Original Article
Utilizing network pharmacology to explore the underlying mechanism of *Cinnamomum cassia* Presl in treating osteoarthritis

Guowei Zhou, Ruoqi Li, Tianwei Xia, Chaoqun Ma, Jirong Shen

Affiliated Hospital of Nanjing University of Chinese Medicine, Nanjing 210029, Jiangsu, China

Received July 30, 2019; Accepted October 8, 2019; Epub December 15, 2019; Published December 30, 2019

**Abstract:** Objective: The network pharmacology method was adopted to establish the relationship between “Ingredients-Disease Targets-Biological Pathway”, screen the potential target of *Cinnamomum cassia* Presl in treating osteoarthritis (OA), and explore the underlying mechanism. Methods: Chemical components and selected targets related to *Cinnamomum cassia* Presl were retrieved from BATMAN-TCM. OA disease targets were searched from TTD, DrugBank, OMIM, GAD, PharmGKB, and DisGeNET databases. Canonical SMILES were obtained from Pubchem, and targets were obtained from SwissTargetPrediction. We constructed a target interaction network graph by using the STRING database and Cytoscape 3.2.1, and screened key targets by using the MCC algorithm of cytoHubba. Enrichment analysis of the GO function and KEGG pathway was conducted using the DAVID database. Results: Eighteen active compounds derived from *Cinnamomum cassia* Presl and 40 intersections between *Cinnamomum cassia* Presl and OA were obtained. Ten key targets were screened by the MCC algorithm, including PTGS2, MAPK8, MMP9, ALB, MMP2, MMP1, SERPINE1, TGFB1, PLAU, and ESR1. The GO analysis consisted of 33 enrichment results, including biological processes (e.g., collagen catabolic process and inflammatory response), cell composition (e.g., extracellular space and extracellular matrix), and molecular function (e.g., heme binding and aromatase activity). A total of 32 pathways were enriched by KEGG, including osteoclast differentiation, arachidonic acid metabolism, hypoxia-inducible factor (HIF)-1, nuclear factor κB (NF-κB), Toll-like receptors (TLRs), and tumor necrosis factor (TNF). Conclusion: This study predicted the main possible mechanism of *Cinnamomum cassia* Presl in treating OA, including anti-inflammation, regulating cellular proliferation, differentiation and apoptosis, and antioxidation, which provided a theoretical basis for exploring active ingredients and experimental research about *Cinnamomum cassia* Presl against OA.

**Keywords:** Network pharmacology, *Cinnamomum cassia* Presl, osteoarthritis, mechanism

**Introduction**

Osteoarthritis (OA) is a chronic disease that occurs in articular cartilage and impacts the ligaments, synovium, articular capsule and subchondral bone, leading to joint deformity and loss of function [1]. OA is a common disease in middle-aged and elderly patients, and the disability rate is > 50% [2]. The etiology of OA is still unclear [3]. Modern medicine can only relieve symptoms and many of them also have side effects [4]. It is of great value to excavate potential active ingredients for the treatment of OA from traditional Chinese medicine. OA is often referred to as “bi zheng” in ancient Chinese medical books. Traditional Chinese medicine often used *Cinnamomum Cassia* to treat OA.

*Cinnamomum Cassia* is the dry bark of *Cinnamomum cassia*. Modern pharmacologic studies have found that cinnamaldehyde and other monomers in *Cinnamomum Cassia* have the following properties: anti-inflammatory; regulating cell proliferation; antioxidant and other therapeutic effects on OA [5, 6]. The toxicity is relatively small. These studies indicated that *Cinnamomum Cassia* have the potential ability to treat OA; however, *Cinnamomum Cassia* has multi-component characteristics, and its mechanism is relatively complex.

As a new research method, network pharmacology breaks away from the traditional research mode of “one target, one disease, one drug” [7]. The systematism of the strategy resonates well with the holistic view of traditional Chinese
Underlying mechanism of *Cinnamomum cassia* Presl against OA

**Methods**

*Active compounds screening of cinnamomum cassia*

BATMAN-TCM (http://bionet.ncpsb.org/batman-tcm), an online bioinformatics analysis database, can be used for target prediction of traditional Chinese medicine components, functional analysis of targets, visualization of related networks, and comparative analysis of traditional Chinese medicine. The active compounds in *Cinnamomum Cassia* were collected from the BATMAN-TCM (http://bionet.ncpsb.org/batman-tcm) database (with “ROUGUI” as the search term) [12].

*Target fishing of cinnamomum cassia*

All active compounds in *Cinnamomum Cassia* were input to the Pubchem database (https://pubchem.ncbi.nlm.nih.gov/) to obtain the Canonical SMILES. SwissTargetPrediction (http://www.swisstargetprediction.ch/) is a network platform that can predict small molecule targets on the basis of the 2D and 3D similarity measurements of ligands [13]. First, the Can-
Underlying mechanism of *Cinnamomum cassia* Presl against OA


**Disease targets database building and collection of cinnamomum cassia-OA common target**

The OA-associated disease targets were searched from TTD (https://db.idrblab.org/ttd/), DrugBank (https://www.drugbank.ca/), OMIM (http://www.omim.org/), GAD (https://geneti-cassiodb.nih.gov/), PharmGKB (https://www.pharmgkb.org/), and DisGeNET (http://www.disgenet.org/web/DisGeNET/menu/home) 6 databases with “osteo-arthritis” as a key word. The common targets of *Cinnamomum Cassia* and OA were obtained.

**Construction of network and analysis**

The active compounds and common targets were put into Cytoscape 3.2.1 to draw “Drug-Compounds-Targets Network”. The common targets were also uploaded to the STRING database (http://string-db.org/) for building a protein interaction network. Next, the MCC algorithm of the cytoHubba plug-in of the Cytoscape 3.2.1 was used to screen key targets [15].

**Enrichment analysis of the go function and kegg pathway**

Database for Annotation, Visualization and Integrated Discovery (DAVID, https://david.ncifcrf.gov/, v6.8) was used for gene ontology (GO) enrichment analysis. Pathway enrichment analysis was accomplished using Kyoto Encyclopedia of Genes and Genomes (KEGG, http://www.kegg.jp/) data obtained from DAVID [16].

**Results**

**Active compounds of cinnamomum cassia**

A total of 18 active herbal ingredients were retrieved from the BATMAN-TCM (Table 1). They are cinnamic aldehyde (cinnamaldehyde), procyanidin B2, coumarin, melilotocarpan A, cinnamyl benzoate, cinnamyl alcohol, anethole, procyanidin B1, procyanidin B5, protocatechuic acid, cinnamyl acetate, procyanidin C1, (-)-epicatechin-3-o-gallate, trans-cinnamic acid, ethyl cinnamate, cinnamic ccid, styrene, and procyanidin B7.

**Prediction and analysis active compound targets of Cinnamomum cassia**

Eight hundred twenty-one targets were obtained, which were reduced to 335 after duplication removal. A total of 3247 targets were screened from the GeneCards database, and 1396 after banishing duplication. There were 169 targets in GeneCards and SwissTarget-Prediction (Figure 2). Then, the “compound-predicted” network was built by Cytoscape 3.2.1 software (Figure 3).

**Collection of OA targets and acquisition of cinnamomum cassia (RG)-OA common targets**

Six hundred four targets were obtained from six databases, and the number was reduced to 531 after removing duplication. Forty common targets were obtained that intersected with *Cinnamomum Cassia* (RG)-associated targets (Figure 4). They are ABCC1, C1R, AKR1C1, CA3, BCHE, CA2, MMP13, CTSK, PLAU, MMP1, SERPINE1, ADAM17, BCL2, TGFβ1, MMP2, MMP9, AR, NFKB1, MAPK8, ALB, PTGS2, ESR1, TLR9, ESRR2, NOS2, CYP19A1, CYP1A1, ACHE, CYP1A2, CYP2C19, ABCB1, PTGS1, CES1, AKR1C3, AKR1B1, PLA2G2A, ALOX5, MPO, TTR, and TDO2.

Forty targets were uploaded to the STRING database to draw the Protein-Protein Interaction (PPI) network (Figure 5A). There were 39 nodes (isolated nodes were removed) and 184 sides (network nodes represent targets, and lines represent the interactions of targets). The average node degree was 9.2. “Cinnamomum Cassia (RG)-Compounds-Targets Network” was drawn by Cytoscape 3.2.1 (Figure 5B). The information on protein interactions was uploaded to the Cytoscape 3.2.1, and the MCC algorithm (the higher the score, the higher the significance) of the cytoHubba plug-in was used to screen out 10 key targets (Figure 5C). Key targets that rank from more importance-to-less importance are PTGS2, MAPK8, MMP9, ALB,
Underlying mechanism of *Cinnamomum cassia* Presl against OA

Table 1. Basic information of 18 candidate compounds of *Cinnamomum cassia*

<table>
<thead>
<tr>
<th>Compound</th>
<th>Pubchem ID</th>
<th>Canonical SMILES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cinnamic Aldehyde/Cinnamaldehyde</td>
<td>637511</td>
<td>C1=CC=C(C=C1)C=CC=0</td>
</tr>
<tr>
<td>Coumarin</td>
<td>323</td>
<td>C1=CC=C2C=C1C=C=C=C2</td>
</tr>
<tr>
<td>Melilotoxcarpan A</td>
<td>4484393</td>
<td>C0C1=CC2=C(C=C1)C3OC3C4=C(C32)C=CC=C4O</td>
</tr>
<tr>
<td>Cinnamyl benzoate</td>
<td>5705112</td>
<td>C1=CC=C(C=C1)C=COC(=O)C2=CC=CC=C2</td>
</tr>
<tr>
<td>Cinnamyl alcohol</td>
<td>5315892</td>
<td>C1=CC=C(C=C1)C=CC0</td>
</tr>
<tr>
<td>Anethole</td>
<td>637563</td>
<td>C0C1=CC=C(C=C1)C=CC0</td>
</tr>
<tr>
<td>Protocatechuic Acid</td>
<td>72</td>
<td>C1=CC(=CC1=C=C1)C=C0</td>
</tr>
<tr>
<td>Cinnamyl acetate</td>
<td>5282110</td>
<td>C(C(=O)O)CC1=CC=C1C=C1</td>
</tr>
<tr>
<td>Trans-Cinnamic Acid</td>
<td>444539</td>
<td>C1=CC=C(C=C1)C=C0</td>
</tr>
<tr>
<td>Ethyl cinnamate</td>
<td>637597</td>
<td>C(C(=O)C)C1=CC=C1C=C1</td>
</tr>
<tr>
<td>Cinnamic Acid</td>
<td>444539</td>
<td>C1=CC=C(C=C1)C=C0</td>
</tr>
<tr>
<td>Styrene</td>
<td>7501</td>
<td>C=C1C=CC=C1</td>
</tr>
<tr>
<td>Procyanidin B7</td>
<td>474541</td>
<td>C1(C(O)C2=CC(=CC=C2)C3(C(C4=CC(=CC(=CC4)O)O)O)O)O)O)O)O)O)O)O</td>
</tr>
</tbody>
</table>

A total of 48 GO enrichment results were obtained during this study, and 33 results were screened out according to $P < 0.05$. The top 20 results were shown in Table 2. They are collagen catabolic process, heme binding, extracellular space, extracellular matrix, aromatase activity, and so on.

Similarly, 36 KEGG enrichment results were obtained in this analysis, and 28 results were collected based on the threshold of $P < 0.05$. The Top 20 results were shown in Figure 6. They are pathways in cancer, toxoplasmosis, Chagas disease, MicroRNAs in cancer, tuberculosis, ovarian steroidogenesis, Leishmaniasis, prolactin signaling pathway, hepato-

Figure 2. Venn diagram of *Cinnamomum cassia* targets. The blue circle represents the targets predicted by the Genecards, and the red circle represents the targets predicted by the SwissTargetPrediction.

MMP2, MMP1, SERPINE1, TGFB1, PLAU, and ESR1.
Figure 3. Compound-predicted targets network. The square nodes represent the compounds of Cinnamomum Cassia, the round nodes represent the targets. The node color changes from orange-to-blue reflect the degree value changes from low-to-high in the network.
Underlying mechanism of *Cinnamomum cassia* Presl against OA

Discussion

According to the investigations, it was suggested that the global age-standardized prevalence of knee OA is 3.8% [1]. With the development of molecular biology, disease-modifying osteoarthritis drugs have become a major focus of research. The positive effects and relatively small toxicity of *Cinnamomum Cassia* in treating OA have attracted the attention of scientists and researchers [5, 6].

Eighteen compounds of *Cinnamomum Cassia* were retrieved from the BATMAN-TCM database in this study. The targets of such compounds were further predicted by Swisstarget prediction and were obtained in combination with GeneCards database retrieval, thus 169 *Cinnamomum Cassia*-associated targets were obtained. OA-associated targets were retrieved from the 6 online databases. A total of 40 intersection targets between OA targets and *Cinnamomum Cassia*-associated targets were obtained. It can be seen from the PPI diagram and the “drug-compounds-targets” diagram that the active compounds of *Cinnamomum Cassia* have more than one targets. Such targets can also interact with multiple compounds. Moreover, 10 key targets can be further obtained from the MCC algorithm of the cytoHubba plug-in of Cytoscape 3.2.1. There are PTGS2, MAPK8, MMP9, ALB, MMP2, MMP1, SERPINE1, TGFβ1, PLAU, and ESR1. The results of KEGG pathway enrichment showed that pathways in cancer, Toxoplasmosis, Chagas disease, microRNAs in cancer, and tuberculosis were the most significant signaling pathways (Figure 3). These pathways are complex and many downstream pathways are included. For example, downstream pathways of cancer signaling pathways include the Wnt signaling pathway, Hedgehog signaling pathway, Notch signaling pathway, MAPK signaling pathway, and vascular endothelial growth factor signaling pathways.

In combination with the common OA signaling pathways and after the exclusion of cancer-associated pathways and other broad pathways, *Cinnamomum Cassia* can be predicted to have effects in treating OA by an anti-inflammatory effect, and mediate cell proliferation, differentiation, and apoptosis, thus promoting the balance between osteoblast (OB) and osteoclast (OC) and the antioxidant effects.

Anti-inflammatory

Enrichment analysis results showed that the inflammation-associated signaling pathways mainly enrich NF-κB, TLRs, TNF, arachidonic acid metabolism-associated signaling pathways and other signaling pathways. Inflammatory factors, such as IL-1β, play an important role in the pathogenesis of OA [17]. IL-1β is an important inflammatory factor causing OA and can aggravate OA-associated inflammation by activating NF-κB and other signaling pathways, leading to an increase in protease expression in the downstream of inflammatory reaction, accelerating the degradation of proteoglycan...
Underlying mechanism of *Cinnamomum cassia* Presl against OA

**Figure 5.** A. Protein-protein interaction. B. Drug-Compound-Target Network. The red squares represent the drug, *Cinnamomum Cassia* (RG); green triangles represent compounds; and yellow rounds represent targets. C. Key Target Proteins. The node color changes from yellow-to-red reflect the MCC value changes from low-to-high in the network.
Underlying mechanism of *Cinnamomum cassia* Presl against OA

and collagen, and damaging articular cartilage [18-23]. The preliminary study of this research group showed that cinnamaldehyde can protect cartilage by mediating signal pathways such as NF-κB to resist IL-1β-induced inflammation on chondrocytes [6]. In addition, type-A procyanidine polyphenols extracted from the bark of *Cinnamomum zeylanicum* was also reported to have disease-modifying potential in animal models of inflammation and arthritis in rats [24]. Youn et al. have reported that cinnamaldehyde suppressed the activation of NF-κB and IRF3 induced by LPS, leading to the decreased expression of target genes, such as cyclooxygenase-2 (COX-2) and Interferon-β (IFN-β), in RAW264.7 macrophages [25].

Mediate cell proliferation, differentiation, apoptosis, and promote the balance between OB and OC

In the enrichment results, such OC differentiation-related pathways, such as HIF-1, and the downstream pathways of cancer signaling pathways, such as the Wnt [26] and MAPK signaling pathways, are also closely correlated to such effects [27]. Wang et al. found that a high concentration of cinnamaldehyde significantly inhibits the growth of synovial fibroblasts in OA patients [28]. Wu et al. found that administration of cinnamaldehyde promotes osteogenesis in ovariectomized rats and differentiation of osteoblast in vitro [29]. The results of western blot supported that cinnamaldehyde promotes the maturation and differentiation of osteoblasts for cinnamaldehyde and apparently enhances the expression of runx2, ocn and col1a1 [29]. Moreover, with the treatment of cinnamaldehyde, the number of osteoclasts in ovariectomized rats is gradually decreased, so bone resorption activity was inhibited [29]. Zhang et al. found that cinnamaldehyde inhibits proliferation and bone resorption of osteoclast induced by dexamethasone, which may be mediated by down-regulation of receptor activator of NF kappaB (RANK) and nuclear factor of activating T cells 1 (NFATc1) mRNA [30].

Antioxidant effects

Previous studies have shown that the mitochondrial dysfunction of chondrocytes and synovial cells in patients with OA produces a large amount of NO and reactive oxygen species, which can cause oxidative damage. According to the previous study, cinnamaldehyde can increase the activity of catalase, superoxide dismutase and glutathione peroxidase, and

<table>
<thead>
<tr>
<th>Category</th>
<th>GO ID</th>
<th>Description</th>
<th>Genes</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BP</td>
<td>GO:0030574</td>
<td>collagen catabolic process</td>
<td>5</td>
<td>8.18E-09</td>
</tr>
<tr>
<td>MF</td>
<td>GO:0020037</td>
<td>heme binding</td>
<td>8</td>
<td>4.07E-08</td>
</tr>
<tr>
<td>CC</td>
<td>GO:0005615</td>
<td>extracellular space</td>
<td>12</td>
<td>1.23E-06</td>
</tr>
<tr>
<td>CC</td>
<td>GO:0031012</td>
<td>extracellular matrix</td>
<td>5</td>
<td>7.00E-05</td>
</tr>
<tr>
<td>MF</td>
<td>GO:0070330</td>
<td>aromatase activity</td>
<td>3</td>
<td>1.54E-04</td>
</tr>
<tr>
<td>MF</td>
<td>GO:0004222</td>
<td>metalloendopeptidase activity</td>
<td>5</td>
<td>2.26E-04</td>
</tr>
<tr>
<td>MF</td>
<td>GO:0004601</td>
<td>peroxidase activity</td>
<td>3</td>
<td>6.68E-04</td>
</tr>
<tr>
<td>BP</td>
<td>GO:0045893</td>
<td>positive regulation of transcription, DNA-templated</td>
<td>5</td>
<td>0.001653109</td>
</tr>
<tr>
<td>MF</td>
<td>GO:0005496</td>
<td>steroid binding</td>
<td>3</td>
<td>0.001704889</td>
</tr>
<tr>
<td>MF</td>
<td>GO:0008270</td>
<td>zinc ion binding</td>
<td>9</td>
<td>0.00338197</td>
</tr>
<tr>
<td>MF</td>
<td>GO:0005506</td>
<td>iron ion binding</td>
<td>4</td>
<td>0.005819584</td>
</tr>
<tr>
<td>MF</td>
<td>GO:0003990</td>
<td>acetylcholinesterase activity</td>
<td>2</td>
<td>0.006506693</td>
</tr>
<tr>
<td>BP</td>
<td>GO:0010469</td>
<td>regulation of receptor activity</td>
<td>2</td>
<td>0.007896778</td>
</tr>
<tr>
<td>MF</td>
<td>GO:0004497</td>
<td>monooxygenase activity</td>
<td>3</td>
<td>0.008190211</td>
</tr>
<tr>
<td>BP</td>
<td>GO:0006979</td>
<td>response to oxidative stress</td>
<td>3</td>
<td>0.008534181</td>
</tr>
<tr>
<td>CC</td>
<td>GO:0072562</td>
<td>blood microparticle</td>
<td>3</td>
<td>0.009127945</td>
</tr>
<tr>
<td>BP</td>
<td>GO:0001666</td>
<td>response to hypoxia</td>
<td>3</td>
<td>0.00916887</td>
</tr>
<tr>
<td>MF</td>
<td>GO:0004666</td>
<td>prostaglandin-endoperoxide synthase activity</td>
<td>2</td>
<td>0.009744585</td>
</tr>
<tr>
<td>BP</td>
<td>GO:0019371</td>
<td>cyclooxygenase pathway</td>
<td>2</td>
<td>0.010515617</td>
</tr>
<tr>
<td>BP</td>
<td>GO:0006954</td>
<td>inflammatory response</td>
<td>4</td>
<td>0.012832271</td>
</tr>
</tbody>
</table>
inhibit the oxidation of chondrocytes. Cinnamomum Cassia polyphenols also have strong reducibility [31]; and the higher the content, the stronger the antioxidant ability [32]. In addition, enrichment analysis results showed that the estrogen, prolactin, sphingolipid, and other signaling pathways indirectly affect the growth and repair of articular cartilage, thus playing a role in treating OA.

Conclusion

In conclusion, the complex mechanism of Cinnamomum Cassia in treating OA with multiple components, targets, and pathways was studied with a theoretical approach in this study by network pharmacology to provide thoughts and a basis for future studies. Due to the limitation of certain conditions, this study had the following shortcomings. First, TCM often uses Cinnamomum Cassia as a decoction, but this study did not consider the absorption and metabolism of monomers in the body. Second, the content of different compounds in Cinnamomum Cassia and the interactions (synergism or antagonism) between monomers after oral administration were not taken into account. Third, the number of Cinnamomum Cassia-OA intersection targets obtained was small. We believe that with the development of technology and in-depth study, the underlying mecha-
Underlying mechanism of *Cinnamomum cassia* Presl against OA

Acknowledgements

We are grateful to all the coworkers and partners in this study. This work was supported by Jiangsu Province Hospital of Chinese Medicine Project (Y2018CX54).

Disclosure of conflict of interest

None.

Abbreviations

OA, Osteoarthritis; TCM, traditional Chinese medicine; NF-kB, nuclear factor-kappa B; MAPKs, mitogen-activated protein kinase; HIF, hypoxia-inducible factor-1; NF-κB, nuclear factor κB; TLRs, Toll-like receptors; TNF, tumor necrosis factor; LPS, lipopolysaccharide; COX-2, cyclooxygenase-2; IFN-β, interferon-β; OB, osteoblast; OC, osteoclast; RANK, receptor activator of NF kappaB; NFATc1, nuclear factor of activating T cells 1.

Address correspondence to: Chaoqun Ma and Jirong Shen, Affiliated Hospital of Nanjing University of Chinese Medicine, Nanjing 210029, Jiangsu, China. E-mail: mcq_1964@sina.com (CQM); joint-66118@sina.com (JRS)

References


[15] Chin CH, Chen SH, Wu HH, Ho CW, Ko MT and Lin CY. cytoHubba: identifying hub objects and...
Underlying mechanism of *Cinnamomum cassia* Presl against OA

13369


