposition, and is a cause of health problems worldwide. In China, *Curculigo orchioides* (CO) has been widely used as a herbal medicine in the prevention and treatment of osteoporosis. However, research on the mechanism of action of CO is still lacking. The aim of this study was to identify the absorbable components, potential targets, and associated treatment pathways of CO using a network pharmacology approach.





Material/Methods: We explored the chemical components of CO and used the five main principles of drug absorption to identify absorbable components. Targets for the therapeutic actions of CO were obtained from the PharmMapper server database. Pathway enrichment analysis was performed using the Comparative Toxicogenomics Database (CTD). Cytoscape was used to visualize the multiple components-multiple target-multiple pathways-multiple disease network for CO.

Results: We identified 77 chemical components of CO, of which 32 components could be absorbed in the blood. These potential active components of CO regulated 83 targets and affected 58 pathways. Data analysis showed that the genes for estrogen receptor alpha (*ESR1*) and beta (*ESR2*), and the gene for 11 beta-hydroxysteroid dehydrogenase type 1, or cortisone reductase (*HSD11B1*) were the main targets of CO. Endocrine regulatory factors and factors regulating calcium reabsorption, steroid hormone biosynthesis, and metabolic pathways were related to these main targets and to ten corresponding compounds.

Conclusions: The network pharmacology approach used in our study has attempted to explain the mechanisms for the effects of CO in the prevention and treatment of osteoporosis, and provides an alternative approach to the investigation of the effects of this complex compound.

MeSH Keywords: **Computational Biology • Herbal Medicine • Osteoporosis**

Full-text PDF: <https://www.medscimonit.com/abstract/index/idArt/904264>

 1919  2  2  26



Background

Osteoporosis is a complex bone disorder with a genetic background that leads to an increased susceptibility to bone fracture resulting in pain and morbidity [1]. The prevalence of osteoporosis increases with age and, in their lifetime, affects up to 30% of women and 12% of men, worldwide [1]. Recently, several treatments have become available for osteoporosis, including estrogen therapy, calcium supplementation, and other hormonal treatments [2, 3].

Chinese herbal medicines have been evaluated for their effects on bone metabolism in preclinical and clinical studies [4]. *Curculigo orchoides* (CO) has been widely used as a traditional Chinese and Indian herbal medicine for osteoporosis [5]. Our previous studies have shown that some components of CO have anti-osteoporotic activity [6]. For example, curculigoside B has been shown to inhibit bone resorption and curculigoside can reverse H_2O_2 -induced stimulation of extracellular signal-regulated kinase 1/2, and nuclear factor- κ B signaling and inhibit p38 mitogen-activated protein kinase activation [6]. Although some mechanisms for the therapeutic action of CO have been previously reported, there are no existing studies that demonstrate the complex mechanisms of action of CO.

CO has been reported to contain saponins, phenols, and phenolic glycosides, triterpenes, triterpenoid glycosides and other compounds [6]. In our previous reports, an ultra-high performance liquid chromatography coupled with electrospray ionization quadrupole time-of-flight tandem mass spectrometry method (UPLC-TOF-MS) was used to identify 45 chemical constituents in CO [6]. However, analysis of several active compounds in CO could not identify the pharmacological targets of CO, possibly because multiple components could hit multiple targets and exert synergistic therapeutic efficacies. Therefore, a comprehensive method that reflects the variation of most components in the crude drug, and more importantly, identifies the targets of the drug, is required.

With the emergence of systems biology, network pharmacology has become a promising paradigm for future drug development [7]. Molecular networks of complex components and multilevel target-based protein and gene interactions can now be constructed for predicting functions of compounds and promoting discovery of active compounds [8]. Thus, the application of network pharmacology could provide new opportunities to understand the interactions between active compounds and relevant targets, which in turn may highlight the mechanisms of action [9]. The aim of this study was to identify the absorbable components, potential targets, and associated treatment pathways of CO using a network pharmacology approach.

Material and Methods

Construction of chemical structures

All chemicals from *Curculigo orchoides* (CO) were collected from the following: ultra-performance liquid chromatography coupled to time-of-flight mass spectrometry (UPLC-TOF-MS) analysis [6]; Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) (<http://lsp.nwsuaf.edu.cn/tcmsp.php>); and literature review. A total of 77 chemicals were identified in CO. And the chemical structures were obtained from the Chemical Book (<http://www.chemicalbook.com>), and presented using ChemDraw software.

Calculation and prediction of absorbable chemical components

ChemDraw software was used to obtain the format of the chemical components. Then, we import the SMILES format into the Molinspiration SMILES website (<http://www.molinspiration.com/>) to calculate the predictive parameters for drug absorption. According to the five principles of drug absorption, a compound could be identified as an absorbable drug if: the hydrogen bond donor (the number of hydrogen atoms attached to the O and N, nOHNH) ≤ 5 ; hydrogen bond acceptor (the number of O and N, nON) ≤ 10 ; fat water partition coefficient (miLogP) ≤ 5 ; and relative molecular mass (MW) ≤ 500 .

Prediction and screening of targets

Using the ChemBio3D Ultra 12.0 informatics system, we transformed the structure of the absorbed components into the MOL2 structure format. To predict possible targets for CO, we imported the components into the public network server of the database of the efficacy group PharmaMapper server website (<http://59.7896.61/pharmmapper/>) to perform reverse docking. The top ten targets of each compound were selected for subsequent study.

The Therapeutic Target Database [10] and Search Tool for the Retrieval of Interacting Genes (STRING) database (version 10.0) (<http://string-db.org>) [11] were used to predict the potential interactions among the targets.

Prediction and screening of pathways and diseases

The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis was performed using the Comparative Toxicogenomics Database (CTD) (<http://ctdbase.org/>) to study the functions and processes that might be altered by the identified targets [12]. The cut-off value for the screening of significant functions and pathways was $P < 0.01$.

The potential targets were connected with the related diseases, which were obtained from the PharmGKB database [13].

Construction of the network

According to the top 40 pathways with their corresponding targets, the diseases, and the components, we constructed a multiple components-multiple target-multiple pathways-multiple disease network using Cytoscape (version 3.4.2; <http://www.cytoscape.org/>). [14] Then, according to the three main targets, we drew a main compounds-main target-main pathway diagram.

Results

Absorption parameters of chemical components

The oral route is a convenient and usual way to deliver drugs to the systemic circulation for patients [15]. In this study, a total of 77 components of *Curculigo orchioi-
des* (CO) were identified. For some natural compounds with poor aqueous solubility, they would be expected to exhibit low efficiency after oral intake, and thereby provide few beneficial therapeutic effects in patients. Therefore, a computer prediction approach was used to calculate the absorption parameters of the identified components.

Table 1 shows the specific absorption parameters of all of the components. The data indicated that there was a total of 32 chemical components that met the five principles of drug absorption.

Target prediction and validation

By importing 32 chemical components that were predicted to be absorbable into the PharmMapper database for directional docking, a total of 83 targets were obtained. These targets were imported into the Comparative Toxicogenomics Database (CTD) database and 58 pathways were obtained that were regulated by CO, with significant differences ($P < 0.01$). The top 40 pathways are listed in Table 2.

Pharmacology network of CO

Using the Cytoscape merge tool, we constructed a pharmacology network for CO, which presented the relationships of the top 40 pathways, targets, diseases, and chemical components. Figure 1 shows the preliminary understanding of the mechanism of CO through this network.

Data analysis showed that the genes for estrogen receptor alpha (*ESR1*) and beta (*ESR2*), and the gene for 11 beta-hydroxysteroid

dehydrogenase type 1, or cortisone reductase (*HSD11B1*) were the main targets of CO. Figure 2 shows the main targets with their corresponding compounds and pathways. Figure 2C shows a molecular docking simulation and that the ten components had strong binding efficiencies with the three main targets of CO.

Discussion

Osteoporosis is characterized by low bone mineral density, leading to increased bone fragility risk [16]. *Curculigo orchioi-
des* (CO) exerts therapeutic effects on osteoporosis. Although this complex Chinese herbal medicine is used for the treatment of various diseases, some questions remain regarding its mechanism of action. In this study, we constructed multilayer networks to predict drug targets in a holistic manner, using a pharmacological drug discovery approach that included identification of the gene-targets and the use of the multiple components-multiple targets-multiple pathways-multiple disease approach. As this study has shown, the availability of large phenotypic and molecular networks may provide an opportunity to study the association between diseases and proteomics datasets.

In this study, data analysis showed that the genes for estrogen receptor alpha (*ESR1*) and beta (*ESR2*), and the gene for 11 beta-hydroxysteroid dehydrogenase type 1, or cortisone reductase (*HSD11B1*) were the main targets of CO. This study also predicted the absorbable chemical components of CO, previously reported to include saponins, phenolic compounds and glycosides [17]. Our previous studies have shown that glycosides from CO could significantly increase bone mineral density, improve the microarchitecture of bone tissue, inhibit the increase of malondialdehyde in serum, and reduce the excretion of urinary calcium in ovariectomized rats [17]. In addition, although the six glycosides, curculigosaponin G, curculigoside B, curculigosaponin E, curculigosaponin J, curculigosaponin L, and curculigoside C, could not be absorbed directly, they could be divided into aglycones, which can be absorbed into the body.

In this study, the validated potential targets for CO were related with seven diseases, including osteoporosis, diabetes, stroke, pancreatitis, myocardial infarction, prostate and breast cancer [18]. Osteoporosis has a relationship with these other six diseases. For example, osteoporosis is one of the major complications of diabetes. Bone marrow mesenchymal stem cells give rise to both osteoblasts and adipocytes, adipokines control energy homeostasis, but also have actions on the skeleton [18]. Patients with chronic pancreatitis may be at an increased risk of low bone density because of malabsorption of vitamin D and calcium, poor diet, pain, alcoholism, and smoking [19]. Prostate

Table 1. Absorption parameters of the components of *Curculigo orchioides* (CO).

| No. | Molecule Name | MW | mLogP | nON | nOHNH | Results |
|-----|---|--------|-------|-----|-------|---------|
| 1 | Palmitic acid | 256.48 | 6.37 | 1 | 2 | × |
| 2 | Neral | 152.26 | 3.19 | 0 | 1 | √ |
| 3 | Sitogluside | 576.95 | 6.34 | 4 | 6 | × |
| 4 | Beta-sitosterol | 414.79 | 8.08 | 1 | 1 | × |
| 5 | (+)-Syringaresinol | 418.48 | 2.1 | 2 | 8 | √ |
| 6 | Stigmasterol | 412.77 | 7.64 | 1 | 1 | × |
| 7 | Oleic acid | 282.52 | 6.84 | 1 | 2 | × |
| 8 | Hyacinthin | 120.16 | 1.52 | 0 | 1 | √ |
| 9 | 3-Methoxyanisole | 138.18 | 1.8 | 0 | 2 | √ |
| 10 | Stearic acid | 284.54 | 7.28 | 1 | 2 | × |
| 11 | Methyl palmitate | 270.51 | 6.62 | 0 | 2 | × |
| 12 | Ethylpalmitate | 284.54 | 6.97 | 0 | 2 | × |
| 13 | Myristic acid | 228.42 | 5.46 | 1 | 2 | × |
| 14 | Pentadecylic acid | 242.45 | 5.91 | 1 | 2 | × |
| 15 | Daturic acid | 270.51 | 6.82 | 1 | 2 | × |
| 16 | ZINC03982454 | 414.79 | 8.08 | 1 | 1 | × |
| 17 | Toluene | 92.15 | 2.32 | 0 | 0 | √ |
| 18 | Tetramethylpyrazine | 136.22 | 0.66 | 0 | 2 | √ |
| 19 | 3,4,5-Trimethoxytoluene | 182.24 | 2.27 | 0 | 3 | √ |
| 20 | Cycloartenol | 426.8 | 7.55 | 1 | 1 | × |
| 21 | Caffeine | 194.22 | -0.1 | 0 | 5 | √ |
| 22 | (E)-6-Methyl-3,5-heptadien-2-one | 152.26 | 2.89 | 0 | 1 | √ |
| 23 | 3,2',4',6'-Tetrahydroxy-4,3'-dimethoxy chalcone | 332.33 | 2.6 | 4 | 7 | √ |
| 24 | 3,3',5,5'-Tetramethoxy-7,9'7',9'-diepoxy-lignan-4,4'-di-O-β-D-glucopyranoside | 742.8 | -1.71 | 8 | 18 | × |
| 25 | Methyl 5-acetyl-1,2,3,5,6-oxatetrazinane-3-carboxylate | 190.19 | -1.6 | 2 | 8 | √ |
| 26 | 4-Methyl heptadecanoic acid | 284.54 | 7.08 | 1 | 2 | × |
| 27 | 4-Acetyl-2-methoxy-5-methyltriacontane | 509.02 | 12.56 | 0 | 2 | × |
| 28 | 5-Methylfurfural | 110.12 | 1.13 | 0 | 2 | √ |
| 29 | Curculigin C | 535.79 | 0.89 | 6 | 11 | × |
| 30 | 2,4,6-Trichloro-3-methoxy-5-methylphenol | 241.5 | 4.03 | 1 | 2 | √ |
| 31 | Curculigosaponin G | 783.12 | 1.58 | 8 | 13 | × |
| 32 | Curculigosaponin G Qt | 474.8 | 4.18 | 3 | 4 | √ |
| 33 | Curculigoside B | 452.45 | 0.55 | 6 | 11 | × |
| 34 | Curculigoside B Qt | 290.29 | 2.45 | 3 | 6 | √ |
| 35 | Curculigoside | 466.48 | 0.8 | 5 | 11 | × |
| 36 | Curcumadiol | 238.41 | 2.56 | 2 | 2 | √ |
| 37 | Curculigoside Qt | 304.32 | 2.7 | 2 | 6 | √ |
| 38 | Tetramethylsuccinamide | 172.26 | -0.79 | 0 | 4 | √ |

Table 1 continued. Absorption parameters of the components of *Curculigo orchoides* (CO).

| No. | Molecule Name | MW | mLogP | nON | nOHNH | Results |
|-----|---|----------|-------|-----|-------|---------|
| 39 | N-acetyl-N-hydroxy-2-carbamic acid methylester | 133.12 | -0.56 | 1 | 5 | √ |
| 40 | 1-Bromo-2-methoxynaphthalene | 237.1 | 3.47 | 0 | 1 | √ |
| 41 | Orcinol glucoside | 286.31 | -0.12 | 5 | 7 | √ |
| 42 | Orcin | 124.15 | 1.78 | 2 | 2 | √ |
| 43 | 2,3,4,7-Tetramethoxyxanthone | 316.33 | 2.9 | 0 | 6 | √ |
| 44 | Lycorine | 287.34 | 0.71 | 2 | 5 | √ |
| 45 | Yuccagenin | 430.69 | 3.67 | 2 | 4 | √ |
| 46 | Corchioside A | 418.44 | -1.36 | 7 | 11 | × |
| 47 | Curculigine A | 531.38 | -0.29 | 7 | 12 | × |
| 48 | 2,4-Dichloro-5-methoxy-3-methylphenol | 207.06 | 3.36 | 1 | 2 | √ |
| 49 | Curculigine B | 501.35 | 0.05 | 6 | 11 | × |
| 50 | Curculigosaponin A | 636.96 | 2.43 | 6 | 9 | × |
| 51 | Curculigosaponin E_qt | 474.8 | 4.18 | 3 | 4 | √ |
| 52 | Curculigosaponin B | 606.93 | 2.94 | 5 | 8 | √ |
| 53 | Curculigosaponin C | 769.09 | 1.2 | 8 | 13 | × |
| 54 | Curculigosaponin D | 799.12 | 0.69 | 9 | 14 | × |
| 55 | Curculigosaponin E | 931.25 | -0.55 | 11 | 18 | × |
| 56 | Curculigosaponin F | 961.28 | -1.06 | 12 | 19 | × |
| 57 | Curculigosaponin J_qt | 474.8 | 4.18 | 3 | 4 | √ |
| 58 | Curculigosaponin G | 783.12 | 1.58 | 8 | 13 | × |
| 59 | Curculigosaponin H | 915.25 | 0.34 | 10 | 17 | × |
| 60 | 2,3,5-Trimethylphenathrene | 220.33 | 5.11 | 0 | 0 | × |
| 61 | Cynanuricoside A_qt | 923.27 | 2.9 | 4 | 16 | × |
| 62 | Curculigosaponin I | 945.28 | -0.17 | 11 | 18 | × |
| 63 | Curculigosaponin J | 1,077.41 | -1.41 | 13 | 22 | × |
| 64 | Curculigosaponin K | 963.3 | -0.88 | 13 | 19 | × |
| 65 | Curculigosaponin L_qt | 476.82 | 4.36 | 4 | 4 | √ |
| 66 | Curculigosaponin L | 785.14 | 1.76 | 9 | 13 | × |
| 67 | Curculigosaponin M | 1,077.41 | -1.19 | 14 | 22 | × |
| 68 | Curculigosaponin M_qt | 458.8 | 5.28 | 3 | 3 | × |
| 69 | Curculigenin A | 474.8 | 4.18 | 3 | 4 | √ |
| 70 | Curculigenin B | 476.82 | 4.36 | 4 | 4 | √ |
| 71 | Curculigenin C | 458.8 | 5.28 | 3 | 3 | × |
| 72 | [5-Hydroxy-2-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxyphenyl]methyl 3-hydroxy-2,6-dimethoxybenzoate | 482.48 | 0.53 | 6 | 12 | × |
| 73 | Curculigoside C_qt | 320.32 | 2.43 | 3 | 7 | √ |
| 74 | 2,6-Dimethoxybenzoic acid | 182.19 | 1.4 | 1 | 4 | √ |
| 75 | Curculigol | 456.83 | 6.59 | 2 | 2 | × |
| 76 | 2-Propyl-1-heptanol | 158.32 | 3.57 | 1 | 1 | √ |
| 77 | DBQ | 220.34 | 3.13 | 0 | 2 | √ |

Table 2. Top 40 KEGG pathways regulated by *Curculigo orchoides* (CO) ($P < 0.01$).

| No | Pathway | Pathway ID | P-value | q-value | Annotated genes quantity |
|----|---|-------------|----------|----------|--------------------------|
| 1 | Metabolic pathways | KEGG: 01100 | 3.99E-10 | 5.98E-08 | 15 |
| 2 | Prostate cancer | KEGG: 05215 | 7.06E-09 | 1.06E-06 | 6 |
| 3 | Progesterone-mediated oocyte maturation | KEGG: 04914 | 3.22E-07 | 4.83E-05 | 5 |
| 4 | Bile secretion | KEGG: 04976 | 1.06E-07 | 1.59E-05 | 5 |
| 5 | Galactose metabolism | KEGG: 00052 | 2.66E-07 | 3.99E-05 | 4 |
| 6 | Purine metabolism | KEGG: 00230 | 1.63E-04 | 0.02446 | 4 |
| 7 | Natural killer cell mediated cytotoxicity | KEGG: 04650 | 8.12E-05 | 0.01217 | 4 |
| 8 | T cell receptor signaling pathway | KEGG: 04660 | 2.69E-05 | 0.00403 | 4 |
| 9 | Insulin signaling pathway | KEGG: 04910 | 8.55E-05 | 0.01283 | 4 |
| 10 | Pancreatic secretion | KEGG: 04972 | 2.41E-05 | 0.00362 | 4 |
| 11 | Hepatitis C | KEGG: 05160 | 6.71E-05 | 0.01006 | 4 |
| 12 | Steroid hormone biosynthesis | KEGG: 00140 | 1.14E-04 | 0.01716 | 3 |
| 13 | Amino sugar and nucleotide sugar metabolism | KEGG: 00520 | 7.89E-05 | 0.01184 | 3 |
| 14 | MAPK signaling pathway | KEGG: 04010 | 0.00997 | 1 | 3 |
| 15 | ErbB signaling pathway | KEGG: 04012 | 4.05E-04 | 0.06079 | 3 |
| 16 | Cell cycle | KEGG: 04110 | 0.00104 | 0.15528 | 3 |
| 17 | Oocyte meiosis | KEGG: 04114 | 8.17E-04 | 0.1225 | 3 |
| 18 | p53 signaling pathway | KEGG: 04115 | 1.73E-04 | 0.02602 | 3 |
| 19 | Wnt signaling pathway | KEGG: 04310 | 0.00211 | 0.3172 | 3 |
| 20 | Focal adhesion | KEGG: 04510 | 0.00437 | 0.65545 | 3 |
| 21 | Regulation of actin cytoskeleton | KEGG: 04810 | 0.00497 | 0.7454 | 3 |
| 22 | Endocrine and other factor-regulated calcium reabsorption | KEGG: 04961 | 6.63E-05 | 0.00994 | 3 |
| 23 | Gastric acid secretion | KEGG: 04971 | 2.22E-04 | 0.03331 | 3 |
| 24 | Alzheimer's disease | KEGG: 05010 | 0.0025 | 0.37548 | 3 |
| 25 | Tuberculosis | KEGG: 05152 | 0.00302 | 0.45366 | 3 |
| 26 | Colorectal cancer | KEGG: 05210 | 1.39E-04 | 0.0208 | 3 |
| 27 | Acute myeloid leukemia | KEGG: 05221 | 9.82E-05 | 0.01472 | 3 |
| 28 | Small cell lung cancer | KEGG: 05222 | 3.44E-04 | 0.0516 | 3 |
| 29 | Non-small cell lung cancer | KEGG: 05223 | 8.35E-05 | 0.01252 | 3 |
| 30 | Primary immunodeficiency | KEGG: 05340 | 2.67E-05 | 0.004 | 3 |
| 31 | Glutathione metabolism | KEGG: 00480 | 0.00299 | 0.44822 | 2 |
| 32 | Folate biosynthesis | KEGG: 00790 | 2.46E-04 | 0.03683 | 2 |
| 33 | Metabolism of xenobiotics by cytochrome P450 | KEGG: 00980 | 0.00565 | 0.84694 | 2 |
| 34 | Drug metabolism – cytochrome P450 | KEGG: 00982 | 0.00595 | 0.89321 | 2 |
| 35 | PPAR signaling pathway | KEGG: 03320 | 0.00595 | 0.89321 | 2 |
| 36 | Apoptosis | KEGG: 04210 | 0.00869 | 1 | 2 |
| 37 | Hedgehog signaling pathway | KEGG: 04340 | 0.00396 | 0.59355 | 2 |
| 38 | VEGF signaling pathway | KEGG: 04370 | 0.00693 | 1 | 2 |
| 39 | NOD-like receptor signaling pathway | KEGG: 04621 | 0.00409 | 0.61306 | 2 |
| 40 | Long-term potentiation | KEGG: 04720 | 0.00549 | 0.82423 | 2 |

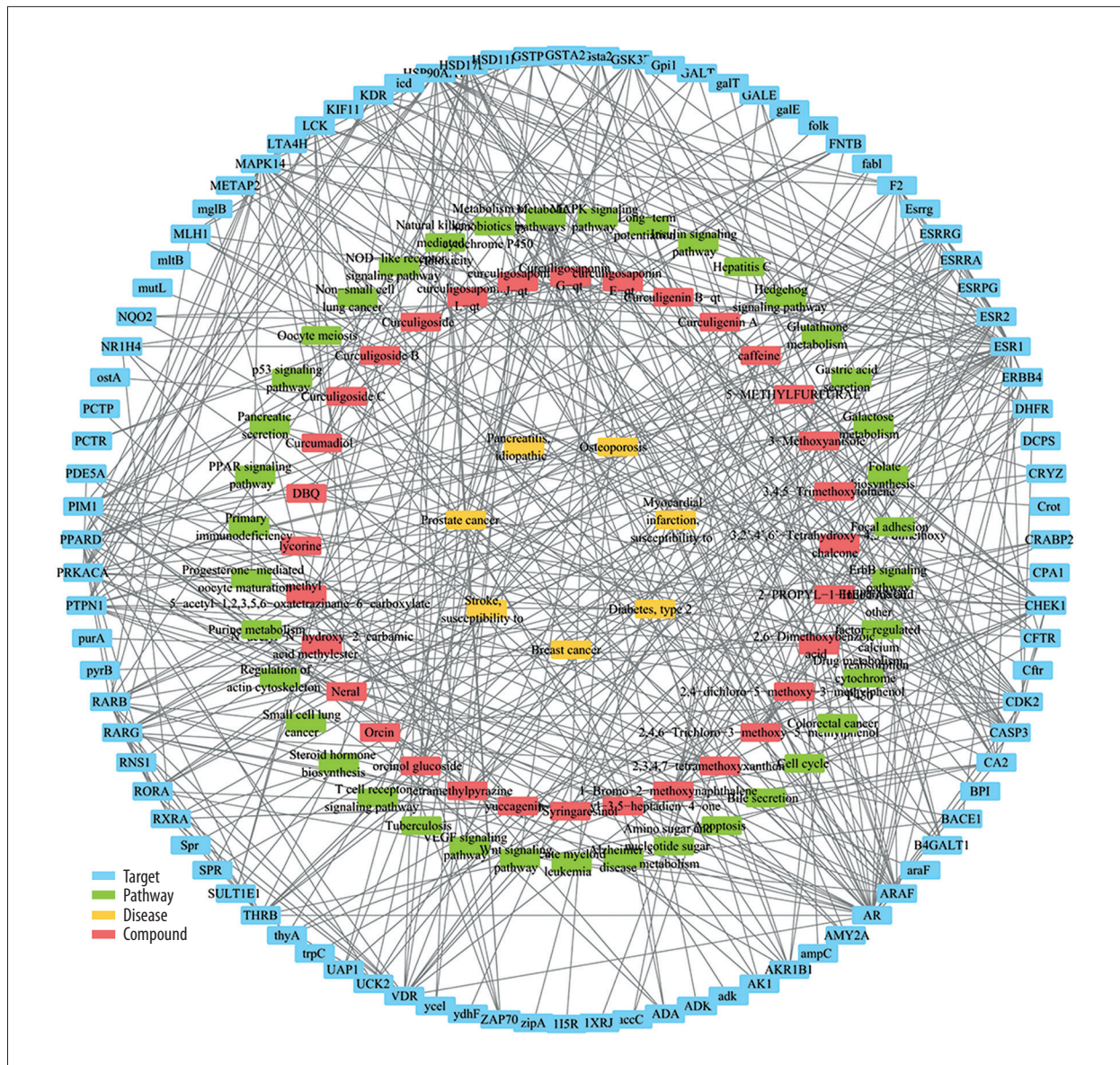


Figure 1. Multiple components-multiple target-multiple pathways-multiple disease network. The yellow circle represents the diseases; the green circle represent the pathways; the red circle represents the components; and the blue circle represents the targets.

and breast cancer patients experience osteoporosis resulting from accelerated loss of bone mineral density caused by their treatment [20]. This suggests that CO might be effective not only in the treatment of primary osteoporosis, but also in the prevention of secondary osteoporosis [21].

Based on illustration of the main targets with their corresponding compounds, we found three major gene targets for CO: *ESR1*, *ESR2*, and *HSD11B1*. *ESR1* and *ESR2* genes encode estrogen receptors, involved in pathological processes including breast cancer, endometrial cancer, and osteoporosis [22]. *ESR1* is expressed in osteoblasts and osteoclasts, and is associated with postmenopausal osteoporosis of the spine in

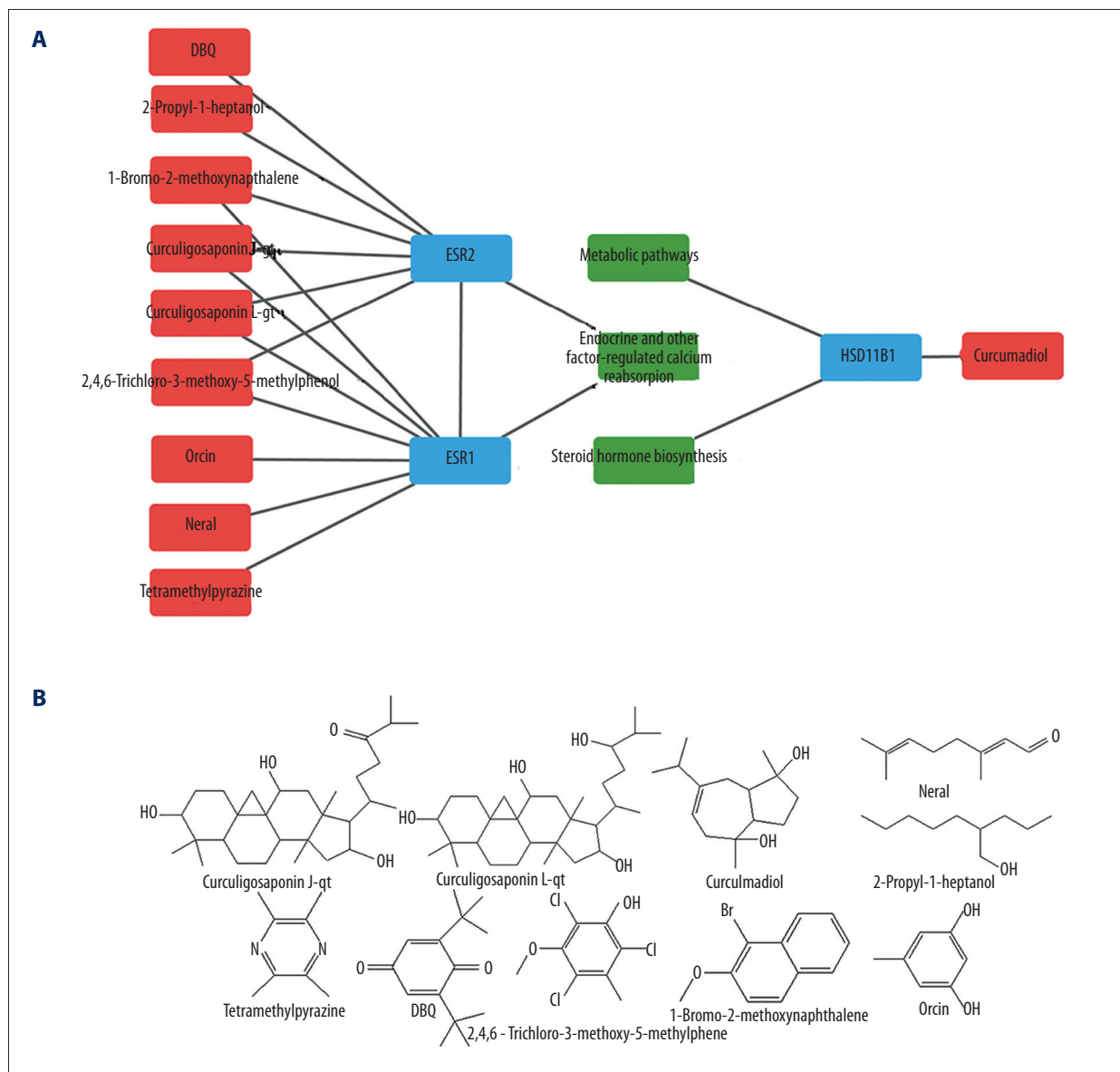
women [22,23]. This gene could be employed as a selection method to identify individuals at increased risk of osteoporosis [23]. In a previous large population-based cohort study, variants in the *ESR2* gene were associated with an increased risk of vertebral fracture in postmenopausal women [24]. The 11 beta-hydroxysteroid dehydrogenase type 1 gene (*HSD11B1*) is a primary regulator catalyzing the reduction of inactive cortisone to active cortisol [25]. Polymorphisms of the 11 β -HSD1 gene have been previously shown to affect the function of the 11 β -HSD1 enzyme and *HSD11B1* polymorphisms have been shown to be predictive of bone mineral density and the risk of bone fracture in postmenopausal women without a clinically apparent hypercortisolemia [25].

In this study, endocrine and other factors regulated calcium reabsorption, steroid hormone biosynthesis, and metabolic pathways were related with the main targets and ten corresponding compounds. Estrogen is an important steroid hormone that is involved in the process of osteoblast differentiation regulated by bone morphogenetic proteins (BMPs) and tumor necrosis factor (TNF)- α [26]. BMPs could increase the sensitivity of estrogen receptors, whereas estrogen differentially regulates BMP-Smad and TNF- α signaling [26].

Ethanol extracts of CO have been shown to possess estrogenic activity. A molecular docking simulation was performed and the results (Figure 2C) showed that the ten components had strong binding efficiencies with the three main targets of CO.

These compounds were considered as the main components that mediated the estrogen-like efficacy of CO.

This is the first report to show the mechanism of CO using a network pharmacology approach, and we successfully predicted the main targets and pathways for CO, providing the basis for further research. This approach would also benefit other studies of Chinese herbal medicines and complex drugs. The findings of this study indicate that CO, a widely used herbal medicine for the treatment of osteoporosis, has its therapeutic effects through multiple targets and multiple pathways. However, it must be stressed that computational models can only provide network data-driven indications for complex therapeutic compounds and the findings of this study should be verified by controlled clinical studies and real-world evidence.



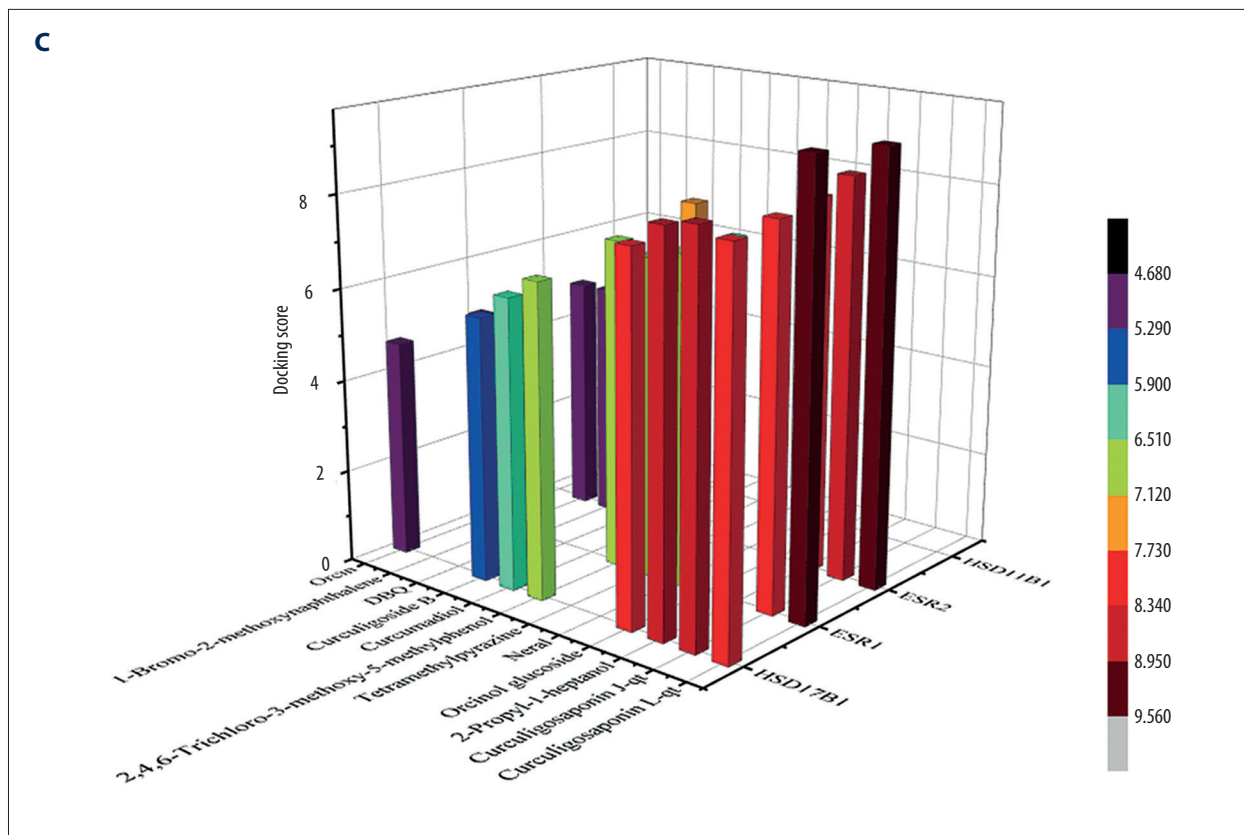


Figure 2. Network of major targets of *Curculigo orchoides* (CO) with corresponding compounds and pathways. (A) The major targets of *Curculigo orchoides* (CO). (B) Chemical structures of the ten compounds. (C) Molecular docking simulation shows that the ten components had strong binding efficiencies with the three main targets of CO.

Conclusions

This study has demonstrated a novel approach for the investigation of the mechanism of action of the Chinese herbal medicine, *Curculigo orchoides* (CO), by combining absorption property screening, target prediction, and network pharmacology.

References:

- Cheng W, Meng H, Xin W et al: Differentiation of bone marrow mesenchymal stem cells in osteoblasts and adipocytes and its role in treatment of osteoporosis. *Med Sci Monit*, 2016; 22: 226–33
- Comas-Calonge A, Figueiredo R, Gay-Escoda C: Surgical treatment vs. conservative treatment in intravenous bisphosphonate-related osteonecrosis of the jaws. Systematic review. *J Clin Exp Dent*, 2017; 9: e302–7
- Wang G, Wang J, Sun D et al: Short-term hypoxia accelerates bone loss in ovariectomized rats by suppressing osteoblastogenesis but enhancing osteoclastogenesis. *Med Sci Monit*, 2016; 22: 2962–71
- Wei X, Xu A, Shen H et al: Qianggu capsule for the treatment of primary osteoporosis: Evidence from a Chinese patent medicine. *BMC Complement Altern Med*, 2017; 17: 108
- Rufus P, Mohamed N, Shuid AN: Beneficial effects of traditional Chinese medicine on the treatment of osteoporosis on ovariectomized rat models. *Curr Drug Targets*, 2013; 14: 1689–93
- He Y, Dong X, Jia X et al: Qualitative and quantitative analysis on chemical constituents from *Curculigo orchoides*, using ultra high performance liquid chromatography coupled with electrospray ionization quadrupole time-of-flight tandem mass spectrometry. *J Pharm Biomed Anal*, 2015; 102: 236–45
- Wei ZJ, Zhou XH, Fan BY et al: Proteomic and bioinformatic analyses of spinal cord injury-induced skeletal muscle atrophy in rats. *Mol Med Rep*, 2016; 14: 165–74
- Xie W, Ji L, Zhao T et al: Identification of transcriptional factors and key genes in primary osteoporosis by DNA microarray. *Med Sci Monit*, 2015; 21: 1333–44
- Yu G, Zhang Y, Ren W, et al: Network pharmacology-based identification of key pharmacological pathways of Yin-huang-Qing-Fei capsule acting on chronic bronchitis. *Int J Chronic Obstr*, 2017; 12: 85–94
- Zhu F, Shi Z, Qin C et al: Therapeutic target database update 2012: A resource for facilitating target-oriented drug discovery. *Nucleic Acids Res*, 2012; 40(D1): D1128–36
- Szklarczyk D, Franceschini A, Wyder S et al: STRING v10: Protein-protein interaction networks, integrated over the tree of life. *Nucleic Acids Res*, 2015; 43: D447–52
- Davis AP, Grondin CJ, Johnson RJ et al: The Comparative Toxicogenomics Database: Update 2017. *Nucleic Acids Res*, 2017; 45: D972–78

13. Whirl-Carrillo M, McDonagh E, Hebert J et al: Pharmacogenomics knowledge for personalized medicine. *Clin Pharmacol Ther*, 2012; 92(4): 414–17
14. Shannon P, Markiel A, Ozier O et al: Cytoscape: A software environment for integrated models of biomolecular interaction networks. *Genome Res*, 2003; 13: 2498–504
15. Wang ZQ, Li JL, Sun YL et al: Chinese herbal medicine for osteoporosis: A systematic review of randomized controlled trails. *Evid-Based Compl Alt Medicine*, 2013; 2013: 356260
16. Li C, Wei G, Gu Q et al: Proliferation and differentiation of rat osteoporosis mesenchymal stem cells (MSCs) after telomerase reverse transcriptase (TERT). *Med Sci Monit*, 2015; 21: 845–54
17. Liu L, Guo YH, Xin HL et al: Antiosteoporotic effects of benzylbenzoate glucosides from *Curculigo orchioides* in ovariectomized rats. *J Chin Integr Med*, 2012; 10(12): 1419–26
18. Sheu Y, Amati F, Schwartz AV et al: Vertebral bone marrow fat, bone mineral density and diabetes: The Osteoporotic Fractures in Men (MrOS) study. *Bone*, 2017; 97: 299–305
19. Hoogenboom SA, Lekkerkerker SJ, Fockens P et al: Systematic review and meta-analysis On the prevalence of vitamin D deficiency in patients with chronic pancreatitis. *Pancreatology*, 2016; 16(5): 800–6
20. Garg A, Leitzel K, Ali S et al: Antiresorptive therapy in the management of cancer treatment-induced bone loss. *Curr Osteoporos Rep*, 2015; 13(2): 73–77
21. Kapoor S: Osteoporosis prevention and beyond: The systemic beneficial effects of *Curculigo orchioides*. *Maturitas*, 2008; 61(4): 371
22. Zhou W, He KH, Chen MH: Correlation between polymorphisms in the estrogen receptor α gene and coronary heart disease: A meta-analysis. *Genet Mol Res*, 2016; 15: 1–8
23. Shang DP, Lian HY, Fu DP et al: Relationship between estrogen receptor 1 gene polymorphisms and postmenopausal osteoporosis of the spine in Chinese women. *Genet Mol Res*, 2016; 15: 1–6
24. Morón FJ, Galán JJ, Ruiz A: Controlled ovarian hyperstimulation pharmacogenetics: A simplified model to genetically dissect estrogen-related disease. *Pharmacogenomics*, 2017; 8(7): 775–85
25. Hwang JY, Lee SH, Kim GS et al: Polymorphisms predicted bone mineral density and fracture risk in postmenopausal women without a clinically apparent hypercortisolemia. *Bone*, 2009; 45(6): 1098–103
26. Lv H, Sun Y, Zhang Y: MiR-133 is involved in estrogen deficiency-induced osteoporosis through modulating osteogenic differentiation of mesenchymal stem cells. *Med Sci Monit*, 2015; 21: 1527–34