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Network pharmacology-based pharmacological mechanism prediction on *Eucommia ulmoides* against rheumatoid arthritis

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Abstract

Rheumatoid arthritis (RA) is a common chronic autoimmune disease characterized by synovial inflammation and progressive joint destruction. Eucommia ulmoides (EU) is a kidney-tonifying Chinese medicine that has been applied to treat RA for decides. The present study aims to explore pharmacological mechanisms of EU against RA using network pharmacology approach. Traditional Chinese Medicine Systems Pharmacology (TCMSP) database was used to screen active ingredients of EU, and their relative targets were fished from UniProt database. RA-related targets were screened from GeneCards database and DisGeNET database. The overlapping genes between EU and RA were identified by Venn diagram, and further analyzed for protein-protein interaction (PPI), Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG). Fifty active ingredients were identified in EU, and corresponded to 207 targets. Meanwhile, 499 targets were closely associated with RA development. A total of 50 overlapping genes between EU and RA were identified, which were regarded as therapeutically relevant. GO enrichment analysis indicated that EU exerted antiRA effects depending on regulating multiple biological processes including inflammatory response, oxidative stress, cell apoptosis and matrix catabolism. Several key pathways such as TNF pathway, IL-17 pathway, T cell receptor pathway, NOD-like receptor pathway and Toll-like receptor pathway, were involved in the above biological processes. Network pharmacology revealed that EU exerts therapeutic effects on RA through multi-ingredients, multi-targets and multi-pathways, which provides basis for its clinical application and promising directions for subsequent research.

Abbreviations: ALB = serum albumin, BC = Betweenness Centrality, BP = Biological Processes, CC = Closeness Centrality, CCL2 = C-C motif chemokine 2, CXCL8 = interleukin-8, DAVID = the Annotation, Visualization and Integrated Discovery, DC = Degree Centrality, DL = drug-likeness, EU = eucommia ulmoides, GO = Gene ontology, KEGG = Kyoto encyclopedia of genes and genomes, IL-1 beta = interleukin-1B, IL-6 = interleukin-6, IL10 = interleukin-10, IL-17 = interleukin-17, JUN = transcription factor AP-1, MMP9 = matrix metalloproteinase-9, OB = oral bioavailability, PPI = protein-protein interaction, PTGS2 = prostaglandin G/H synthase 2, RA = rheumatoid arthritis, TCM = traditional Chinese medicine, TCMSP = Traditional Chinese Medicine System Pharmacology, TNF = tumor necrosis factor, VCAM1 = vascular cell adhesion protein 1, VEGFA = vascular endothelial growth factor A.

Keywords: *Eucommia ulmoides*, rheumatoid arthritis, network pharmacology, traditional Chinese medicine

YY and ZT contributed equally to this work.

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The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request. All our main data used to support the findings of this study have been deposited by the corresponding author. The datasets supporting the conclusions of this article are available in public database from TCMSP, UniProt, GeneCards, DisGeNET, String, DAVID, KEGG.

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1. Introduction

Rheumatoid arthritis (RA) is a common chronic autoimmune disease with typical clinical symptoms: tender, swollen and morning stiffness.[1] With the progression of RA, the affected joints can be physical deformity and disabilities.[2] It is estimated that 1% of worldwide population were affected by RA, resulting in a severe social burden.[3] Currently, the treatment guidelines for RA are consist of nonsteroidal antiinflammatory drugs, glucocorticoids and disease-modifying antirheumatic drugs.[4] Although these drugs clinically alleviate the symptoms of RA, their long-term use will cause a cascade of side effects on neuronal, gastrointestinal, and immune systems.[5] Therefore, it is imperative to explore safe and effective therapeutic strategies for RA.

Nowadays, traditional Chinese medicine (TCM) is widely applied in various chronic diseases due to its constitution of safe natural herbs. Eucommia ulmoides (EU) also called Du-Zhong, a typical kidney-tonifying herb, has been largely reported in treatment of RA alone or mixed in prescriptions.[6] EU has an effective inhibition of synovitis in RA animal models and can prevent cartilage destruction.[7,8] The antiRA effects of EU is well clarified from the TCM theory of "kidney governing bone". RA occurs due to the deficiency of kidney, while EU nourishes kidney to protect bone joints. Nevertheless, the modern pharmacological mechanism of EU against RA remains largely unclear.

Unlike the single-target drug with a clear mechanism, there are multi-ingredients and multi-targets in herbs. The traditional research strategy of "one drug, one target" can not meet the needs to study all the contained ingredients as a whole.[9] Network pharmacology is a novel approach integrated pharmacology, biology, bioinformatics and computer science.[10] With the aid of "Medicine - Target - pathway- Disease" research mode and abundant database information, network pharmacology provides a systematic and integrative understanding on pharmacologic action of TCM/herbs.[11] Thus, more and more researches used network pharmacology to elucidate the mechanism of TCM/herbs on diseases.[12,13] In the present study, we tried to explore the mechanism of EU against RA through network pharmacology method. The flowchart of our study is shown in Figure 1.

2. Material and Methods

Ethical approval was waived or not necessary, all procedures performed in studies do not involve human participants or annimals.

2.1. Collection of active ingredients of EU

The chemical ingredients of EU were collected from the Traditional Chinese Medicine Systems Pharmacology (TCMSP) database (http://tcmspw.com/tcmsp.php), a common pharmacological platform contained 499 herbs registered in Chinese pharmacopoeia (2015) and related chemical ingredients.[14] Oral bioavailability (OB) and druglikeness (DL) were set as the main parameters to screen the active ingredients according to the absorption, distribution, metabolism and excretion process of oral drugs. OB, a major pharmacokinetic parameter of oral drugs, respects the proportion of drug delivery to the systemic circulation. DL is defined as the compound properties including solubility and chemical stability, and is used to evaluated whether a compound is similar to existing drugs. In the present study, we set OB ≥ 30% and DL ≥ 0.18 to select the candidate active ingredients of EU.[15]

2.2. Targets prediction of active ingredients

We screened the active ingredient-related targets using the prediction system developed by the TCMSP, and transformed the target name to a standard gene symbols and ID in the Uniport database (https://www.uniprot.org/) with the filter of "Homo sapiens" and "swiss-prot reviewed".

2.3. RA-related targets

RA-related target genes were collected from the DisGeNET database (https://www.disgenet.org/) and GeneCards database (https://www.genecards.org/) with the keyword "rheumatoid arthritis". After filtered with score > 0.1 in DisGeNET and score > 10 in GeneCards and removed the duplicated genes, we finally obtained a total of 449 RA-related targets.

2.4. Venn diagram

Venny 2.1.0 (https://bioinfogp.cnb.csic.es/tools/venny/) was used to identify the overlapping targets between ingredient targets and disease targets. A total of 50 overlapping target genes were obtained for further bioinformatic analyses.

2.5. Protein-protein interaction (PPI)

With the parameters of "Homo sapiens" and "confidence score > 0.4", the overlapping genes of EU and RA were analyzed in STRING (https://string-db.org/) to create a protein-protein interaction (PPI) network. The node-node data exported from STRING database were analyzed their interconnection network using the visualized software Cytoscape 3.7.1. Three topological parameters including degree centrality (DC), betweenness centrality (BC) and closeness centrality (CC) were used to calculate the pivotal node.

2.6. GO enrichment analysis

Gene ontology (GO) enrichment analysis was performed on the overlapping genes by importing them into the Annotation, Visualization and Integrated Discovery (DAVID, https://david.ncifcrf.gov/home.jsp, version 6.8) database. We detailedly analyzed 3 subitems including molecular function, cellular component and biological process (BP) with the filter of FDR > 0.5. The information of top 30 BP terms were listed in a bubble diagram according to the ascending order of log *P*-value.

2.7. KEGG enrichment analysis

KEGG enrichment analysis was performed on the overlapping genes by importing them into the Kyoto Encyclopedia of Genes and Genomes (KEGG, https://www.kegg.jp/) database. Based on the descending order of gene number enriched in each pathway, we listed the top 30 signaling pathways in a bar diagram.

3. Network construction

The Cytoscape 3.7.1 software was used to establish the following visualized graphs of networks: 1. Herb-Ingredient-Target-Disease network. 2. Target-Pathway network.

4. Results

4.1. Prediction of active ingredients and target genes of EU

Through searching "Eucommia ulmoides" in the TCMSP database and setting the thresholds with DL ≧ 0.18 and OB ≧ 30%,

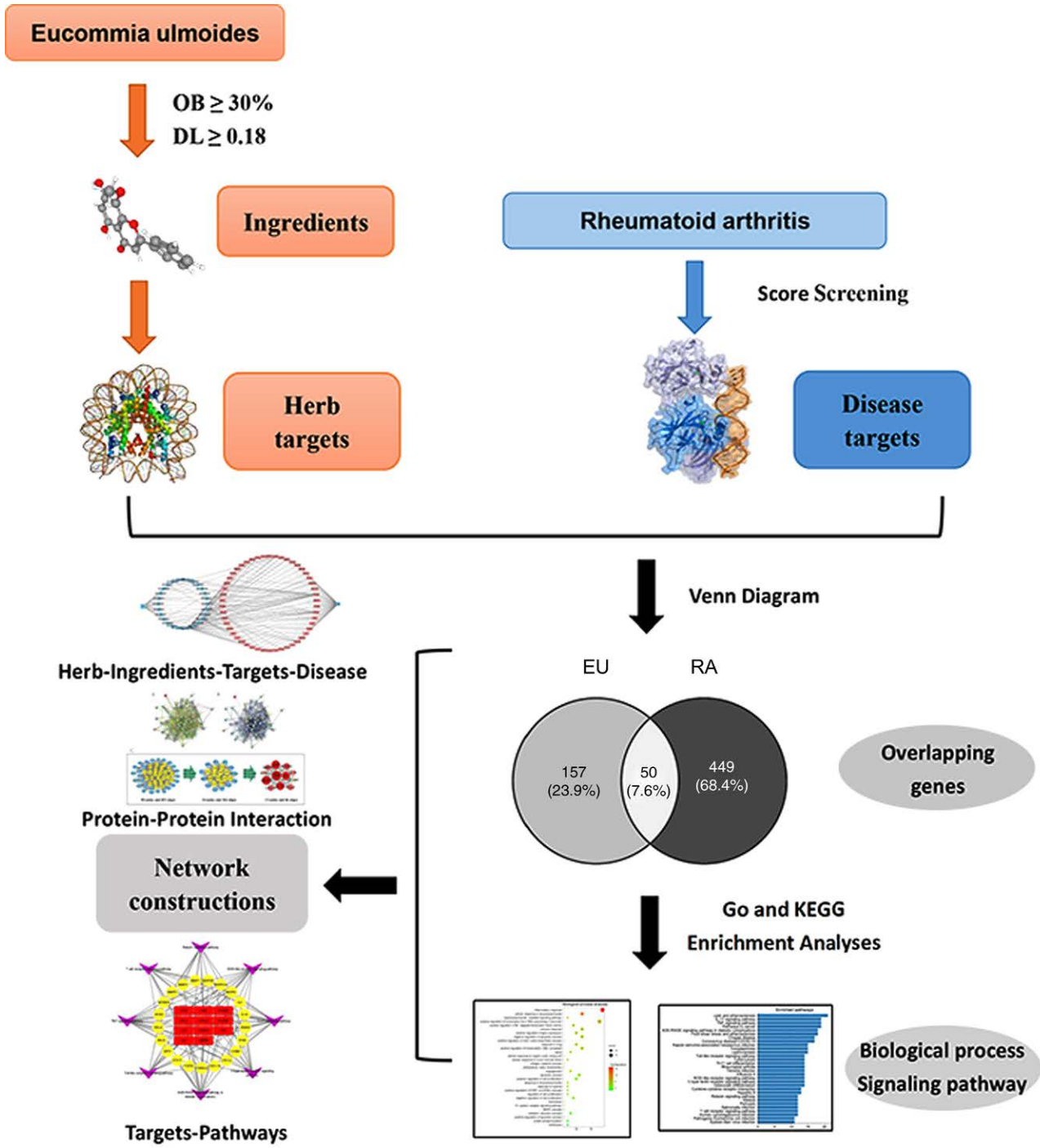

Figure 1. The flow diagram of network pharmacology-based research for investigating potential pharmacological mechanism of EU against RA.

a total of 28 active ingredients were identified in EU. These active ingredients were used to fish the related targets through using the target prediction system. After inputting the related target name into the Uniprot database, we finally obtained 207 target genes of EU.

4.2. Overlapping targets between EU and RA

Meanwhile, we searched "rheumatoid arthritis" in GeneCards database and DisGeNet database with the thresholds of given scores, and obtained 499 target genes. Through collecting the co-part between EU and RA in the Venn diagram (Fig. 2), we identified a total of 50 overlapping genes that were regarded as the therapeutic targets of EU against RA. Moreover, a Herb-Ingredient-Target-Disease network was constructed (Fig. 3). Based on the edge number connected target nodes and ingredient nodes, we identified the top 4 ingredients, MOL000098 (quercetin, OB = 46.43, DL = 0.28), MOL000422 (kaempferol, OB = 41.88, DL = 0.24), MOL002773 (beta-carotene, OB = 37.18, DL = 0.58) and MOL000358 (beta-sitosterol, OB = 36.91, DL = 0.75) that were connected with 43, 19, 7 and 6 overlapping genes, respectively. Therefore, these 4 active ingredients with high edge number played a critical role in the antiRA effects of EU.

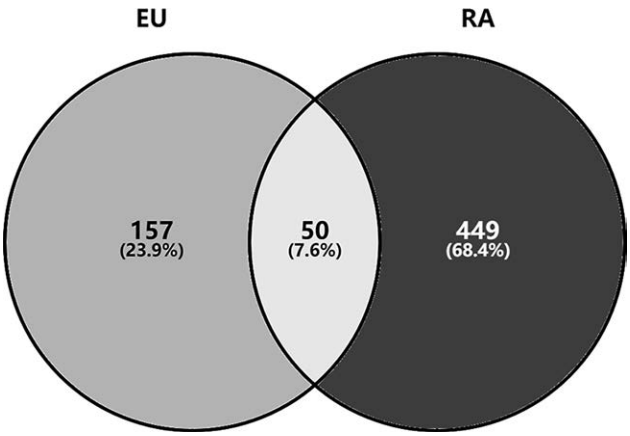

Figure 2. The Venn diagram of target genes identified in EU and RA. The overlap part indicates the potential therapeutic targets through which EU treats RA.

4.3. PPI network and hub therapeutic targets

Then, we imported these 50 overlapping genes into STRING database to establish a PPI network. According to the strength of evidence level or confidence level, a PPI network with 50 nodes and 692 edges was plotted (Fig. 4A, B). Furthermore, 3 parameters including Degree, Betweenness, and Closeness were used to select the pivot nodes. After screening with the thresholds of Degree ≥ 20, Betweenness ≥ 0.003 and Closeness ≥ 0.700, we obtained 24 nodes and 264 edges. Through screening with the thresholds of Degree ≥ 30, Betweenness ≥ 0.008 and Closeness ≥ 0.800, 12 nodes and 66 edges were identified. Thus, the remained genes, tumor necrosis factor (TNF), serum albumin (ALB), vascular endothelial growth factor A (VEGFA), interleukin-6 (IL-6), interleukin-1 beta (IL1B), prostaglandin G/H synthase 2 (PTGS2), matrix metalloproteinase-9 (MMP9), interleukin-8 (CXCL8), C-C motif chemokine 2 (CCL2), interleukin-10 (IL10), transcription factor AP-1 (JUN), vascular cell adhesion protein 1 (VCAM1) were considered as the hub therapeutic targets of EU (Fig. 4C). Their detail information were presented in Table 1 according to the descending order of Degree.

4.4. Biological processes of EU for RA treatment

GO enrichment analysis on these 50 overlapping genes was performed in the DAVID database. After screening with the thresholds of FDR < 0.05, a total of 113 GO items were obtained, and BP subset occupied a primary position (Fig. 5A). Thus, a bubble diagram were build to list the contents of top 30 BP terms (Fig. 5B). As the diagram shown, these 30 BP terms could be summarized into 4 category, inflammatory response, oxidative stress, cell apoptosis and matrix catabolism. In the aspect of inflammatory response, inflammatory response (GO:0006954), cellular response to lipopolysaccharide (GO:0071222), lipopolysaccharide-mediated signaling pathway (GO:0031663), positive regulation of NF-kappaB transcription factor activity (GO:0051092), immune response (GO:0006955), cellular response to tumor necrosis factor (GO:0071356), response to lipopolysaccharide (GO:0032496) were involved. In the aspect of oxidative stress, positive regulation of nitric oxide biosynthetic process (GO:0045429), angiogenesis (GO:0001525), response to hypoxia (GO:0001666) and oxidation-reduction process (GO:0055114) were involved. The category of cell apoptosis contained positive regulation of apoptotic process (GO:0043065), apoptotic process (GO:0006915), negative regulation of apoptotic process (GO:0043066), and matrix catabolism category included collagen catabolic process (GO:0030574), extracellular matrix disassembly (GO:0022617) and proteolysis (GO:0006508). These findings indicated that EU exacts therapeutic effects on RA possibly through a multi-biological process synergetic way.

4.5. Signaling pathways of EU for RA treatment

To predict the potential signaling pathways involved in the antiRA effects of EU, these 50 overlapping genes were performed with KEGG enrichment analysis. Based on the descending order of count number contained in each pathway, we listed the top 30 pathways (Fig. 6A), among which IL-17 signaling

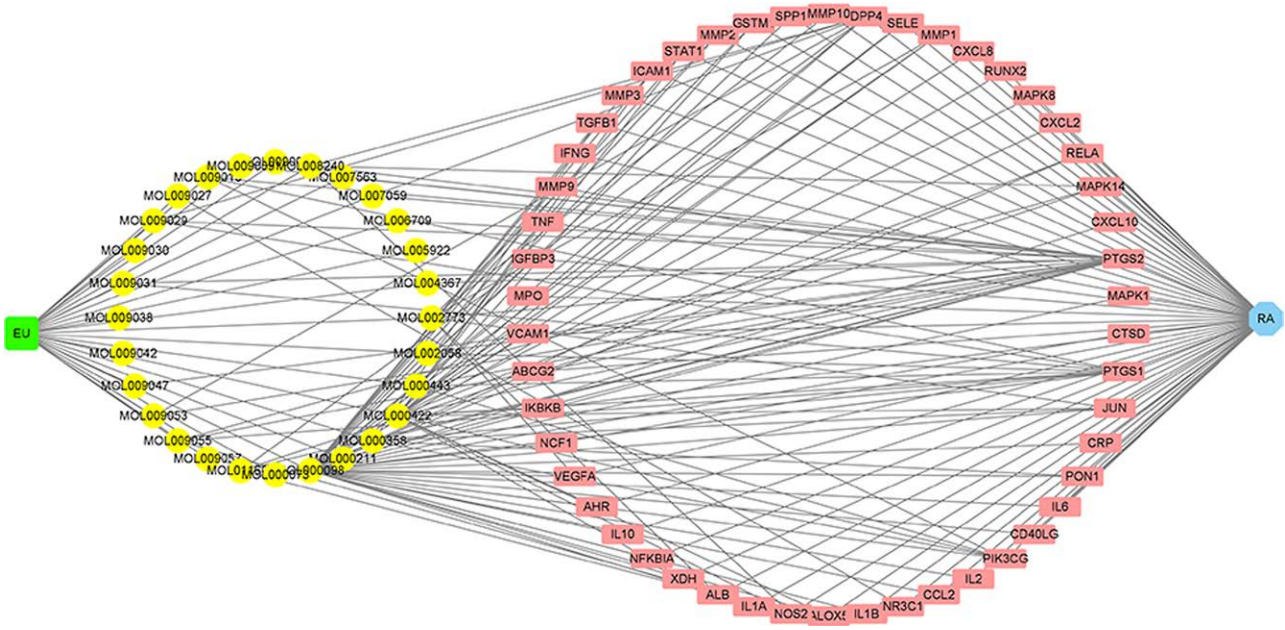

Figure 3. Herb-Ingredient-Target-Disease network construction. The green quadrate node represents EU, the yellow round nodes represent the ingredients, the pink oblong nodes represent targets and the blue hexagon node represents RA.

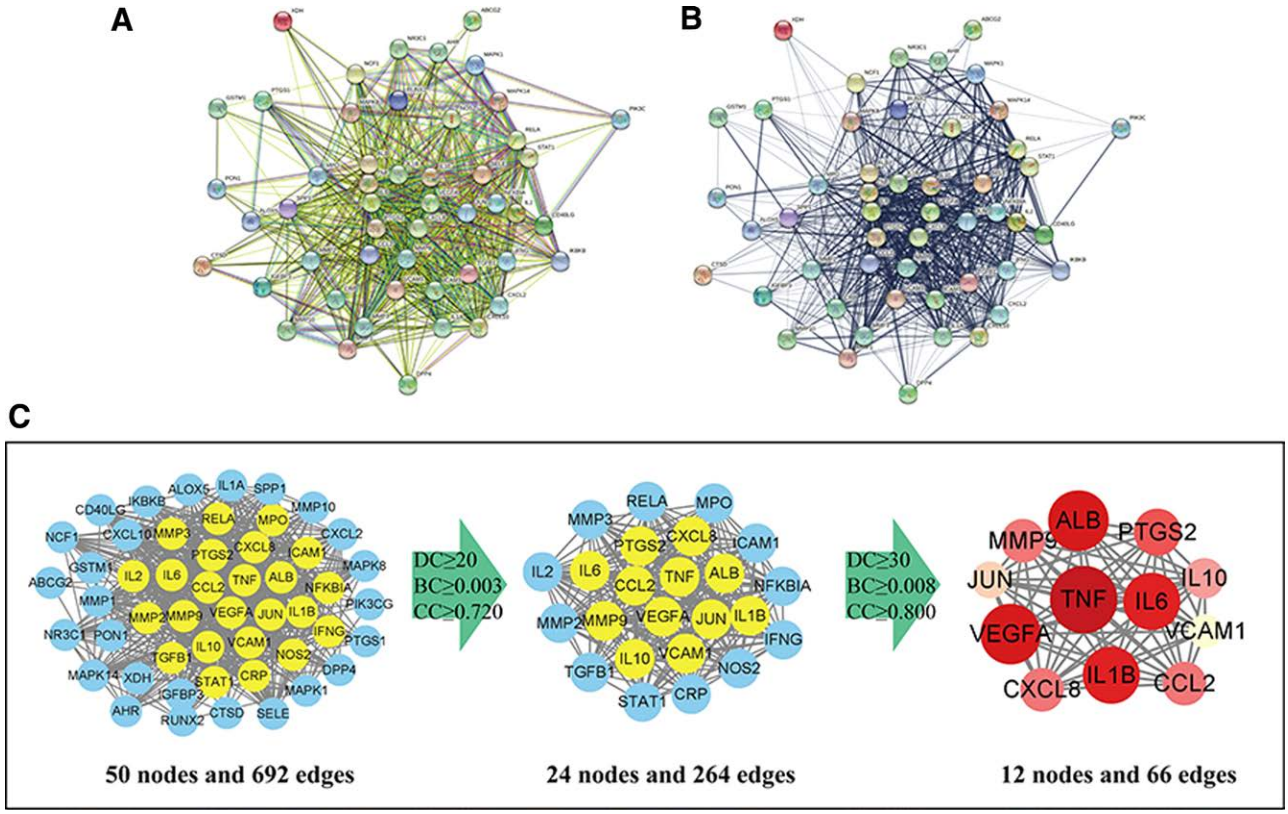

Figure 4. Construction and screening of PPI network. (A) PPI networks constructed using String database. Line colors and line thickness indicates the type of interactive evidence and the confidence level of the supporting data, respectively. (B) The topological screening process on PPI network with Degree Centrality (DC), Betweenness Centrality (BC), and Closeness Centrality (CC). The third image indicated that the node with higher DC value represents bigger size and more brilliant color.

Table 1

Information of 12 hub targets.

Uniprot ID	Gene symbol	Protein name	Degree
P01375	TNF	Tumor necrosis factor	48
B7WNR0	ALB	Serum albumin	46
P15692	VEGFA	Vascular endothelial growth factor A	46
P05231	IL-6	Interleukin-6	45
P01584	IL1B	Interleukin-1 beta	45
P35354	PTGS2	Prostaglandin G/H synthase 2	42
P14780	MMP9	Matrix metalloproteinase-9	41
P10145	CXCL8	Interleukin-8	41
P13500	CCL2	C-C motif chemokine 2	41
P22301	IL10	Interleukin-10	40
P05412	JUN	Transcription factor AP-1	39
P19320	VCAM1	Vascular cell adhesion protein 1	38

pathway, TNF signaling pathway, AGE-RAGE signaling pathway in diabetic complications, Toll-like receptor signaling pathway, NOD-like receptor signaling pathway, C-type lectin receptor signaling pathway, Relaxin signaling pathway and T cell receptor signaling pathway were involved in the above biological processes. Furthermore, a Targets-Pathway network was conducted to present the therapeutic targets enriched in the pathways (Fig. 6B).

5. Discussion

RA is a chronic and erosive autoimmune disease characterized by synovial inflammation and progressive joint structure destruction.[16] The current treatments have certain effects on alleviating joint swelling and pain at early stage of RA, but they are difficult to reverse disease progression once joint destruction appears.[17] In addition, the side effects caused by long-time use of antiRA drugs are calling for more safer and more effective therapeutic agents. EU is a valuable TCM medicine, and has shown curative effects on RA both in clinic and animal.[6–8] In this study, we comprehensively explored its underlying mechanisms against RA through network pharmacology approach.

In Herb-Ingredient-Target-Disease network analysis, the 4 major ingredients including quercetin, kaempferol, beta-carotene and beta-sitosterol were identified. According to the current literature, quercetin can delay the progression of RA through reducing inflammatory cytokines, promoting apoptosis of inflammatory synoviocytes, and protecting inflammatory

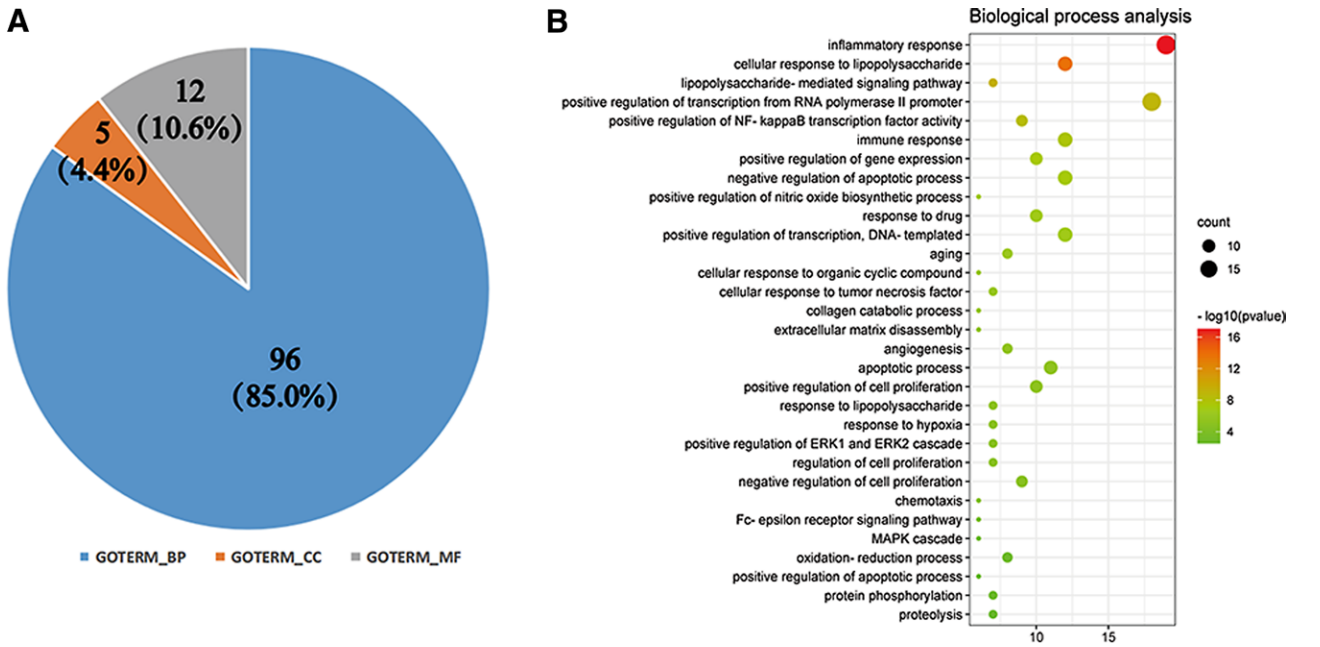

Figure 5. GO enrichment analysis on 50 overlapping genes. (A) The percentage of Three types of GO items (GOTRIM_BP: biological process; GOTRIM_MF: molecular function; GOTRIM_CC: cellular component) in DAVID database. (B) The bubble diagram of the 31 BP items (FDR > 0.5) according to descending order of *P* value.

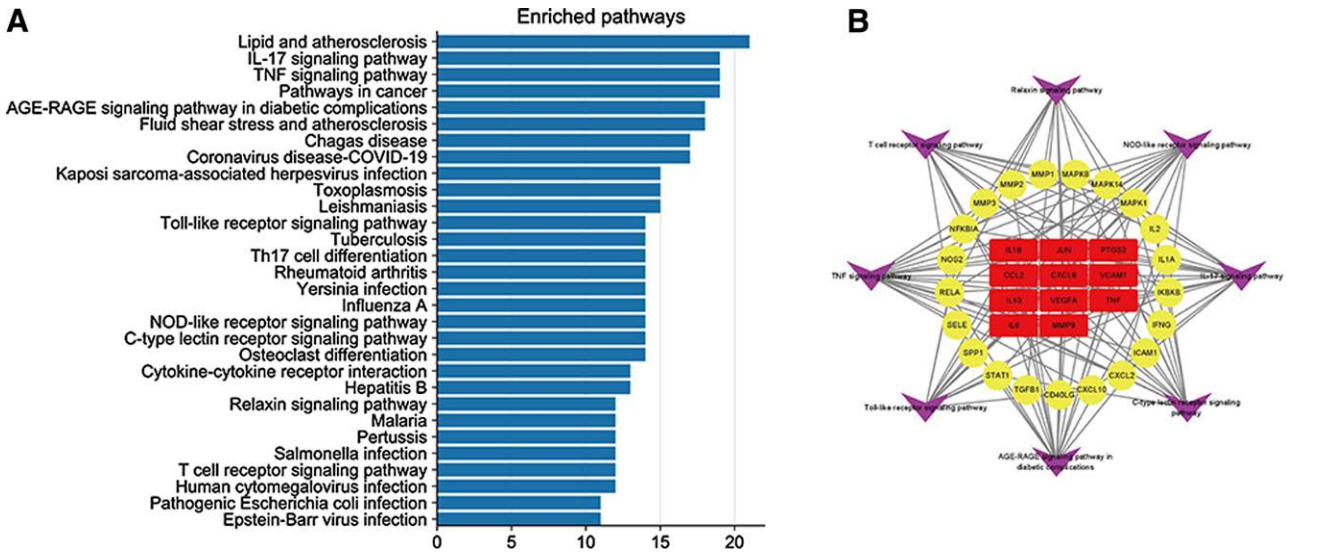

Figure 6. KEGG enrichment analysis and Target-Pathway network construction. (A) Details of the top 30 pathways in KEGG database. (B) Network between 8 key pathways and 32 therapeutic targets (inner rectangle represents 11 hub targets, middle ring represents 21 common targets,outer ring represents 8 KEGG pathways).

damage of articular cartilage[18–20]; Kaempferol has effects on inhibiting harmful immune responses of RA, such as cytokine mediated synoviocytes proliferation, migration and invasion[21,22]; Beta-carotene is regarded as a potent antioxidant that can combat the injury of synoviocytes and chondrocytes caused by oxidative stress in RA patients and animals[23–25]; Beta-sitosterol not only exhibits antiinflammatory effects but also inhibits macrophages-mediated cartilage damage.[26,27] These active ingredients are the key material foundation of EU in RA treatment.

The Venn diagram revealed 50 overlapping genes that were potential therapeutic targets of EU against RA. After PPI topological screening with Degree, Betweeness and Closeness, we finally obtained 12 hub targets that were TNF, ALB, VEGFA, IL-6, IL1B, PTGS2, MMP9, CXCL8, CCL2, IL10, JUN, and VCAM1. According to the results of GO and KEGG enrichment analyses, we speculated that EU treats RA possibly associated with the regulation of several biological processes and signaling pathways.

5.1. Inflammatory response

Persistent synovial inflammation is one of most prominent features of RA, and plays an important role in the progressive damage of affected joint.[28,29] Chemokines including CXCL8 and CCL2, mediate the migration of inflammatory cells into the synovium.[30] Massive inflammatory cytokines such as TNF, IL-1, IL-6, and IL10 were secreted and fully filled in synovial

fluid, eventually lead to irreversible cartilage degradation and bone erosions.[31] In addition, the chemokines and cytokines can deplete B cells and control stimulation of T cells, accelerating the progression of RA.[31] Biological drugs of cytokines inhibitors effectively decrease the inflammatory response of RA patients.[32,33] In the present study, TNF, IL-1, IL-6, IL10, CXCL8, and CCL2 were identified as the key therapeutic targets through PPI screening. Target-Pathway network further reveled the above genes were enriched in inflammation-related TNF pathway, IL-17 pathway and T cell receptor pathway. Consistently, Wang *et al* found that ethanol extract of EU inhibited serum and tissue levels of TNF and IL-1 in RA rat model.[8] Also, Xing *et al* reported that EU could alleviate joint destruction of RA through inhibiting TNF, IL-6 and IL-17 mediated inflammatory infiltration.[6] Therefore, the antiRA effects of EU partly depends on inhibiting inflammatory response.

5.2. Oxidative stress

Oxidative stress is an important part of RA pathophysiology, as the interaction between immune response and endogenous antigens subsequently induces the production of reactive oxygen species.[34] Moreover, a vicious cycle forms between oxidative stress and synovial inflammation in RA, with the progression of one aggravating the other, eventually destroying joint cartilage.[35] Quercetin, one of major ingredients of EU, has resistant effects on oxidative stress.[36] TNF-α, IL-2 and IL-10 identified as the hub therapeutic targets have been reported participate in the process of oxidative stress.[37,38] Target-Pathway network analysis further reveled these above genes were enriched in T cell receptor pathway and NOD-like receptor pathway, and these immune-related signaling can directly regulate oxidative stress. Thus, through present network pharmacology analyses and literature screening, it can be recognized that inhibition of oxidative stress might be a potential mechanism of EU for RA treatment.

5.3. Cell apoptosis and matrix catabolism

In RA progression, proliferated inflammatory synovium caused by cytokines (TNF, IL-1, IL-6, IL10) and chemokines (CXCL8, CCL2) gradually invade into cartilage, eventually resulting in an irreversible destruction of cartilage.[39] Most patients have to receive a joint replacement surgery at late stage of RA.[40] Chondrocyte apoptosis and cartilaginous matrix catabolism are pathological bases in cartilage destruction.[41] They also present inseparable correlations as chondrocytes the solely living cells in cartilage can product extracellular matrix. In return, cartilaginous matrix provides biochemical support, nutrients and oxygen for the resident chondrocytes. Toll-like receptor 4 (TLR4) has been shown to be expressed in joint cartilage, and inhibition of TLR signaling can alleviate cartilage degeneration through reducing chondrocyte apoptosis and matrix catabolism.[42] In this study, GO and KEGG enrichment analyses revealed that 6 items of biological process were categorized into cell apoptosis and matrix catabolism, and the involvement of Toll-like receptor (TLR) pathway in therapeutic mechanism of EU against RA. In addition, decreased synoviocyte apoptosis is considered as another key factor causing extensive invasion of inflammatory synovium in RA,[43] and EU provides joint protection for RA through promoting synoviocyte apoptosis.[7] Therefore, we have reasons to believe, influence of cell apoptosis and matrix catabolism is an important part of mechanism for EU treating RA.

There are some limitations in this study. As these active ingredients of EU are obtained from computer predictions, its exact pharmacokinetic profile in bodies still needs to be detected by using liquid chromatography/tandem mass spectrometry. Bioenrichment analyses reveals several potential targets, biological processes and signaling pathways of EU against RA, while subsequent animal and cellular studies need be performed to validate its exact mechanisms.

6. Conclusions

Through network pharmacology, we systemically predict 50 therapeutic targets of EU. The identification of the major biological processes (inflammatory response, oxidative stress, cell apoptosis and matrix catabolism) and the key pathways (TNF pathway, IL-17 pathway, T cell receptor pathway, NOD-like receptor pathway, and Toll-like receptor pathway) indicates that EU treats RA in a direct or indirect synergy way of multi-targets, muti-biological processes and multi-pathways. Although subsequent validations are needed to determine the exact mechanism of EU, our present study provide promising directions for future research.

Author contributions

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Xu.Supervision: Yonggan Ying, Shuijun Zhang.Validation: Yonggan Ying.Writing-original draft:
Yonggan Ying, Chenjie Xia.Writing-review & editing: Yonggan Ying, Shuijun Zhang.

References

[1] Burmester GR, Pope JE. Novel treatment strategies in rheumatoid arthritis. Lancet. 2017;389:2338–48.
[2] Boissier MC, Semerano L, Challal S, et al. Rheumatoid arthritis: from autoimmunity to synovitis and joint destruction. J Autoimmun. 2012;39:222–8.
[3] Nemtsova MV, Zaletaev DV, Bure IV, et al. Epigenetic changes in the pathogenesis of rheumatoid arthritis. Front Genet. 2019;10:570.
[4] Smolen JS, Aletaha D, McInnes IB. Rheumatoid arthritis. Lancet. 2016;388:2023–38.
[5] Feng X, Chen Y. Drug delivery targets and systems for targeted treatment of rheumatoid arthritis. J Drug Target. 2018;26:845–57.
[6] Xing YY, Wang JY, Wang K, et al. Inhibition of rheumatoid arthritis using bark, leaf, and male flower extracts of eucommia ulmoides. Evid Based Complement Alternat Med. 2020;2020:3260278.
[7] Zhang Y, Wang JY, Wang H, et al. An alcohol extract prepared from the male flower of Eucommia ulmoides Oliv. promotes synoviocyte apoptosis and ameliorates bone destruction in rheumatoid arthritis. Chin Med. 2021;16:113.
[8] Wang JY, Chen XJ, Zhang L, et al. Comparative studies of different extracts from Eucommia ulmoides Oliv. against Rheumatoid Arthritis in CIA Rats. Evid Based Complement Alternat Med. 2018;2018:7379893.
[9] Luo TT, Lu Y, Yan SK, et al. Network pharmacology in research of Chinese medicine formula: methodology, application and prospective. Chin J Integr Med. 2020;26:72–80.
[10] Hopkins AL. Network pharmacology. Nat Biotechnol. 2007;25:1110–1.
[11] Zhang R, Zhu X, Bai H, et al. Network pharmacology databases for traditional Chinese medicine: review and assessment. Front Pharmacol. 2019;10:123.
[12] Song X, Zhang Y, Dai E, et al. Prediction of triptolide targets in rheumatoid arthritis using network pharmacology and molecular docking. Int Immunopharmacol. 2020;80:106179.
[13] Guo K, Wang T, Luo E, et al. Use of network pharmacology and molecular docking technology to analyze the mechanism of action of velvet antler in the treatment of postmenopausal osteoporosis. Evid Based Complement Alternat Med. 2021;2021:7144529.
[14] Liu Y, Zhang J, Liu X, et al. Investigation on the mechanisms of guiqi huoxue capsule for treating cervical spondylosis based on network pharmacology and molecular docking. Medicine (Baltim). 2021;100:e26643.

[15] Zhu N, Hou J. Exploring the mechanism of action Xianlingubao Prescription in the treatment of osteoporosis by network pharmacology. Comput Biol Chem. 2020;85:107240.
[16] Smolen JS, Aletaha D, Barton A, et al. Rheumatoid arthritis. Nat Rev Dis Primers. 2018;4:18001.
[17] Sparks JA. Rheumatoid arthritis. Ann Intern Med. 2019;170:ITC11–16.
[18] Yuan K, Zhu Q, Lu Q, et al. Quercetin alleviates rheumatoid arthritis by inhibiting neutrophil inflammatory activities. J Nutr Biochem. 2020;84:108454.
[19] Gokhale JP, Mahajan HS, Surana SJ. Quercetin loaded nanoemulsion-based gel for rheumatoid arthritis: in vivo and in vitro studies. Biomed Pharmacother. 2019;112:108622.
[20] Kim HR, Kim BM, Won JY, et al. Quercetin, a plant polyphenol, has potential for the prevention of bone destruction in rheumatoid arthritis. J Med Food. 2019;22:152–61.
[21] Pan D, Li N, Liu Y, et al. Kaempferol inhibits the migration and invasion of rheumatoid arthritis fibroblast-like synoviocytes by blocking activation of the MAPK pathway. Int Immunopharmacol. 2018;55:174–82.
[22] Liu W, Fan Y, Tian C, et al. Deciphering the molecular targets and mechanisms of HGWD in the treatment of rheumatoid arthritis via network pharmacology and molecular docking. Evid Based Complement Alternat Med. 2020;2020:7151634.
[23] Costenbader KH, Kang JH, Karlson EW. Antioxidant intake and risks of rheumatoid arthritis and systemic lupus erythematosus in women. Am J Epidemiol. 2010;172:205–16.
[24] Aryaeian N, Djalali M, Shahram F, et al. Beta-Carotene, Vitamin E, MDA, glutathione reductase and arylesterase activity levels in patients with active rheumatoid arthritis. Iran J Public Health. 2011;40:102–9.
[25] Darlington LG, Stone TW. Antioxidants and fatty acids in the amelioration of rheumatoid arthritis and related disorders. Br J Nutr. 2001;85:251–69.
[26] Liu R, Hao D, Xu W, et al. β-Sitosterol modulates macrophage polarization and attenuates rheumatoid inflammation in mice. Pharm Biol. 2019;57:161–8.
[27] Guo Q, Li L, Zheng K, et al. Imperatorin and β-sitosterol have synergistic activities in alleviating collagen-induced arthritis. J Leukoc Biol. 2020;108:509–17.
[28] Liu H, Zhu Y, Gao Y, et al. NR1D1 modulates synovial inflammation and bone destruction in rheumatoid arthritis. Cell Death Dis. 2020;11:129.
[29] McInnes IB, Schett G. Pathogenetic insights from the treatment of rheumatoid arthritis. Lancet. 2017;389:2328–37.
[30] Szekanecz Z, Pakozdi A, Szentpetery A, et al. Chemokines and angiogenesis in rheumatoid arthritis. Front Biosci (Elite Ed). 2009;1:44–51.
[31] García-Hernández MH, González-Amaro R, Portales-Pérez DP. Specific therapy to regulate inflammation in rheumatoid arthritis: molecular aspects. Immunotherapy. 2014;6:623–36.
[32] Chaabo K, Kirkham B. Rheumatoid arthritis - Anti-TNF. Int Immunopharmacol. 2015;27:180–4.
[33] Ogata A, Kato Y, Higa S, et al. IL-6 inhibitor for the treatment of rheumatoid arthritis: a comprehensive review. Mod Rheumatol. 2019;29:258–67.
[34] Smallwood MJ, Nissim A, Knight AR, et al. Oxidative stress in autoimmune rheumatic diseases. Free Radic Biol Med. 2018;125:3–14.
[35] Kaur G, Sharma A, Bhatnagar A. Role of oxidative stress in pathophysiology of rheumatoid arthritis: insights into NRF2-KEAP1 signalling. Autoimmunity. 2021;54:385–97.
[36] Shen P, Lin W, Deng X, et al. Potential implications of quercetin in autoimmune diseases. Front Immunol. 2021;12:689044.
[37] Šteňová E, Bakošová M, Lauková L, et al. Biological Anti-TNF-α therapy and markers of oxidative and Carbonyl stress in patients with rheumatoid arthritis. Oxid Med Cell Longev. 2021;2021:5575479.
[38] Cacciapaglia F, Anelli MG, Rizzo D, et al. Influence of TNF-α inhibition on oxidative stress of rheumatoid arthritis patients. Reumatismo. 2015;67:97–102.
[39] Lin YJ, Anzaghe M, Schülke S. Update on the pathomechanism, diagnosis, and treatment options for rheumatoid arthritis. Cells. 2020;9:880.
[40] Young BL, Watson SL, Perez JL, et al. Trends in joint replacement surgery in patients with rheumatoid arthritis. J Rheumatol. 2018;45:158–64.
[41] Malemud CJ. Chondrocyte apoptosis in rheumatoid arthritis: is preventive therapy possible. Immunotherapy (Los Angel). 2015;1:102.
[42] Ding Y, Wang L, Zhao Q, et al. MicroRNA-93 inhibits chondrocyte apoptosis and inflammation in osteoarthritis by targeting the TLR4/NF-κB signaling pathway. Int J Mol Med. 2019;43:779–90.
[43] Wang JY, Yuan Y, Chen XJ, et al. Extract from Eucommia ulmoides Oliv. ameliorates arthritis via regulation of inflammation, synoviocyte proliferation and osteoclastogenesis in vitro and in vivo. J Ethnopharmacol. 2016;194:609–16.