

# The role of autophagy in the treatment of osteoporosis by Chinese medicines (natural)

Yu Zhou<sup>1,2#</sup>, Xin Li<sup>3#</sup>, Yang Chen<sup>4</sup>, Liqi Ng<sup>2</sup>, Swastina Nath Varma<sup>2</sup>, Chao-Zong Liu<sup>2</sup>, Qing Gong<sup>5\*</sup>, Cheng-Liang Yin<sup>6\*</sup>

<sup>1</sup>Foot and Ankle Surgery, Chongqing Orthopedic Hospital of Traditional Chinese Medicine, Chongqing 400012, China. <sup>2</sup>Institute of Orthopaedic and Musculoskeletal Science, Royal National Orthopaedic Hospital, London HA74LP, UK. <sup>3</sup>Jilin Ginseng Academy, Changchun University of Chinese Medicine, Changchun 130117, China. <sup>4</sup>Department of Endocrinology, Chongqing Hospital of Traditional Chinese Medicine, Chongqing 400011, China. <sup>5</sup>Institute of Traditional Chinese Medicine, Changchun University of Chinese Medicine, Changchun 130117, China. <sup>6</sup>Faculty of Medicine, Macau University of Science and Technology, Macau 999078, China.

#These authors contributed equally to this work and are co-first authors for this paper.

\*Corresponding to: Cheng-Liang Yin, Faculty of Medicine, Macau University of Science and Technology, Weilong Road, Macau 999078, China. E-mail: chengliangyin@163.com; Qing Gong, Institute of Traditional Chinese Medicine, Changchun University of Chinese Medicine, No. 1035 Boshuo Road, Changchun 130117, China. E-mail: czgongqing@126.com.

## Author contributions

Zhou Y and Li X contributed equally to this work. Gong Q, Yin CL conceived the idea, Zhou Y and Li X wrote the manuscript. Ng LQ, Varma SN and Liu CZ helped modify the language and the revision. Chen Y and Li X collected the literature. Gong Q and Liu CZ helped supervise the research and contribute to the final draft of the paper. All authors have read and approved the manuscript.

## Competing interests

The authors declare no conflicts of interest.

## Acknowledgments

The authors would like to thank the financial support from the Science and Technology Development Program of Jilin Provincial Science and Technology Department, (No. 20210101200JC). The funding source had the role in design, execution and decision to submit the manuscript for publication. And we also would like to thank Yin-Rui Wang of Sichuan Normal University for her help in drawing our figure.

## Peer review information

Traditional Medicine Research thanks Xiao-Bing Jiang, Bo Li, and other anonymous reviewers for their contribution to the peer review of this paper.

## Abbreviations

OP, osteoporosis; BMSCs, bone marrow mesenchymal cells; OBs, osteoblasts; OCs, osteoclasts; TCM, traditional Chinese medicine; Cis A, cistanoside A; OVX, ovariectomize; TRAP, anti-tartrate acid phosphatase; ALP, alkaline phosphatase; LC3-II, protein 2 light chain 3; BMD, bone mineral density; Cur, curculigoside; OCN, osteocalcin;  $\beta$ -Ecd,  $\beta$ -Ecdysterone; PRED, prednisolone; GC, glucocorticoid; TMP, tetramethylpyrazine; Kae, kaempferol; OCPs, osteoclast precursors; RSV, Resveratrol; TBIL, Timosaponin BII; DG, Pueraria Lobata; MC3T3-E1, mouse osteoblast cell line.

## Citation

Zhou Y, Li X, Chen Y, et al. The role of autophagy in the treatment of osteoporosis by Chinese medicines (natural). *Tradit Med Res.* 2023;8(10):58. doi: 10.53388/TMR20230503002.

Executive editor: Xin-Yi Yang.

Received: 03 May 2023; Accepted: 20 June 2023; Available online: 04 July 2023.

© 2023 By Author(s). Published by TMR Publishing Group Limited. This is an open access article under the CC-BY license. (<https://creativecommons.org/licenses/by/4.0/>)

## Abstract

Osteoporosis is one of the common orthopaedic diseases, characterised by increased bone fragility due to reduced bone mass and microstructural degeneration, posing a great threat to patients' quality of life and safety. In recent years, Chinese medicine (natural) has had a unique advantage in the treatment of osteoporosis and has shown good efficacy. Autophagy is an inherent cellular survival mechanism for the removal and recycling of damaged proteins and organelles and plays an important role in maintaining the stability of the intracellular environment and organ function. Therefore, this article aims to provide a comprehensive review of these Chinese medicines (natural) for the treatment of osteoporosis through autophagy. They have been intensively studied and reported to have effects such as promoting osteogenesis and anti-bone resorption. The Chinese medicines include plants such as *Cistanche deserticola*, *Epimedium*, *Curculigo orchoides Gaertn*, *Achyranthes bidentata Blume*, *Leonurus japonicus Houtt*, *Ginseng*, *Chuanxiong Rhizome*, *Eucommia ulmoides*, *Morindae Officinalis Radix*, *Curcuma longa*, *Polygoni Cuspidati Rhizoma et Radix*, *Anemarrhena asphodeloides Bunge*, *Salvia miltiorrhiza Bge* and *Pueraria Lobata*, thus providing evidence for the use of alternative herbal therapies for the effective treatment of osteoporosis.

**Keywords:** osteoporosis; traditional Chinese medicine; autophagy; herb; review

**Highlights**

1. Traditional Chinese medicine has attracted the attention of physicians and researchers in China and abroad due to its unique advantages in the treatment of a wide range of diseases. Natural herbs have now been proven to be effective in the treatment of many orthopaedic conditions, including osteoporosis, osteoarthritis and other conditions. Osteoporosis is a common systemic skeletal disorder with a high global prevalence, causing a significant impact on the daily life of patients and in severe cases even life-threatening.
2. Autophagy is a method of biodegradation unique to eukaryotic cells and likewise it is a mechanism of cell survival. Recent studies have shown that these natural herbal therapies appear to have therapeutic modulation of osteoporosis's autophagy by promoting bone formation and reducing unbalanced bone resorption, thereby improving bone mineral density and biomechanical properties and reducing bone microarchitectural degradation.
3. We have reviewed the current mechanisms by which natural herbal medicines can act against osteoporosis by stimulating autophagy through their components or compounds, including: *Cistanche deserticola*, *Epimedium*, *Curculigo orchoides* Gaertn, *Achyranthes bidentata* Blume, *Leonurus japonicus* Houtt, *Ginseng*, *Chuanxiong* Rhizome, *Eucommia ulmoides*, *Morindae Officinalis* Radix, *Curcuma longa*, *Polygoni Cuspidati Rhizoma et Radix*, *Anemarrhena asphodeloides* Bunge, *Salvia miltiorrhiza* Bge and *Pueraria Lobata*. We hope to provide the reader with further research ideas.

**Background**

Osteoporosis (OP) is a common systemic skeletal disorder caused by an imbalance in the remodelling process where bone resorption exceeds bone formation [1, 2]. Its pathological features are dominated by a reduction in mineralized bone mass and destruction of bone tissue microstructure, which subsequently leads to increased bone fragility and fracture risk. The prevalence of osteoporosis worldwide is over 15% in people aged 50 and over 70% in people aged 80 and above, causing a great impact on the daily life of patients and even endangering their lives in serious cases [3–5].

The pathogenesis of OP can be attributed to a variety of mechanisms, such as ageing, oxidative stress, oestrogen deficiency and osteoimmunological abnormalities, which have not yet been fully elucidated and ultimately result in the disruption of bone homeostasis and hence the development of the disease [6–10]. The mechanisms regulating bone homeostasis are divided into 2 main aspects: bone resorption and bone formation that are influenced by a variety of cells. Bone marrow mesenchymal cells (BMSCs) are subpopulation of mesodermal cells derived from bone marrow, adipose tissue, umbilical cord blood and synovial membranes, and have a strong ability to differentiate into osteoblasts, adipocytes, etc [11–13]. They can also secrete a variety of osteogenic factors and vascular growth factors to promote bone regeneration. BMSCs also play a critical role in bone metabolism [14]. It has been suggested that reduced bone formation is a key component of the pathogenesis of OP, and those osteoblasts (OBs), which are found in bone tissue and are the direct bearers of bone formation, are the cells that receive stimuli to exert their effects and eventually differentiate into mature osteoblasts, whose main role is to form the bone matrix and regulate the proliferation and differentiation of osteoclasts in a variety of ways [15–18]. Osteoclasts (OCs) are derived from the monocyte-macrophage lineage and are primarily responsible for regulating the bone resorption aspects of bone tissue metabolism in the body. It is a terminal large multinucleated cell whose main function is the resorption of old bone mass, a role that is achieved mainly through the secretion of matrix metalloproteinases and the histone enzymes K, H<sup>+</sup> and Cl<sup>-</sup> [19, 20].

Macrophage colony-stimulating factor was found to stimulate osteoclast precursor cells to bind to the corresponding macrophage-colony-stimulating factor and induce RANK expression, thereby stimulating the corresponding receptor to mobilise downstream signalling factors to promote osteoclast production, which is closely related to bone remodelling and bone repair [21]. The OBs and OCs are in a mutually supportive and balanced relationship, and their abnormal function plays an important role in the development of OP, so only when they reach relative homeostasis can the bone mass of the body appear normal. Osteocytes are ancient cells that make up approximately 95% of the adult skeleton. Osteocytes differentiate from OBs, and when OBs produce osteoid on the bone surface at a smaller rate than neighbouring cells, they become encapsulated in osteoid and slowly differentiate into osteocytes [22, 23]. A key feature of osteocytes is their ability to regulate the function of OBs and OCs [24]. The luminal tubule system is the ideal network for transmitting biochemical signals from deeply embedded osteocytes to bone surface OBs, thereby allowing osteocytes to influence the activity of OBs, and similar osteocytes are effective supporters of OCs formation and activation in vitro [25–27].

Autophagy is a biodegradation method unique to eukaryotic cells and is also a cell survival mechanism. It is a catabolic and energy-generating process that degrades damaged organelles, abnormal proteins, pathogenic microorganisms and other materials, promoting the “recycling” of cellular components and thus providing energy to “starving” cells, as well as promoting protein renewal metabolism and maintaining intracellular homeostatic functions [28–30]. Autophagy is a dynamic, multi-step process that can be divided into four parts: Initiation, Nucleation-Elongation-Maturation, Fusion and Degradation [31–33].

Traditional Chinese medicine (TCM) has received much attention from national and international researchers in recent years due to its unique advantages in the treatment of a wide range of diseases. For example, many natural herbal medicines are effective in the treatment of a variety of orthopaedic conditions, including osteoporosis and osteoarthritis of the knee [34–37]. Recent studies have shown that natural herbal remedies have a modulating effect on osteoporosis, promoting a balanced relationship between bone formation and breakdown by regulating bone autophagy, thereby improving bone mineral density and biomechanical properties, and reducing bone microstructural degeneration. Ancient Chinese medical texts, for example, also contain many authoritative classical formulas such as Qing E Wan, Er Zhi Wan, Zuo Gui Wan and You Gui Wan, to name but a few. In a study by Qing E Wan in the treatment of postmenopausal women with osteoporosis, it was found that the treatment group significantly increased the patients' bone mineral bone mineral density (BMD), osteocalcin and bone alkaline phosphatase activities, and significantly decreased the levels of serum matrix metalloproteinase-2, bone cross-linked C-telopeptides of type I collagen, urine bone cross-linked N-telopeptides of type I collagen were significantly reduced, indicating that Qing E Wan has anti-postmenopausal osteoporosis effects. In a 6-month study of Er Zhi Wan for osteoporosis, significant improvements in BMD and serum E2 were found in patients in the treatment group [38]. In a related study by Zuo Gui Wan and You Gui Wan, 2 Chinese herbal compound formulas were found to increase BMD to varying degrees in the treatment of different types of OP, while also improving serum bone-related markers and osteogenesis-related markers to varying degrees [39, 40]. In summary, TCM has been shown to be effective and safe in anti-OP [41–43]. TCM has studied a variety of cellular pathways anti-OP, but there are few summaries of studies on autophagy.

Therefore, the keywords “osteoporosis”, “autophagy”, “natural herbal medicine” and “compounds of natural herbal medicine” were used. A literature search was conducted in PubMed. The literature related to bone quality was screened and drugs with anti-osteoporosis and autophagic effects were included. Studies unrelated to osteoporosis and autophagy were excluded, and in addition, drugs

with defects in experimental design were excluded. In summary, this paper discusses the role of herbal medicines targeting the regulation of autophagy in the treatment of osteoporosis and their potential regulatory mechanisms.

### Chinese Medicine (Natural)

#### *Cistanche deserticola*

*Cistanche deserticola* is a Chinese herbal medicine commonly used in traditional medicine. It is the dried fleshy stem with scaly leaves of *Cistanche deserticola* Y.C. Ma or *Cistanche tubulosa* (Schenk) Wight of the family *Lietangiidae* [44]. According to recent research, *Cistanche deserticola* is a Chinese medicine containing a variety of chemical components, such as phenylethanol glycosides, cyclic enol ether terpenoids, lignans, sugars and other chemical components, which have a wide range of pharmacological effects, mainly used for neuroprotection, immunomodulation, anti-aging, anti-osteoporosis, hepatoprotective, etc [45–51]. Cistanoside A (Cis A) is a phenylethanol glycoside extracted from *Cistanche deserticola*, a component with mainly antioxidant activity and anti-inflammatory properties, also used in the treatment of osteoporosis [52–55]. Cis A increased bone strength, bone mineral density and improved bone trabecular microarchitecture in ovariectomized (OVX) mice as an anti-osteoporotic effect, while serum biochemical analysis of bone formation and bone resorption markers showed that Cis A decreased the activity of bone resorption markers such as anti-tartrate acid phosphatase (TRAP), deoxyypyridinoline and histone proteinase K, and increased the activity of bone formation markers alkaline phosphatase (ALP) and bone Gla-protein. The mechanism of Cis A was found to down-regulate TRAP 6, RANKL protein levels and NF- $\kappa$ B signalling cascade, up-regulate OPG protein expression levels and PI3K/Akt signalling cascade and improve the OPG/RANKL expression ratio. It was demonstrated that it could inhibit osteoclastogenesis and promote osteoblastogenesis through TRAF6-mediated coordinated inhibition of NF- $\kappa$ B and stimulation of the PI3K/Akt pathway, thus acting as an anti-osteoporosis agent. The protein quantification of Protein 2 light chain 3 (LC3-II) is widely used to examine and evaluate the autophagic activity of cells and has been used as a marker of autophagy, while ATG 7 and Beclin 7 proteins, also involved in autophagy, have been shown to be involved in the mineralization of osteoblast lines [56, 57]. In a study of the effect of Cis A on primary osteoblasts, it was found that Cis A (10  $\mu$ M) promoted the mineralization of primary osteoblasts, while the expression of Beclin-1 and LC3 II/I increased in Cis A cultured primary osteoblasts, demonstrating that Cis A promotes the induction of autophagy in primary osteoblasts and, through autophagy, the differentiation of primary osteoblasts [58]. The Wnt/ $\beta$ -catenin pathway has been reported in many studies of autophagy in tumour diseases, and studies of the mechanism of osteogenesis in primary osteoblasts by Cis A, it was found that inhibition of autophagy can affect both differentiation and mineralization of primary osteoblasts [59–61]. Further studies revealed that Cis A-treated primary osteoblasts upregulated the expression levels of Beclin-1 and LC3, indicating that Cis A induced primary osteogenic autophagy. The authors then found that primary osteoblast differentiation and mineralization as well as autophagy were blocked by blocking the Wnt/ $\beta$ -catenin pathway inhibitor (Dickkopf-1). The involvement of Cis A in the Wnt/ $\beta$ -catenin pathway was further confirmed by protein blotting to induce autophagy, down-regulate apoptosis and promote osteogenesis. In general, TCM has the advantage of being multi-component and multi-acting, with the different components of *Cistanche deserticola* having their own effects, of which Cis A has been shown to have anti-OP effects in vitro and in vivo, either through autophagy or anti-inflammatory effects, which is in line with the characteristics of TCM.

#### *Epimedium*

*Epimedium* is a deciduous or evergreen perennial herb of the family *Berberidaceae*, commonly used in Chinese medicine, with flavonoids as the main active ingredients, such as epimedeside, epimedeside A,

epimedeside B and epimedeside C. It has a wide range of medicinal uses [62, 63]. As Chinese medicine continues to develop and advance, research on *Epimedium* is becoming more and more advanced and it has been found that *Epimedium* and its active ingredients can treat a variety of diseases such as reproductive, neurological and bone related diseases [64–67]. A study reported that the water extract of *Epimedium* could prevent bone loss, and showed that treatment with water extract of *Epimedium* could restore estradiol levels in OVX rats, promote bone formation and inhibit bone resorption, and show its anti-osteoporotic effect by BMD and bone strength [68]. In another study on osteoporosis disease, water extract of *Epimedium* was found to increase BMSCs proliferation and osteogenic differentiation and to increase the mRNA expression levels of osteogenic differentiation-related markers such as Alp, Col1a1 and Runx2 [69]. Further, in vivo studies also showed that the whole body, humerus, lumbar spine, and femur of OVX rats treated with *Epimedium* extract significantly increased BMD compared to the model group, and the Bone volume/Tissue volume of the femur was higher than that of the OVX group, as well as the 3D imaging and HE staining results showed that *Epimedium* extract helped restore the normal microstructure of bone. Analysis of sequencing results revealed that Atg4B is one of the important target genes of miR-27a-5p and also an important factor involved in the autophagy process [70]. Protein and mRNA expression studies of autophagy-related factors LC3 and Beclin1 revealed that treatment with water extract of *Epimedium* up-regulated the expression of important autophagy-related mRNAs and proteins as well as down-regulated p6257 to promote osteogenesis and regulate bone transformation markers to prevent osteoporosis. However, the specific components that cause autophagy need to be further investigated.

#### *Curculigo orchioides gaertn*

*Curculigo orchioides gaertn* is a perennial herb of the genus *Cynomorium* in the family *Lithospermum* [71]. It is the only tonic herb recorded in the TCM as a toxic herb. Its main chemical constituents include phenols and phenolic glycosides, lignans and lignan glycosides, triterpenes and triterpene glycosides, etc. It has antioxidant, anti-inflammatory and anti-osteoporosis effects [72–74]. It has been shown that curculigoside (Cur) can reduce the induction of RAW264.7 cells into osteoclasts and that bone resorption leads to the destruction of the bone matrix and the release of Ca<sup>+</sup> and collagen degradation products [75]. It has also been shown to reduce the release of relevant substrates from cell culture systems and has been shown to inhibit bone resorption by osteoclasts through studies of experimental bone fragment thickness and F-actin ring and several data. The validation of relevant bone resorption marker proteins, such as NFATC1 and C-fos, also confirmed that Cur can inhibit bone resorption to slow down the process of osteoporosis. Similarly, oxidative stress caused by iron overload is an important factor in primary osteoporosis, and previous studies have reported that iron overload in vivo inhibits osteoblast proliferation and differentiation and promotes osteoclastogenesis [76–80]. Studies have shown that Cur can reduce iron-induced bone loss in iron overload mouse models and mouse osteoblast cell line (MC3T3-E1) cells, in addition to improving femoral BMD and mechanical properties, reducing serum levels of IL-6 and TRACP-5b, and increasing osteocalcin (OCN) levels [80]. The assay of MC3T3-E1 cells and liver-related antioxidant enzyme markers in mice after induction confirmed that Cur prevents bone loss mainly by antagonizing iron overload-induced oxidative damage. Autophagy is a self-repair mechanism to prevent cell death in the presence of oxidative stress [81]. It was found that the protein expression of LC3 and Beclin1 in cells induced by cynarin treatment increased, while the expression level of IGFR was reduced, as well as the phosphorylation of Akt, p66 and FoxO1, which was also confirmed by immunohistochemistry. In the study of oxidative stress-related mechanisms, it was found that Cur could reverse the increase in p53 expression and decrease in Nrf2 expression in the Akt downstream pathway due to iron overload, suggesting that Cur mediates IGFR/Akt pathway autophagy to promote osteogenesis under high oxidative stress. In conclusion, the cynarin in *Curculigo orchioides gaertn* may act

as an anti-OP agent through autophagy, inhibition of OCs and iron overload, thereby reducing bone resorption.

#### ***Achyranthes bidentata* Blume**

*Achyranthes bidentata* Blume is the dried root of *Achyranthes bidentata* Blume in the Amaranth family. The main chemical components are polysaccharides, saponins and steroids, as well as a small amount of organic acids, flavonoids, velvet ketones and other active ingredients, with anti-tumor, immunomodulatory, anti-osteoporosis and other effects, in TCM has a history of more than 2,000 years of medicinal use [82–88].  $\beta$ -Ecdysterone ( $\beta$ -Ecd) is an estrogenic analogue derived from *Achyranthes bidentata* Blume [89]. It was shown that  $\beta$ -Ecd inhibited prednisolone (PRED)-induced bone loss in osteoporotic rats [90]. The treatment with 10 mg/kg  $\beta$ -Ecd improved BMD levels and bone microarchitecture in osteoporotic rats as analysed by micro-CT. Serum tests showed that the treatment alleviated the significant reduction in serum calcium and phosphorus levels caused by PRED induction in rats, while serum TRAP was suppressed [91]. Since the mechanism of PRED-induced osteoporosis is thought to be glucocorticoid-induced apoptosis, studies on the anti-apoptotic factor Bcl-2, the pro-apoptotic factor caspase-3 and the autophagy-related regulatory proteins Beclin-1, ATG5 and LC3II/I67 revealed that  $\beta$ -Ecd inhibited apoptosis and induced autophagy to treat bone loss in rats. Similarly, in mouse studies,  $\beta$ -Ecd was found to inhibit the expression levels of serum CTX-1, a marker of glucocorticoid (GC)-induced bone resorption in osteoporotic mice, increase OCN levels and improve BMD as well as bone microarchitecture [92–94]. Bone strength measurements also showed that the  $\beta$ -Ecd treatment significantly increased the maximum load, ultimate stress and toughness of the femur in mice. To investigate the mechanism of bone formation, experimental studies with BMSCs revealed that  $\beta$ -Ecd can stimulate osteogenic differentiation and increase the mRNA expression levels of Runx2 and Bglap1. To explore whether  $\beta$ -Ecd mediates autophagy, a focal RT-PCR gene array for autophagy was performed in vivo and in vitro experiments were performed to validate autophagy-related proteins, and it was found that  $\beta$ -Ecd can prevent bone loss through autophagy. In conclusion,  $\beta$ -Ecd in *Achyranthes bidentata* Blume treats bone loss by inhibiting apoptosis and inducing autophagy, serves the purpose of anti-OP.

#### ***Leonurus japonicus* Houtt**

*Leonurus japonicus* Houtt is the fresh or dried above-ground whole herb of *Leonurus japonicus* Houtt, family Labiatae [95]. Modern research has shown that *Leonurus japonicus* Houtt contains alkaloids, diterpenes, ferulic acid, volatile oil, flavonoids, polysaccharides and other chemical constituents [95, 96]. It has various pharmacological effects such as anti-thrombotic, menstrual disorders, anti-inflammatory and analgesic etc [97–100]. It is widely used in the clinical treatment of various diseases and is a commonly used herbal medicine. Leonurine has been shown to be the main bioactive component of *Leonurus japonicus* Houtt, with antioxidant and anti-inflammatory effects [101, 102]. In a study of osteogenic differentiation of Leonurine-treated rat BMSCs, 10  $\mu$ M Leonurine was found to be non-significantly toxic to BMSCs and favoured the proliferation of BMSCs [103]. In ALP staining and Alizarin Red S staining at 6 and 14 days, 10  $\mu$ M of Leonurine significantly promoted osteogenic differentiation of BMSCs. To further investigate the mechanism, BMSCs treated with 10  $\mu$ M Leonurine significantly upregulated osteogenic markers such as RUNX2, OPG and other mRNA and protein levels, as well as upregulated autophagy-related factors ATG7 and LC II/I and downregulated P62 protein expression levels. Based on previous studies that found Leonurine to be closely associated with the PI3K/Akt/mTOR pathway, it was found that Leonurine down-regulates phosphorylated PI3K/AKT/mTOR and inhibits PI3K/Akt/mTOR signalling pathway activity [104]. This confirmed that Leonurine-activated autophagy promotes osteoblast differentiation by regulating the PI3K/Akt/mTOR pathway.

#### **Ginseng**

*Panax ginseng* is a perennial herb that belongs to the Araliaceae family, which is one of the common traditional Chinese herbs [105]. Modern pharmacological research has found that ginseng components are mainly concentrated in saponins, polysaccharides and volatile components, as well as other compounds [106, 107]. It has anti-aging, anti-diabetic, anti-atherosclerotic, anti-osteoarthritic, anti-tumour amongst other pharmacological effects [108–112]. Ginsenoside is the main constituent of ginseng [113]. Ginsenoside Rg3 was found to inhibit the differentiation of RAW264.7 cells into osteoblasts, as well as to promote the mineralization and osteogenic differentiation of MC3T3-E1 cells, indicating a potential therapeutic effect of Rg3 on osteoporosis [114, 115]. In a study of Rg3 treatment in OVX rats, it was found that Rg3 significantly improved BMD and the thickness, number and density of femoral trabeculae in OVX rats, and increased the protein expression levels of osteogenesis-related markers such as OCN, OPN, COL1A1 and Runx2 [116]. In addition to this, the expression of autophagy-related proteins, such as LC3 II/I and Beclin1, was also upregulated and the expression level of p62 protein was downregulated. The mechanism was investigated by finding that Rg3 could promote p-AMPK expression and inhibit p-p70S6K expression. Also in the study of MC3T3-E1 cells, 1–20  $\mu$ M/L Rg3 was found to have no toxic effect on the cells, and 20  $\mu$ M/L Rg3 was found to significantly increase the mineralization capacity of MC3T3-E1 by alizarin red staining assay. In order to verify the mechanism in OVX rats, in the next experimental study using MC3T3-E1 cells, it was found that 10 and 20  $\mu$ M/L Rg3 significantly upregulated the protein expression levels of osteogenesis-related markers as well as p-AMPK, and downregulated p-p70S6K protein expression. The effect of Rg3 on AMPK/mTOR signalling was also found to be dose-dependent. The results indicated that Ginsenoside Rg3 enhances autophagy by mediating the AMPK/mTOR signalling pathway and thus prevents osteoporosis. The ginsenosides in *Ginseng* have anti-OP effects by inhibiting the differentiation of OCs and promoting the differentiation of OBs, and Ginsenoside Rg3 has anti-OP effects by mediating the AMPK/mTOR signalling pathway through autophagy.

#### **Chuanxiong Rhizome**

Chuanxiong Rhizome, the dried rhizome of *Ligusticum chuanxiong* Hort, in the TCM is a blood activator and blood stasis remover. Its chemical composition is mainly volatile oil, alkaloids, polysaccharides, etc [117–120]. It has various pharmacological activities on the cardiovascular system, liver and kidney system, nervous system and other systems, mainly analgesic, anti-inflammatory, antioxidant, anti-tumour, anti-coagulant and other effects [121–127]. In the study of the action of tetramethylpyrazine (TMP), an extract of Chuanxiong, on BMSCs and GC-induced rats, it was found that 10  $\mu$ M–200  $\mu$ M TMP inhibited GC-induced induction of apoptosis in BMSCs, inhibited Caspase-3 activity, and had no toxic effect on cells. As it has been shown in previous studies on BMSCs in osteoporosis, glucocorticoids induce autophagy in BMSCs to prevent an increase in bone loss [128, 129]. Therefore, in further studies, it was found that 50  $\mu$ M TMP increased the formation of autophagic vesicles as well as increasing the protein expression level of LC3 II/I, and this result was confirmed by immunofluorescence experiments. It was also found that the 3-MA group inhibited the autophagy of BMSCs after TMP treatment and increased TUNEL-positive cells and caspase-3 activity in studies with autophagy inhibitors and autophagy activators. The Rapamycin group promoted autophagy in BMSCs but was not found to enhance autophagy after TMP treatment, therefore it was inferred that TMP and rapamycin promote autophagy by the same mechanism of action. Due to the importance of the AMPK/mTOR signalling pathway in autophagy, it was experimentally confirmed that TMP can upregulate p-AMPK and downregulate p-mTOR protein expression levels to mediate the AMPK/mTOR signalling pathway to induce autophagy in BMSCs [130–131]. In vivo experiments in GC-induced rats also showed that TMP could improve BMD and bone microarchitecture deterioration caused by glucocorticoids, and in vitro studies in BMSCs collected from the model group at 12 weeks confirmed that BMSCs treated with TMP had more autophagic vesicles

and higher LC3-II/I protein expression levels than the model group, validated in vitro experiments [129]. These demonstrated that TMP mediates the activation of autophagy by the AMPK/mTOR signalling pathway to ameliorate GC-induced osteoporosis.

#### ***Eucommia ulmoides***

*Eucommia ulmoides* is the dried bark of *Eucommia ulmoides* Oliv [132]. *Eucommia ulmoides* is rich in cyclic enol ether terpenes, lignans, flavonoids, phenylpropanoids, polysaccharides and other active ingredients, which have significant advantages in lowering blood pressure, blood lipids, blood sugar and preventing osteoporosis [133]. kaempferol (Kae) is one of the active ingredients of the flavonoids of *Eucommia* extract and has a wide range of pharmacological effects, while the flavonoids are molecular targets of autophagy and play an important role in humans [134–136]. When studying the effect of Kae on osteoclasts, it was found that 50  $\mu$ M Kae inhibited the expression levels of osteoclast-associated factors, such as TRAP6 and c-Fos, and activated and up-regulated the expression levels of autophagy-related proteins, such as Beclin-1 and LC3, in RAW263.7 cells [137]. In studies related to osteoclast differentiation and bone resorption, Kae was found to reduce RANKL (50ng/mL) induced osteoclast production in Raw264.7 cells and significantly inhibited their osteoclastic capacity [137, 138]. Meanwhile, Kae could inhibit the expression of RANKL-induced NFATc1, and other osteoclast-associated markers mRNA, thus further confirming its inhibition of osteoclastogenesis, and found that the mechanism was related to extracellular regulated protein kinases and C-Jun N-terminal kinase inactivation. In the study of Kae on autophagy, it was found that Kae could upregulate the expression levels of the pro-apoptotic proteins including caspase-9 and caspase-3, as well as inhibit autophagy-related markers, suggesting that Kae could be used to treat the disease by inhibiting autophagy and thereby reducing osteoclast differentiation and osteoclastic capacity.

#### ***Morindae Officinalis Radix***

*Morindae Officinalis Radix* comes from the dried root of *Morinda officinalis* How, a plant of the genus *Bacopa*, family *Cyperaceae*, and is one of the “Four Southern Medicines” of China. Its main chemical components include anthraquinones, cyclic enol ether terpenoids, sugars, amino acids, organic acids, flavonoids and trace elements, which have anti-dementia anti-osteoporosis and anti-inflammatory effects [139–145]. Monotropein is an active component of cyclic enol ether terpenoids. In studies on primary osteoblasts, it was found that 0.08  $\mu$ M–0.2  $\mu$ M monotropein had no toxic effect on OCs and inhibited H<sub>2</sub>O<sub>2</sub>-induced oxidative stress in pro-apoptotic cells [146]. Due to the close correlation between autophagy and antioxidants, in further experiments, autophagy-related markers were investigated and it was found that 0.08  $\mu$ M Monotropein significantly upregulated LC3II/I and Beclin-1 protein expression levels, and through the increase in autophagic flux, it could be confirmed that Monotropein prevented osteoblast apoptosis through autophagy. The Akt pathway normally mediates oxidative stress in osteoblasts while the mTOR pathway normally mediates autophagic protection after oxidative stress in osteoblasts [147, 148]. In the study of its mechanism, it was found that Monotropein stimulated the phosphorylation of Akt and mTOR to increase the expression of autophagy-related proteins and that the mediation of both activators by Akt activator (SC79) and mTOR activator (MYH1485) blocked the effect of autophagy induced by Monotropein. It was finally confirmed that Monotropein could mediate the Akt/mTOR signalling pathway to induce autophagy in osteoblasts to reduce oxidative stress [146]. In conclusion, Monotropein in *Morindae Officinalis Radix* mediates the Akt/mTOR signalling pathway to act as an anti-inflammatory and anti-oxidant via autophagy, thereby inhibiting apoptosis and thus acting as an anti-OP.

#### ***Curcuma longa***

*Curcuma longa* is a common TCM which comes from the dried rhizome of *Curcuma longa* L., a plant in the ginger family [149]. Its main chemical components are phenols and terpenoids, as well as small

amounts of alkaloids and sterols, which have antioxidant, anti-inflammatory and anti-tumour effects [150–156]. Curcumin, a polyphenol with a small relative molecular mass, is the most active component of turmeric and is safe and non-toxic [157, 158]. Previous studies have shown that curcumin is considered an autophagy activator and has the ability to modulate autophagy in a variety of cells [159–161]. In studies on osteoporosis, curcumin was found to exert a direct autophagic effect on osteoclast precursors (OCPs), with a drug-dependent increase [162]. Since RANKL-induced OCPs can also promote autophagy, curcumin inhibited RANKL-mediated autophagy in OCPs when 15  $\mu$ M curcumin treatment was added, as revealed by the autophagy index as well as Beclin-1 expression levels. In the same study on osteoclasts, curcumin was found to inhibit osteoclast proliferation through inhibition of autophagy, and by using gene-silencing techniques on autophagy genes (Atg5, Atg7, Beclin-1), it was found that curcumin may mediate osteoclast inhibition of autophagy through Atg7/Beclin-1. In the in vivo study, curcumin improved the tibial microarchitecture of the OVX model rats and the results were reconfirmed by H&E staining, while significantly suppressing serum osteoclast-associated activity marker levels and improving serum osteoblast cell-associated marker (ALP) levels. Further studies on osteoclastogenesis inhibitor (TRAP3) revealed that curcumin could inhibit osteoclastogenesis by alleviating osteoclast-induced degradation of TRAP3 and improving the formation of autophagic lysosomes [163]. This demonstrates that curcumin can inhibit osteoclastogenesis by mediating autophagy to regulate the degradation of TRAP3, thereby preventing osteoporosis.

#### ***Polygoni Cuspidati Rhizoma et Radix***

*Polygoni Cuspidati Rhizoma et Radix* is the dried rhizome and roots of *Polygonum cuspidatum* Sieb. et Zucc, which has been used for thousands of years in China for the treatment and prevention of diseases [164]. The chemical composition includes compounds such as quinones, stilbene, flavonoids and phenylpropanoids, which can be used to treat inflammation, hyperlipidaemia, nerve damage, cardiovascular disease, etc [165–170]. Resveratrol (RSV) is one of the important constituents of *Polygoni Cuspidati Rhizoma et Radix*, and in previous reports on osteoporosis, RSV improved BMD in rats with osteoporosis models, as well as reducing femoral porosity in the proximal epiphysis [171]. Serum ALP and OCN increased compared to the control group but decreased compared to the model group. The immunohistochemical results showed a significant increase in OCN and SIRT1 expression and improved trabecular architecture in the RSV-treated group. The silent information regulator of transcription1 has a role in regulating cellular defence against oxidative stress and cell survival [172]. In vivo correlation experiments revealed that the RSV treatment group resulted in increased expression of LC3 and Beclin-1 proteins and down-regulation of Akt phosphorylation and mTOR phosphorylation. Further in vitro studies revealed that RSV could increase SIRT1 expression in dexamethasone-treated MC3T3-E1 cells in a drug-dependent as well as time-dependent manner. Resveratrol was also found to significantly increase the expression of Beclin-1 and Atg7, as well as to down-regulate Akt phosphorylation and mTOR phosphorylation in both treated dexamethasone and untreated MC3T3-E1 cells, validating the above experiments [171]. The autophagy promoted by RSV was blocked by Rapamycin (mTOR inhibitor) and LY294002 (PI3K inhibitor) interventions and related findings, demonstrating that Resveratrol prevents osteoporosis by mediating the PI3K/Akt/mTOR pathway to promote autophagy. In another study, 0.1  $\mu$ M–5  $\mu$ M RSV was found to promote the proliferation of MC3T3-E1 and BMSCs cells, as well as differentiation towards OB [173]. Assays of autophagy-related markers revealed that LC3-II/I and ATG-7 protein levels were significantly increased after RSV treatment. Activation of autophagy by RSV was blocked as demonstrated by experiments related to the autophagy inhibitors 3M and bafilomycin. In summary, RSV in *Polygoni Cuspidati Rhizoma et Radix* can act as an anti-OP through autophagy.

#### ***Anemarrhena asphodeloides* Bunge**

*Anemarrhena asphodeloides* Bunge is the dried rhizome of *Anemarrhena asphodeloides* Bge, family Liliaceae [174]. It contains active ingredients such as saponins, diphenylpyrazones, lignans, alkaloids, polysaccharides and trace elements, which have anti-platelet thrombotic, Alzheimer's disease, anti-inflammatory and neuroprotective effects [175–181]. Timosaponin BII (TBII) is one of the ingredients of *Anemarrhena asphodeloides* Bunge, where previous studies reporting on TBII and osteoporosis found that TBII could affect diabetic Goto-Kakizaki rats bone trabecular microstructure and showed a drug-dependent increase, with the high dose group almost reaching control levels [182]. Similarly, the results of its anti-apoptosis-related protein study revealed that TBII inhibited the upregulation of Bax and Bcl2 protein levels in primary osteoblasts after high glucose treatment. The results of the same anti-apoptosis-related protein study showed that TBII inhibited the upregulation of Bax and Bcl2 protein levels in primary osteoblasts after high glucose treatment. It was confirmed by ALP staining that TBII drug-dependently inhibited high glucose-induced osteoblast apoptosis and also promoted osteoblast differentiation. In the study of the mechanism, it was found that the expression level of Beclin 1 was significantly upregulated in the TBII-treated group compared with the model group at the tibial stem end of the scale, while the levels of autophagy markers LC3II/I and Beclin 1 protein were drug-dependently upregulated in the in vitro experiments. However, observation under transmission electron microscopy revealed more autophagic vesicles in osteoblasts treated with a high sugar environment than in osteoblasts not treated with hyperglycemia, suggesting that TBII can stimulate osteoblast autophagy under hyperglycemia conditions. It was likewise confirmed through studies that an autophagy inhibitor can inhibit the autophagy of TBII and that an autophagy activator, rapamycin can enhance the autophagy of TBII, which can improve osteoblast apoptosis through autophagy. On the other hand, TBII down-regulated p-mTOR levels in the tibial stem scales of Goto-Kakizaki rats, as well as reduced p-I $\kappa$ B expression and NF $\kappa$ B nuclear translocation in osteoblasts after hyperglycemia induced. In addition, NF $\kappa$ B overexpression eliminated the increase in LC3II/I and Beclin1 in high glucose osteoblasts after TBII treatment, suggesting that TBII may prevent osteoporosis by inhibiting mTOR/NF $\kappa$ B to activate autophagy [182].

#### **Salvia miltiorrhiza Bge and Pueraria Lobata**

*Salvia miltiorrhiza* Bge is the dried root and rhizome of *Salvia miltiorrhiza* Bge. of the genus *Salvia* in the family Labiatae [183]. *Salvia miltiorrhiza* Bge has anti-clotting, anti-inflammatory, anti-osteoporosis and anti-tumour effects due to its chemical composition of tansy ketones, tannic acid, volatile oils and polysaccharides, and has become a commonly used herbal medicine in Chinese compound formulations and related preparations [184–189]. *Pueraria Lobata* is the dried tuberous root of *Pueraria Lobata* (Wild.) Ohwi or *Pueraria thomsonii* Benth, a perennial legume, which is rich in a variety of chemical constituents, mainly isoflavones, triterpenoids, saponins, alkaloids, coumarins and other compounds, with effects on improving cardiovascular and cerebrovascular diseases, anti-diabetes, anti-inflammatory and immune protection [190–196]. Previous herb pair reports found that aqueous Extract of *Salvia miltiorrhiza* Bge and *Pueraria Lobata* (DG) improved BMD, bone mineral content and bone microarchitecture in the OVX model and reduced bone loss in rats [197]. Morphological observations revealed that femur type II collagen, TRAP-positive area and Cathepsin K-positive area were also significantly restored after treatment with aqueous extract compared to the model group. Serum RANKL was significantly downregulated in the treatment group, while serum ALP was not significantly different. In addition, DG treatment significantly reduced the serum blood urea nitrogen and creatinine release levels in OVX rats and improved the kidney damage caused by DG. In vitro experiments revealed that DG inhibited the differentiation of RAW264.7 cells into osteoclasts and reduced the protein expression levels of osteoclast-specific proteins such as RANK, NFATc1 and c-Fos. When the mechanism was investigated, it was found that osteoclasts showed autophagy under

RANKL stimulation, which was most pronounced on day 5, and autophagic vesicles appeared. Analysis of autophagy-related markers revealed that RANKL-induces osteoclast Beclin-1 and LC3B protein expression levels were significantly upregulated, as well as P62 protein expression levels were downregulated, but all of these were improved by DG treatment. Similarly, DG also inhibited the oxidative stress response stimulated by RANKL. It was demonstrated that DG prevented osteoporosis by inhibiting osteoclast autophagy and oxidative stress.

#### **Discussion**

In summary, as the ageing structure of society rises, osteoporosis leads to a range of conditions that can have a great impact on the quality of life and health of patients. Chinese medicine (natural) has received much attention from scholars at home and abroad for its strong medicinal activity, multi-effects, multi-targeting and low toxic side effects. Along with continuous research, the mechanisms of its monomer and formulae have been increasingly explored.

The process of bone formation and bone resorption is normally regulated by a variety of cells and factors in a dynamic balance. Previous studies have found that osteoblasts in BMSCs are mainly regulated by the WNT signalling pathway, which is essential for new bone formation. Activation of  $\beta$ -catenin by frizzled proteins and low-density lipoprotein receptor 5/6 promotes the activation of the WNT signalling pathway by transcription factors such as Runt-related transcription factor 2, which promotes the differentiation of MSCs into osteoblasts [198–200]. On the other hand, secreted-frizzled related proteins, sclerostin and Dickkopf-1 inhibit the activation of  $\beta$ -catenin proteins, thereby suppressing the differentiation of BMSCs-mediated WNT into osteoblasts [201]. The main components of the bone matrix, such as OCN, ALP and type I collagen, are mainly secreted by osteoblasts before the mineralization of calcium phosphate in the form of hydroxyapatite takes place. Osteoclasts are key to the bone resorption process and are regulated by a variety of signals, of which the RANK-RANKL regulatory axis is the most important. RANKL ligands, secreted by osteoblasts and activated immune T cells, bind to the RANK receptors of osteoclasts and activate the relevant enzymes to promote bone resorption [202, 203]. OPG is a natural inhibitor of RANKL ligands and can reduce bone resorption by osteoclasts [204]. Similarly, macrophage colony-stimulating factor can increase the osteoclastic capacity of osteoclasts by upregulating the expression of the RANK receptor [205]. In addition to this, senescence-induced upregulation of all systemic inflammatory factors such as TNF- $\alpha$ , IL-1, IL-6, IL-17 and IFN- $\gamma$ , are also involved in the process of bone remodelling, while IL-4, IL-10 etc. have the opposite effect to the above [206–209]. It is well known that a single herb has multiple components, many of which also have the ability to regulate bone-specific matrix proteins, related transcription factors and signalling pathways to promote osteoblast proliferation and differentiation [210]. The herbs or chemicals contained in the herbs described in this article have anti-inflammatory effects, so this could be one of the bases for herbs against osteoporosis.

It has been confirmed that osteoporosis is also closely related to multiple factors such as cellular scorching and DNA damage [211, 212]. Autophagy plays a similar role as a “double-edged sword” in the mechanism of cellular damage. On one hand, autophagy is an important regulatory system for homeostasis in vivo, both in terms of degrading misfolded proteins and selectively degrading damaged organelles to inhibit apoptosis, as well as being involved in the apoptotic process. On the other hand, when autophagy is prolonged, proteins and organelles that are essential for basic homeostasis and cell survival may be over-degraded, leading to further cell death. Thus, to the extent that autophagy degrades cells, autophagy is both a cell survival mechanism and a cell death pathway [213–215]. At the same time, autophagic structures act as hubs for the spatial organisation of recycling and synthesis processes in secretory cells and abnormal autophagic function can cause many diseases. Studies have shown that the upregulation of autophagy promotes the

transformation of osteoblasts to osteocytes, allowing cells to adapt to a more hypoxic environment, increasing cell survival and thus preventing bone loss [216]. These suggest that maintaining appropriate levels of autophagy in the body can play a key protective role against the accumulation of reactive oxygen species caused by various factors while studies of autophagy on osteoclasts have found that the regulation of autophagy on osteoclasts is bidirectional and atypical [217–220]. The causal relationship between changes in autophagy levels and osteoporosis formation is not well understood

and further research and exploration of the role of the autophagic pathway in bone homeostasis are needed.

Chinese medicines (natural) contain compounds that may be effective in activating/inhibiting autophagy to treat osteoporosis, and this review documents the available evidence for their potential biopharmacological effects and possible mechanisms of action. In vivo and in vitro summaries of the anti-osteoporotic effects of the natural herbs reviewed in this paper are presented in Table 1 and Table 2 respectively. Chinese medicines (natural) appear to act to prevent

**Table 1 Summary of an in vivo autophagy study of Chinese medicines (natural) treatments for OP**

Chinese medicines	Compound	Animal models	Beneficial results	References
<i>Epimedium</i>	Water extract of Epimedium	OVX rats	BMD, microarchitecture, serum P, Ca, BGP and E2↑.	[42]
<i>Curculigo orchoides gaertn</i>	Curculigoside	Iron-overload mice	Femoral BMD, biomechanical parameters, microarchitecture, antioxidant, serum OCN↑, serum IL-6, TNF- $\alpha$ , TRACP-5b↓.	[53]
<i>Achyranthes bidentata Blume</i>	$\beta$ -Ecdysterone (10 mg/kg)	The subcutaneous implantation of PRED into SD rats	BMD, microarchitecture, autophagy, Runx2, BMP2↑, serum ALP, TRAP, serum Ca, P, apoptosis, RANKL ↓.	[63]
	$\beta$ -Ecdysterone (0.5 mg/kg)	Slow release pellets of prednisolone (GC) were implanted into mice	Serum Ca, CTX-1, BMD, microarchitecture biomechanical parameters, autophagy↑, serum CTX-1↓	[67]
<i>Ginseng</i>	Ginsenoside Rg3	OVX rats	BMD, microarchitecture, serum ALP, autophagy, OCN, OPN, COL1A1, Runx2↑, serum TRAP↓.	[89]
<i>Chuanxiong Rhizome</i>	Tetramethylpyrazine	GC-induced rats	BMD, microarchitecture, autophagy↑, apoptosis↓.	[101]
<i>Curcuma longa</i>	Curcumin	OVX mice and OVX rats	Microarchitecture, serum ALP, autophagy↑, serum TRAP-5b↓.	[135]
<i>Anemarrhena asphodeloides Bunge</i>	Timosaponin BII	GK rats	microarchitecture, autophagy↑	[155]
<i>Salvia miltiorrhiza Bge and Pueraria Lobata</i>	Aqueous Extract of Salvia miltiorrhiza Bunge-Radix Puerariae	OVX rats	BMD, BMC, femoral porosity, Collagen II, serum OPG↑, TRAP, Cathepsin K, serum RNKL, BUN, Creatinine↓.	[170]

They enhance the BMD and biomechanical parameters of bones in osteoporosis model animals and regulate the dynamic metabolism of bone formation and bone resorption through autophagy. OP, osteoporosis; PRED, prednisolone; SD, sprague dawley; GK, Goto-Kakizaki; GC, glucocorticoid; OVX, ovariectomize.

**Table 2 Summary of an in vitro autophagy study of Chinese Medicines (natural) treatments for OP**

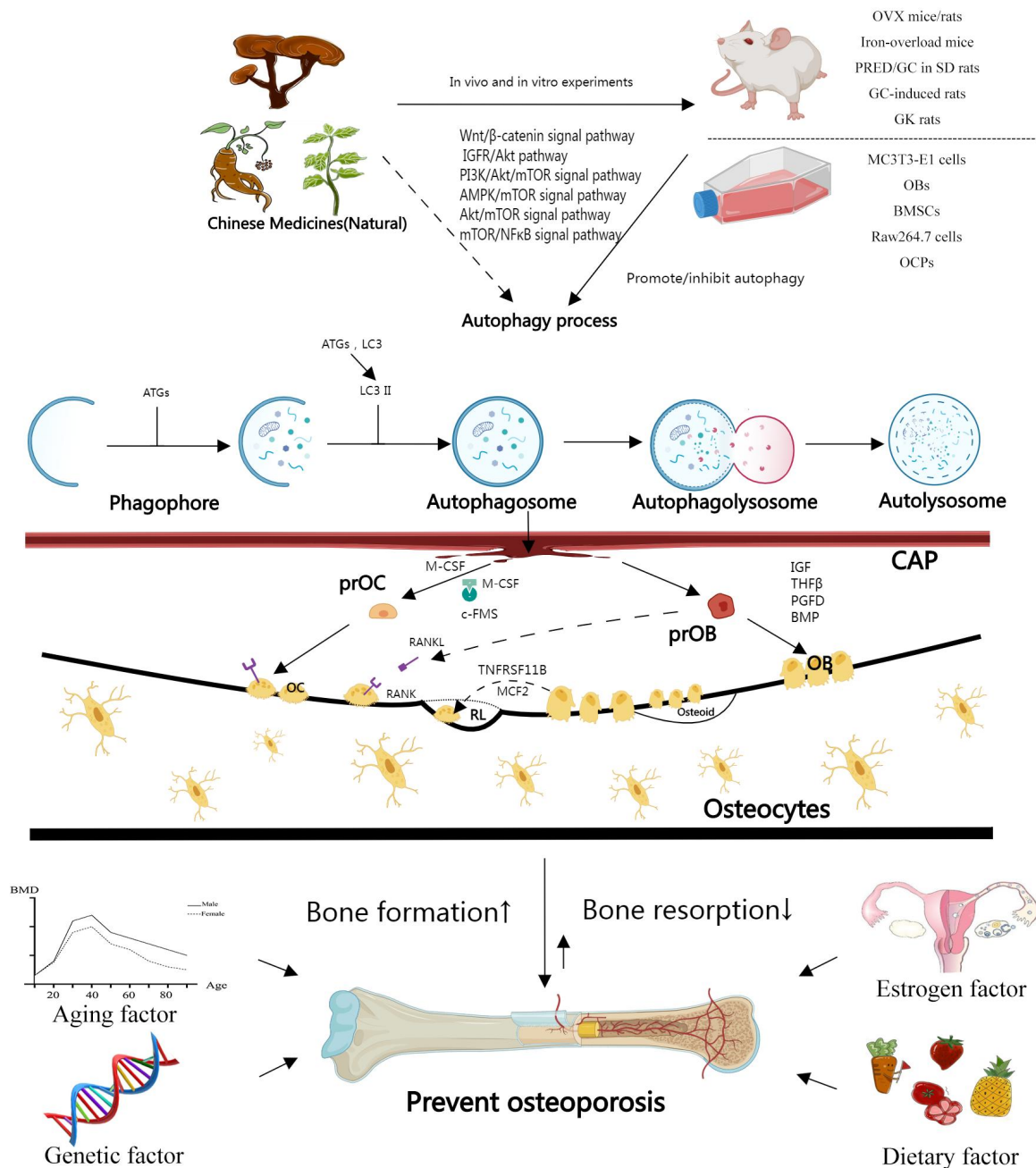
Chinese Medicines	Compound	Cellular models	Beneficial results	References
<i>Cistanche deserticola</i>	Cistanoside A	OBs	Differentiation, mineralization, autophagy↑, apoptosis↓.	[31]
<i>Epimedium</i>	Water extract of Epimedium	BMSCs	Proliferation, differentiation, autophagy↑, apoptosis↓.	[42]
<i>Curculigo orchoides gaertn</i>	Curculigoside	MC3T3-E1 cells	Mineralization, antioxidant, autophagy↑, apoptosis↓.	[53]
<i>Achyranthes bidentata Blume</i>	$\beta$ -Ecdysterone	BMSCs	Autophagy, differentiation↑.	[67]
<i>Leonurus japonicus Houtt</i>	Leonurine	BMSCs	Proliferation, differentiation, autophagy↑.	[76]
<i>Chuanxiong Rhizome</i>	Tetramethylpyrazine	BMSCs	Autophagy↑, induced toxicity, apoptosis↓.	[101]
<i>Eucommia ulmoides</i>	Kaempferol	Raw 264.7 cells	Apoptosis, osteoclastogenesis, bone resorption, autophagy↓.	[110]
<i>Morinda Officinalis Radix</i>	Monotropein	OBs	Antioxidant, Anti-apoptotic, autophagy↑.	[119]
<i>Curcuma longa</i>	Curcumin	OCPs	Autophagy↑, proliferation↓.	[135]
<i>Anemarrhena asphodeloides Bunge</i>	Timosaponin BII	OBs	Cellular activity, antioxidant, anti-apoptotic↑.	[155]
<i>Salvia miltiorrhiza Bge and Pueraria Lobata</i>	Aqueous Extract of Salvia miltiorrhiza Bunge-Radix Puerariae	Raw 264.7 cells	differentiation, RANK, NFATc1, c-Fos, autophagy, oxidative stress↓.	[170]

They prevent bone loss by promoting osteoblast proliferation and differentiation as well as inhibiting RAW263.7 cell differentiation through autophagy.

bone loss by activating autophagy to promote bone formation activity and preventing apoptosis in osteoblasts, including BMSCs, OBs and MC3T3-E1 cells. Some of these herbs can protect them from oxidative stress damage or prevent inflammation by inhibiting inflammation-related factors, among others. In addition, compounds of certain herbs may also achieve attenuation of osteoclast production or inhibition of osteoclast osteoclastic function through regulation of autophagy, thereby potentially reducing the imbalance between bone formation by osteoblasts and bone resorption by osteoclasts (Figure 1). As shown in Table 3, we summarise the signalling pathways that

appear to mediate the anti-osteoporotic effects of the Chinese medicines (natural) reviewed herein through the activation of autophagy.

Chinese medicines (natural) in this review are classical medicines for the treatment of osteoporosis through autophagy. It is well known that TCM is empirical medicine [221]. Based on the rich experience of clinical practice and the theory of TCM, herbal medicines are classified into different categories according to their efficacy. The 14 natural herbs mentioned above are mainly used to tonify kidney yang and invigorate blood, and are part of the basic theory of traditional



**Figure 1 Chinese medicines (natural) mediate autophagy to intervene in bone remodelling.** Natural herbs have been shown to mediate multiple pathways to produce autophagy through in vivo or in vitro experiments. Through oral administration of herbal medicines, after a series of actions into the bloodstream, they enter the bone surface via autophagy-mediated ProOC or proOB through the CAP, and after autophagy, they may differentiate into OB and osteoblasts (OCTES) and OC cells respectively, which may change their role. Under the influence of local factors such as osteoblasts (MCF2, TNFSF11), osteoclast differentiation and resorption are promoted and at these sites, resorption voids (RL) are formed. Another secreted factor, TNFRSF11B/osteoprotegerin (tumour necrosis factor receptor superfamily, member 11b), inhibits osteoclast-mediated bone resorption by acting as a physiological inhibitor of TNFSF11, while autophagy may be present throughout, acting to prevent osteoporosis. CAP, capillaries; OB, osteoblast; OC, osteoclast; ProOC, pro-osteoclasts; proOB, pro-osteoblasts.

**Table 3 Summary of possible pathways involved in autophagy in the treatment of osteoporosis with Chinese medicines (natural)**

Access routes involved	Chinese Medicines	Compound	References
Wnt/ $\beta$ -catenin signal pathway	<i>Cistanche deserticola</i>	Cistanoside A	[31]
IGFR/Akt pathway	<i>Curculigo orchioides gaertn</i>	Curculigoside	[53]
PI3K/Akt/mTOR pathway	<i>Leonurus japonicus Houtt</i>	Leonurine	[76]
	Chuanxiong Rhizome	Tetramethylpyrazine	[101]
Akt/mTOR signal pathway	<i>Morindae Officinalis Radix</i>	Monotropein	[119]
mTOR/NF $\kappa$ B signal pathway	<i>Anemarrhena asphodeloides Bunge</i>	Timosaponin BII	[155]

Chinese medicine, which states that “kidney governing bones” and “invigorates blood to dispel blood stasis” [222]. The kidneys in TCM are responsible for bone growth, development and repair, and yang tonics are also classically used in TCM to treat osteoporosis and have been experimentally shown to be beneficial in improving bone formation [74, 223, 224]. In research, it has been found that blood-activating drugs can help to remove blood stasis by antiplatelet aggregation, prolonging plasma prothrombin time and lowering plasma fibrinogen levels [118, 225]. In addition, blood-activating herbs can also promote angiogenesis and the growth of new tissue [226]. This confirms what TCM advocates, that blood-activating herbs can help to remove blood stasis and create new tissue. It is well known that osteoporosis is closely related to the imbalance between OB and OC, and related inflammatory factors, such as IL-1 and TNF- $\alpha$ , have important effects on the activity and function of OB and OC. For example, IL-1 mediates OC production and inhibits its apoptosis through activation of the RANK/RANKL/OPG Signaling pathway leading to increased bone resorption, disrupting the balance between bone resorption and bone formation, and promoting the development of OP [227–229]. In addition, IL-1 promotes OP progression by regulating the Wnt pathway and leads to the reduced bone formation by inhibiting OB activity [230]. TNF- $\alpha$  induces increased RANKL secretion, while RANK and RANKL binding recruits TNF receptor-related factors and activates signalling pathways such as NF- $\kappa$ B, MAPK and AKT, enhancing OC activity and bone resorption, so inflammatory factors also play an important role [231–233]. It has been found that in addition to the already described effects of tonifying Yang and invigorating blood, the anti-inflammatory effects of the above natural herbs are also significant [234]. By inhibiting inflammatory factors, they may activate or inhibit the relevant signalling pathways, thereby regulating the onset and development of OP and acting as a treatment for osteoporosis. However, there are still few relevant studies and further research is needed.

Chinese medicine has shown good results and great potential in the treatment of osteoporosis, but there is still work to be done on autophagy and osteoporosis. Secondly, the mechanism of action of a single clear component of Chinese medicine on osteoporosis is not very clear but may have multiple mechanisms of interaction, and further research is still needed. At this stage, there are more studies on the pharmacological effects of Chinese medicine on osteoporosis, but fewer studies on clinical applications and smaller sample sizes, which will require larger sample sizes and increased follow-up times in the future. Although there are still many problems to be solved, as the research on the molecular mechanism of action of Chinese medicine on osteoporosis continues to deepen, it will provide more effective ways to treat osteoporosis in the future.

### Conclusion

In summary, Chinese Medicines (natural) may act against osteoporosis by stimulating autophagy through their components or compounds. More in-depth studies and reports on the safety, efficacy and potential of multi-components and multi-compounds of herbal medicines are needed to provide more evidence for candidates to carry out more

theoretical basis for beneficial and safer prevention and treatment of osteoporosis.

### References

- Noh JY, Yang Y, Jung H. Molecular Mechanisms and Emerging Therapeutics for Osteoporosis. *Int J Mol Sci* 2020;21(20):7623. Available at: <http://doi.org/10.3390/ijms21207623>
- Ensrud KE, Crandall CJ. Osteoporosis. *Ann Intern Med* 2017;167(3):ITC17. Available at: <http://doi.org/10.7326/AITC201708010>
- Zanker J, Duque G. Osteoporosis in Older Persons: Old and New Players. *J Am Geriatr Soc* 2018;67(4):831–840. Available at: <http://doi.org/10.1111/jgs.15716>
- Khatib J, Stote K, Gosmanov AR. Utility of Dxa Screening for Diagnosis of Osteoporosis in Us Veterans Aged 70 Years and Older. *J Investig Med* 2018;66(2):298–303. Available at: <http://doi.org/10.1136/jim-2017-000557>
- Briggs AM, Cross MJ, Hoy DG, et al. Musculoskeletal Health Conditions Represent a Global Threat to Healthy Aging: A Report for the 2015 World Health Organization World Report on Ageing and Health. *Gerontologist* 2016;56(Suppl2):S243–255. Available at: <http://doi.org/10.1093/geront/gnw002>
- Lane NE. Epidemiology, etiology, and diagnosis of osteoporosis. *Am J Obstet Gynecol* 2006;194(2):S3–11. Available at: <http://doi.org/10.1016/j.ajog.2005.08.047>
- Stein E, Shane E. Secondary osteoporosis. *Endocrinol Metab Clin N Am* 2003;32(1):115–34. Available at: [http://doi.org/10.1016/S0889-8529\(02\)00062-2](http://doi.org/10.1016/S0889-8529(02)00062-2)
- Kimball JS, Johnson JP, Carlson DA. Oxidative Stress and Osteoporosis. *J Bone Joint Surg Am* 2021;103(15):1451–1461. Available at: <http://doi.org/10.2106/JBJS.20.00989>
- Li L, Wang Z. Ovarian Aging and Osteoporosis. *Adv Exp Med Biol* 2018:199–215. Available at: [http://doi.org/10.1007/978-981-13-1117-8\\_13](http://doi.org/10.1007/978-981-13-1117-8_13)
- Fischer V, Haffner-Luntzer M. Interaction between bone and immune cells: Implications for postmenopausal osteoporosis. *Semin Cell Dev Biol* 2022;123:14–21. Available at: <http://doi.org/10.1016/j.semcdb.2021.05.014>
- Qadir A, Liang S, Wu Z, Chen Z, Hu L, Qian A. Senile Osteoporosis: The Involvement of Differentiation and Senescence of Bone Marrow Stromal Cells. *Int J Mol Sci* 2020;21(1):349. Available at: <http://doi.org/10.3390/ijms21010349>
- Zhang L, Zheng YL, Wang R, Wang XQ, Zhang H. Exercise for osteoporosis: A literature review of pathology and mechanism. *Front Immunol* 2022;13. Available at: <http://doi.org/10.3389/fimmu.2022.1005665>
- Shares BH, Busch M, White N, Shum L, Eliseev RA. Active mitochondria support osteogenic differentiation by stimulating  $\beta$ -catenin acetylation. *J Biol Chem* 2018;293(41):16019–16027.

- Available at:  
<http://doi.org/10.1074/jbc.RA118.004102>
14. Chen G, Wang S, Long C, et al. PiRNA-63049 inhibits bone formation through Wnt/ $\beta$ -catenin signaling pathway. *Int J Biol Sci* 2021;17(15):4409–4425. Available at:  
<http://doi.org/10.7150/ijbs.64533>
  15. Brunetti G, D'Amato G, Chiarito M, et al. An update on the role of RANKL–RANK/osteoprotegerin and WNT- $\beta$ -catenin signaling pathways in pediatric diseases. *World J Pediatr* 2018;15(1):4–11. Available at:  
<http://doi.org/10.1007/s12519-018-0198-7>
  16. Zhang Z, Wen H, Yang X, et al. Stimuli and Relevant Signaling Cascades for NFATc1 in Bone Cell Homeostasis: Friend or Foe? *Curr Stem Cell Res Ther* 2019;14(3):239–243. Available at:  
<http://doi.org/10.2174/1574888X14666181205122729>
  17. Zhang L, Lin Y, Zhang X, Shan C. Research progress of exosomes in orthopedics. *Front Genet* 2022;13. Available at:  
<http://doi.org/10.3389/fgene.2022.915141>
  18. Chatziravdeli V, Katsaras GN, Lambrou GI. Gene Expression in Osteoblasts and Osteoclasts Under Microgravity Conditions: A Systematic Review. *Curr Genomics* 2019;20(3):184–198. Available at:  
<http://doi.org/10.2174/1389202920666190422142053>
  19. Bellavia D, Dimarco E, Costa V, et al. Flavonoids in Bone Erosive Diseases: Perspectives in Osteoporosis Treatment. *Trends Endocrinol Metab* 2021;32(2):76–94. Available at:  
<http://doi.org/10.1016/j.tem.2020.11.007>
  20. Sato Y, Sakai H, Kobayashi Y, Shibasaki Y, Sasaki T. Bisphosphonate administration alters subcellular localization of vacuolar-type H(+)-ATPase and cathepsin K in osteoclasts during experimental movement of rat molars. *Anat Rec* 2000;260(1):72–80. Available at:  
[http://doi.org/10.1002/1097-0185\(20000901\)260:1 < 72::AID-AR80 > 3.0.CO;2-2](http://doi.org/10.1002/1097-0185(20000901)260:1 < 72::AID-AR80 > 3.0.CO;2-2)
  21. ROSS FP. M-CSF, c-Fms, and Signaling in Osteoclasts and their Precursors. *Ann NY Acad Sci* 2006;1068(1):110–116. Available at:  
<http://doi.org/10.1196/annals.1346.014>
  22. Qin L, Liu W, Cao H, Xiao G. Molecular mechanosensors in osteocytes. *Bone Res* 2020;8(1). Available at:  
<http://doi.org/10.1038/s41413-020-0099-y>
  23. Datta HK, Ng WF, Walker JA, Tuck SP, Varanasi SS. The cell biology of bone metabolism. *J Clin Pathol* 2008;61(5):577–587. Available at:  
<http://doi.org/10.1136/jcp.2007.048868>
  24. Dallas SL, Prideaux M, Bonewald LF. The Osteocyte: An Endocrine Cell ... and More. *Endocr Rev* 2013;34(5):658–690. Available at:  
<http://doi.org/10.1210/er.2012-1026>
  25. Heino TJ, Hentunen TA, Väänänen HK. Conditioned medium from osteocytes stimulates the proliferation of bone marrow mesenchymal stem cells and their differentiation into osteoblasts. *Exp Cell Res* 2004;294(2):458–468. Available at:  
<http://doi.org/10.1016/j.yexcr.2003.11.016>
  26. Raheja LF, Genetos DC, Yellowley CE. Hypoxic osteocytes recruit human MSCs through an OPN/CD44-mediated pathway. *Biochem Biophys Res Commun* 2008;366(4):1061–1066. Available at:  
<http://doi.org/10.1016/j.bbrc.2007.12.076>
  27. Zhao S, Kato Y, Zhang Y, Harris S, Ahuja SS, Bonewald LF. MLO-Y4 Osteocyte-Like Cells Support Osteoclast Formation and Activation. *J Bone Miner Res* 2002;17(11):2068–2079. Available at:  
<http://doi.org/10.1359/jbmr.2002.17.11.2068>
  28. Xiao L, Xiao Y. The Autophagy in Osteoimmunology: Self-Eating, Maintenance, and Beyond. *Front Endocrinol* 2019;10. Available at:  
<http://doi.org/10.3389/fendo.2019.00490>
  29. Mizushima N, Komatsu M. Autophagy: Renovation of Cells and Tissues. *Cell* 2011;147(4):728–741. Available at:  
<http://doi.org/10.1016/j.cell.2011.10.026>
  30. Yang Z, Klionsky DJ. Mammalian autophagy: core molecular machinery and signaling regulation. *Curr Opin Cell Biol* 2010;22(2):124–131. Available at:  
<http://doi.org/10.1016/j.ceb.2009.11.014>
  31. Yin X, Zhou C, Li J, et al. Autophagy in bone homeostasis and the onset of osteoporosis. *Bone Res* 2019;7(1). Available at:  
<http://doi.org/10.1038/s41413-019-0058-7>
  32. Li X, Xu J, Dai B, Wang X, Guo Q, Qin L. Targeting autophagy in osteoporosis: From pathophysiology to potential therapy. *Ageing Res Rev* 2020;62:101098. Available at:  
<http://doi.org/10.1016/j.arr.2020.101098>
  33. Wang T, He H, Liu S, et al. Autophagy: A Promising Target for Age-related Osteoporosis. *Curr Drug Targets* 2019;20(3):354–365. Available at:  
<http://doi.org/10.2174/1389450119666180626120852>
  34. Wang N, Xu P, Wang X, et al. Integrated pathological cell fishing and network pharmacology approach to investigate main active components of Er-Xian decoction for treating osteoporosis. *J Ethnopharmacol* 2019;241:111977. Available at:  
<http://doi.org/10.1016/j.jep.2019.111977>
  35. Mukwaya E, Xu F, Wong MS, Zhang Y. Chinese herbal medicine for bone health. *Pharm Biol* 2014;52(9):1223–1228. Available at:  
<http://doi.org/10.3109/13880209.2014.884606>
  36. Hou PW, Liu SC, Tsay GJ, Tang CH, Chang HH. The Traditional Chinese Medicine “Hu-Qian-Wan” Attenuates Osteoarthritis-Induced Signs and Symptoms in an Experimental Rat Model of Knee Osteoarthritis. *Evid Based Complement Alternat Med* 2022;2022:5367494. Available at:  
<http://doi.org/10.1155/2022/5367494>
  37. Wang M, Liu L, Zhang CS, et al. Mechanism of Traditional Chinese Medicine in Treating Knee Osteoarthritis. *J Pain Res* 2020;13:1421–1429. Available at:  
<http://doi.org/10.2147/JPR.S247827>
  38. Xie YM, Liu H, Jiang JJ, et al. Clinical practice guideline for postmenopausal osteoporosis with traditional Chinese medicine. *Zhongguo Zhong Yao Za Zhi* 2021;46(22):5992–5998. Chinese Available at:  
<http://doi.org/10.19540/j.cnki.cjcm.20210709.501>
  39. Zhang ND, Han T, Huang B-K, et al. Traditional Chinese medicine formulas for the treatment of osteoporosis: Implication for antiosteoporotic drug discovery. *J Ethnopharmacol* 2016;189:61–80. Available at:  
<http://doi.org/10.1016/j.jep.2016.05.025>
  40. Wenxiong L, Kuaiqiang Z, Zhu L, et al. Effect of Zuogui pill and Yougui pill on osteoporosis: a randomized controlled trial. *J Tradit Chin Med* 2018;38(1):33–42. Available at:  
<http://doi.org/10.1016/j.jtcm.2018.01.005>
  41. Li J, Fu SF, Yang Y, An R, Liu HY, Mao HP. Clinical practice of traditional Chinese medicine for the treatment of postmenopausal osteoporosis: a literature review. *Climacteric* 2022;25(6):562–569. Available at:  
<http://doi.org/10.1080/13697137.2022.2102894>
  42. Wang J, Xue JS, Huang S. Recent Advancements in Prevention and Treatment of Osteoporosis with Traditional Chinese Medicine: A Long Way from Lab Bench to Bedside. *Curr Mol Pharmacol* 2023;16(3):321–330. Available at:  
<http://doi.org/10.2174/1874467215666220414145641>
  43. Chen G, Guan Y, Ye X, et al. Effects of bushen qiangu method for primary osteoporosis. *Medicine (Baltimore)* 2020;99(24):e20697. Available at:  
<http://doi.org/10.1097/MD.00000000000020697>
  44. Liu WJ, Cao Y, Song QQ, et al. Chemical characterization for flowers and lignified stems of *Cistanche deserticola*. *Zhongguo Zhong Yao Za Zhi* 2018;43(18):3708–3714. Chinese Available at:  
<http://doi.org/10.19540/j.cnki.cjcm.20180612.001>
  45. An C, Pu X, Wang Q, Zhang H. *Cistanche* extracts ameliorates

- the neurotoxicity induced by hydrogen peroxide in new mutant DJ-1-transfected neuroblastoma cellular models. *Brain Behav* 2019;9(7):e01304. Available at: <http://doi.org/10.1002/brb3.1304>
46. Feng SS, Yang XM, Weng X, Wang B, Zhang A. Aqueous extracts from cultivated *Cistanche deserticola* Y.C. Ma as polysaccharide adjuvant promote immune responses via facilitating dendritic cell activation. *J Ethnopharmacol* 2021;277:114256. Available at: <http://doi.org/10.1016/j.jep.2021.114256>
  47. Liu B, Shi J, Li Z, et al. Study on Neuroendocrine-Immune Function of *Cistanche deserticola* and Its Rice Wine Steaming Products in Glucocorticoid-Induced Rat Model. *Evid Based Complement Alternat Med* 2020;2020:5321976. Available at: <http://doi.org/10.1155/2020/5321976>
  48. Shen CY, Jiang JG, Yang L, Wang DW, Zhu W. Anti-ageing active ingredients from herbs and nutraceuticals used in traditional Chinese medicine: pharmacological mechanisms and implications for drug discovery. *Br J Pharmacol* 2016;174(11):1395–1425. Available at: <http://doi.org/10.1111/bph.13631>
  49. Wang F, Tu P, Zeng K, Jiang Y. Total glycosides and polysaccharides of *Cistanche deserticola* prevent osteoporosis by activating Wnt/ $\beta$ -catenin signaling pathway in SAMP6 mice. *J Ethnopharmacol* 2021;271:113899. Available at: <http://doi.org/10.1016/j.jep.2021.113899>
  50. Song D, Cao Z, Liu Z, et al. *Cistanche deserticola* polysaccharide attenuates osteoclastogenesis and bone resorption via inhibiting RANKL signaling and reactive oxygen species production. *J Cell Physiol* 2018;233(12):9674–9684. Available at: <http://doi.org/10.1002/jcp.26882>
  51. Yuan P, Li J, Aipire A, et al. *Cistanche tubulosa* phenylethanoid glycosides induce apoptosis in H22 hepatocellular carcinoma cells through both extrinsic and intrinsic signaling pathways. *BMC Complement Altern Med* 2018;18(1). Available at: <http://doi.org/10.1186/s12906-018-2201-1>
  52. Wang XM, Wang JF, Guan HY, et al. Comparison of the Chemical Profiles and Antioxidant Activities of Different Parts of Cultivated *Cistanche deserticola* Using Ultra Performance Liquid Chromatography-Quadrupole Time-of-Flight Mass Spectrometry and a 1,1-Diphenyl-2-picrylhydrazyl-Based Assay. *Mol* 2017;22(11):2011. Available at: <http://doi.org/10.3390/molecules22112011>
  53. Xiong Q, Kadota S, Tani T, Namba T. Antioxidative Effects of Phenylethanoids from *Cistanche deserticola*. *Bio Pharm Bull* 1996;19(12):1580–1585. Available at: <http://doi.org/10.1248/bpb.19.1580>
  54. Xiong Q, Tezuka Y, Kaneko T, et al. Inhibition of nitric oxide by phenylethanoids in activated macrophages. *Eur J Pharmacol* 2000;400(1):137–144. Available at: [http://doi.org/10.1016/S0014-2999\(00\)00354-X](http://doi.org/10.1016/S0014-2999(00)00354-X)
  55. Xu XX, Zhang ZZ, Wang WP, Yao HQ, Ma XQ. Therapeutic Effect of *Cistanche* A on Bone Metabolism of Ovariectomized Mice. *Mol* 2017;22(2):197. Available at: <http://doi.org/10.3390/molecules22020197>
  56. Klionsky DJ, Cuervo AM, Seglen PO. Methods for Monitoring Autophagy from Yeast to Human. *Autophagy* 2007;3(3):181–206. Available at: <http://doi.org/10.4161/auto.3678>
  57. Nollet M, Santucci-Darmanin S, Breuil V, et al. Autophagy in osteoblasts is involved in mineralization and bone homeostasis. *Autophagy* 2014;10(11):1965–1977. Available at: <http://doi.org/10.4161/auto.36182>
  58. Chen T, Gao F, Luo D, et al. *Cistanche* A promotes osteogenesis of primary osteoblasts by alleviating apoptosis and activating autophagy through involvement of the Wnt/ $\beta$ -catenin signal pathway. *Ann Transl Med* 2022;10(2):64–64. Available at: <http://doi.org/10.21037/atm-21-6742>
  59. Fan Q, Yang L, Zhang X, et al. Autophagy promotes metastasis and glycolysis by upregulating MCT1 expression and Wnt/ $\beta$ -catenin signaling pathway activation in hepatocellular carcinoma cells. *J Exp Clin Cancer Res* 2018;37(1):9. Available at: <http://doi.org/10.1186/s13046-018-0673-y>
  60. Gordon MD, Nusse R. Wnt Signaling: Multiple Pathways, Multiple Receptors, and Multiple Transcription Factors. *J Biol Chem* 2006;281(32):22429–22433. Available at: <http://doi.org/10.1074/jbc.R600015200>
  61. Pérez-Plasencia C, López-Urrutia E, García-Castillo V, Trujano-Camacho S, López-Camarillo C, Campos-Parra AD. Interplay Between Autophagy and Wnt/ $\beta$ -Catenin Signaling in Cancer: Therapeutic Potential Through Drug Repositioning. *Front Oncol* 2020;10. Available at: <http://doi.org/10.3389/fonc.2020.01037>
  62. Yang XH, Li L, Xue YB, Zhou XX, Tang JH. Flavonoids from *Epimedium pubescens*: extraction and mechanism, antioxidant capacity and effects on CAT and GSH-Px of *Drosophila melanogaster*. *PeerJ* 2020;8:e8361. Available at: <http://doi.org/10.7717/peerj.8361>
  63. Wang Z, Wang D, Zhen W, Zhang J, Peng S. The effect of icariin on bone metabolism and its potential clinical application. *Osteoporos Int* 2018;29(3):535–544. Available at: <http://doi.org/10.1007/s00198-017-4255-1>
  64. Chuang H, Bharath Kumar V, Day CH, et al. *Epimedium* promotes steroidogenesis by CREB activation-mediated mitochondrial fusion in endosulfan treated leydig cells. *Environ Toxicol* 2021;36(9):1873–1879. Available at: <http://doi.org/10.1002/tox.23307>
  65. Niu H, Wang M, Ma D, et al. *Epimedium* flavonoids improve cognitive impairment and white matter lesions induced by chronic cerebral hypoperfusion through inhibiting the Lingo-1/Fyn/ROCK pathway and activating the BDNF/NRG1/PI3K pathway in rats. *Brain Res* 2020;1743:146902. Available at: <http://doi.org/10.1016/j.brainres.2020.146902>
  66. Liu S, Huang Y, Wang C, Tian S, Xu Y, Ge J. *Epimedium* protects steroid-induced avascular necrosis of femoral head in rats by inhibiting autophagy. *Exp Ther Med October* 2018;16(6):5047–5052. Available at: <http://doi.org/10.3892/etm.2018.6827>
  67. Zu Y, Mu Y, Li Q, Zhang ST, Yan HJ. Icariin alleviates osteoarthritis by inhibiting NLRP3-mediated pyroptosis. *J Orthop Surg Res* 2019;14(1). Available at: <http://doi.org/10.1186/s13018-019-1307-6>
  68. Liu H, Xiong Y, Wang H, et al. Effects of water extract from *epimedium* on neuropeptide signaling in an ovariectomized osteoporosis rat model. *J Ethnopharmacol* 2018;221:126–136. Available at: <http://doi.org/10.1016/j.jep.2018.04.035>
  69. Li X, Chen R, Li Y, et al. miR-27a-5p—Abundant Small Extracellular Vesicles Derived From *Epimedium*-Preconditioned Bone Mesenchymal Stem Cells Stimulate Osteogenesis by Targeting Atg4B-Mediated Autophagy. *Front Cell Dev Biol* 2021;9. Available at: <http://doi.org/10.3389/fcell.2021.642646>
  70. DeSelm CJ, Miller BC, Zou W, et al. Autophagy Proteins Regulate the Secretory Component of Osteoclastic Bone Resorption. *Dev Cell* 2011;21(5):966–974. Available at: <http://doi.org/10.1016/j.devcel.2011.08.016>
  71. 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. Available at: <http://doi.org/10.1016/j.jep.2013.03.066>
  72. Hejazi II, Khanam R, Mehdi SH, et al. Antioxidative and anti-proliferative potential of *Curculigo orchoides* Gaertn in oxidative stress induced cytotoxicity: In vitro, ex vivo and in

- silico studies. *Food Chem Toxicol* 2018;115:244–259. Available at: <http://doi.org/10.1016/j.fct.2018.03.013>
73. Tan S, Xu J, Lai A, et al. Curculigioside exerts significant anti-arthritis effects in vivo and in vitro via regulation of the JAK/STAT/NF- $\kappa$ B signaling pathway. *Mol Med Rep* 2019;19(3):2057–2064. Available at: <http://doi.org/10.3892/mmr.2019.9854>
  74. He J, Li X, Wang Z, et al. Therapeutic Anabolic and Anticatabolic Benefits of Natural Chinese Medicines for the Treatment of Osteoporosis. *Front Pharmacol* 2019;10. Available at: <http://doi.org/10.3389/fphar.2019.01344>
  75. Liu M, Liu S, Zhang Q, et al. Curculigioside attenuates oxidative stress and osteoclastogenesis via modulating Nrf2/NF- $\kappa$ B signaling pathway in RAW264.7 cells. *J Ethnopharmacol* 2021;275:114129. Available at: <http://doi.org/10.1016/j.jep.2021.114129>
  76. Cheng Q, Zhang X, Jiang J, et al. Postmenopausal Iron Overload Exacerbated Bone Loss by Promoting the Degradation of Type I Collagen. *Biomed Res Int* 2017;2017:1345193. Available at: <http://doi.org/10.1155/2017/1345193>
  77. Kim B-J, Ahn SH, Bae SJ, et al. Iron overload accelerates bone loss in healthy postmenopausal women and middle-aged men: A 3-year retrospective longitudinal study. *J Bone Miner Res* 2012;27(11):2279–2290. Available at: <http://doi.org/10.1002/jbmr.1692>
  78. Messer JG, Kilbarger AK, Erikson KM, Kipp DE. Iron overload alters iron-regulatory genes and proteins, down-regulates osteoblastic phenotype, and is associated with apoptosis in fetal rat calvaria cultures. *Bone* 2009;45(5):972–79. Available at: <http://doi.org/10.1016/j.bone.2009.07.073>
  79. Xiao W, Beibei F, Guangsi S, et al. Iron overload increases osteoclastogenesis and aggravates the effects of ovariectomy on bone mass. *J Endocrinol* 2015;226(3):121–134. Available at: <http://doi.org/10.1530/JOE-14-0657>
  80. Zhang Q, Zhao L, Shen Y, et al. Curculigioside Protects against Excess-Iron-Induced Bone Loss by Attenuating Akt-FoxO1-Dependent Oxidative Damage to Mice and Osteoblastic MC3T3-E1 Cells. *Oxid Med Cell Longev* 2019;2019:1–14. Available at: <http://doi.org/10.1155/2019/9281481>
  81. Ezzat S, Louka ML, Zakaria ZM, Nagaty MM, Metwaly RG. Autophagy in osteoporosis: Relation to oxidative stress. *J Cell Biochem* 2018;120(2):2560–2568. Available at: <http://doi.org/10.1002/jcb.27552>
  82. Yang L, Jiang H, Yan M, et al. UHPLC-MS/MS Quantification Combined with Chemometrics for Comparative Analysis of Different Batches of Raw, Wine-Processed, and Salt-Processed Radix *Achyranthis Bidentatae*. *Mol* 2018;23(4):758. Available at: <http://doi.org/10.3390/molecules23040758>
  83. Yang L, Jiang H, Yan M, et al. Comparison of pharmacokinetics of phytoecdysones and triterpenoid saponins of monomer, crude and processed Radix *Achyranthis Bidentatae* by UHPLC-MS/MS. *Xenobiotica* 2019;50(6):677–684. Available at: <http://doi.org/10.1080/00498254.2019.1579946>
  84. Zhao BT, Jeong SY, Moon DC, Son KH, Son JK, Woo MH. High performance liquid chromatography used for quality control of *Achyranthis Radix*. *Arch Pharm Res* 2012;35(8):1449–1455. Available at: <http://doi.org/10.1007/s12272-012-0815-2>
  85. Hong G, Lee JY, Kang H, et al. Inhibition of Osteoarthritis-Related Molecules by Isomucronulatol 7-O- $\beta$ -d-glucoside and Ecliptasaponin A in IL-1 $\beta$ -Stimulated Chondrosarcoma Cell Model. *Mol* 2018;23(11):2807. Available at: <http://doi.org/10.3390/molecules23112807>
  86. Liu Z, Wang X, Ou S, Arowolo M, Hou DX, He J. Effects of *Achyranthes bidentata* Polysaccharides on Intestinal Morphology, Immune Response, and Gut Microbiome in Yellow Broiler Chickens Challenged with *Escherichia coli* K88. *Polymers (Basel)* 2018;10(11):1233. Available at: <http://doi.org/10.3390/polym10111233>
  87. Zhang S, Zhang Q, Zhang D, Wang C, Yan C. Anti-osteoporosis activity of a novel *Achyranthes bidentata* polysaccharide via stimulating bone formation. *Carbohydr Polym* 2018;184:288–298. Available at: <http://doi.org/10.1016/j.carbpol.2017.12.070>
  88. Yan C, Zhang S, Wang C, Zhang Q. A fructooligosaccharide from *Achyranthes bidentata* inhibits osteoporosis by stimulating bone formation. *Carbohydr Polym* 2019;210:110–118. Available at: <http://doi.org/10.1016/j.carbpol.2019.01.026>
  89. BOO KH, LEE D, JEON GL, et al. Distribution and Biosynthesis of 20-Hydroxyecdysone in Plants of *Achyranthes japonica* Nakai. *Biosci Biotechnol Biochem* 2010;74(11):2226–2231. Available at: <http://doi.org/10.1271/bbb.100410>
  90. Tang Y, Yue Z, Xin D, et al.  $\beta$ -Ecdysterone promotes autophagy and inhibits apoptosis in osteoporotic rats. *Mol Med Rep* 2017;17(1):1591–1598. Available at: <http://doi.org/10.3892/mmr.2017.8053>
  91. Huang Y, Bo Y, Wu X, et al. An intergated serum and urinary metabonomic research based on UPLC-MS and therapeutic effects of Gushudan on prednisolone-induced osteoporosis rats. *J Chromatogr B* 2016;1027:119–130. Available at: <http://doi.org/10.1016/j.jchromb.2016.05.019>
  92. Chotiyanwong P, McCloskey EV. Pathogenesis of glucocorticoid-induced osteoporosis and options for treatment. *Nat Rev Endocrinol* 2020;16(8):437–447. Available at: <http://doi.org/10.1038/s41574-020-0341-0>
  93. Liu W, Dai N, Wang Y, et al. Role of autophagy in cadmium-induced apoptosis of primary rat osteoblasts. *Sci Rep* 2016;6(1). Available at: <http://doi.org/10.1038/srep20404>
  94. Dai W, Jiang L, Lay YAE, et al. Prevention of glucocorticoid induced bone changes with beta-ecdysone. *Bone* 2015;74:48–57. Available at: <http://doi.org/10.1016/j.bone.2015.01.001>
  95. Shang X, Pan H, Wang X, He H, Li M. *Leonurus japonicus* Houtt.: Ethnopharmacology, phytochemistry and pharmacology of an important traditional Chinese medicine. *J Ethnopharmacol* 2014;152(1):14–32. Available at: <http://doi.org/10.1016/j.jep.2013.12.052>
  96. Garran TA, Ji R, Chen JL, et al. Elucidation of metabolite isomers of *Leonurus japonicus* and *Leonurus cardiaca* using discriminating metabolite isomerism strategy based on ultra-high performance liquid chromatography tandem quadrupole time-of-flight mass spectrometry. *J Chromatogr A* 2019;1598:141–153. Available at: <http://doi.org/10.1016/j.chroma.2019.03.059>
  97. Xiong L, Zhou QM, Peng C, et al. Bis-spirolabdane diterpenoids from *Leonurus japonicus* and their anti-platelet aggregative activity. *Fitoterapia* 2015;100:1–6. Available at: <http://doi.org/10.1016/j.fitote.2014.11.003>
  98. Li Y, Lin Y, Liu X, et al. Leonurine: From Gynecologic Medicine to Pleiotropic Agent. *Chin J Integr Med* 2019;26(2):152–160. Available at: <http://doi.org/10.1007/s11655-019-3453-0>
  99. Jin M, Li Q, Gu Y, et al. Leonurine suppresses neuroinflammation through promoting oligodendrocyte maturation. *J Cell Mol Med* 2018;23(2):1470–1485. Available at: <http://doi.org/10.1111/jcmm.14053>
  100. Du YY, Chen ZX, Liu MY, et al. Leonurine Regulates Treg/Th17 Balance to Attenuate Rheumatoid Arthritis Through Inhibition of TAZ Expression. *Front Immunol* 2020;11. Available at: <http://doi.org/10.3389/fimmu.2020.556526>

101. Loh KP, Qi J, Tan BKH, Liu XH, Wei BG, Zhu YZ. Leonurine Protects Middle Cerebral Artery Occluded Rats Through Antioxidant Effect and Regulation of Mitochondrial Function. *Stroke* 2010;41(11):2661–2668. Available at: <http://doi.org/10.1161/STROKEAHA.110.589895>
102. Zhang Y, Guo W, Wen Y, et al. SCM-198 attenuates early atherosclerotic lesions in hypercholesterolemic rabbits via modulation of the inflammatory and oxidative stress pathways. *Atherosclerosis* 2012;224(1):43–50. Available at: <http://doi.org/10.1016/j.atherosclerosis.2012.06.066>
103. Zhao B, Peng Q, Poon EHL, et al. Leonurine Promotes the Osteoblast Differentiation of Rat BMSCs by Activation of Autophagy via the PI3K/Akt/mTOR Pathway. *Front Bioeng Biotechnol* 2021;9. Available at: <http://doi.org/10.3389/fbioe.2021.615191>
104. Yuan FL, Xu RS, Jiang DL, et al. Leonurine hydrochloride inhibits osteoclastogenesis and prevents osteoporosis associated with estrogen deficiency by inhibiting the NF- $\kappa$ B and PI3K/Akt signaling pathways. *Bone* 2015;75:128–137. Available at: <http://doi.org/10.1016/j.bone.2015.02.017>
105. Xu W, Choi HK, Huang L. State of Panax ginseng Research: A Global Analysis. *Mol* 2017;22(9):1518. Available at: <http://doi.org/10.3390/molecules22091518>
106. Wang CQ, Yi LW, Zhao L, et al. 177 Saponins, Including 11 New Compounds in Wild Ginseng Tentatively Identified via HPLC-IT-TOF-MSn, and Differences among Wild Ginseng, Ginseng under Forest, and Cultivated Ginseng. *Mol* 2021;26(11):3371. Available at: <http://doi.org/10.3390/molecules26113371>
107. Yang Y, Yang Y, Qiu H, et al. Localization of constituents for determining the age and parts of ginseng through ultraperformance liquid chromatography quadrupole/time of flight-mass spectrometry combined with desorption electrospray ionization mass spectrometry imaging. *J Pharm Biomed Anal* 2021;193:113722. Available at: <http://doi.org/10.1016/j.jpba.2020.113722>
108. Phu HT, Thuan DTB, Nguyen THD, Posadino AM, Eid AH, Pintus G. Herbal Medicine for Slowing Aging and Aging-associated Conditions: Efficacy, Mechanisms and Safety. *Curr Vasc Pharmacol* 2020;18(4):369–393. Available at: <http://doi.org/10.2174/1570161117666190715121939>
109. Chen W, Balan P, Popovich DG. Review of Ginseng Anti-Diabetic Studies. *Mol* 2019;24(24):4501. Available at: <http://doi.org/10.3390/molecules24244501>
110. Zhang Q, Liu J, Duan H, Li R, Peng W, Wu C. Activation of Nrf2/HO-1 signaling: An important molecular mechanism of herbal medicine in the treatment of atherosclerosis via the protection of vascular endothelial cells from oxidative stress. *J Adv Res* 2021;34:43–63. Available at: <http://doi.org/10.1016/j.jare.2021.06.023>
111. Aravinthan A, Hossain MA, Kim B, et al. Ginsenoside Rb1 inhibits monoiodoacetate-induced osteoarthritis in postmenopausal rats through prevention of cartilage degradation. *J Ginseng Res* 2021;45(2):287–294. Available at: <http://doi.org/10.1016/j.jgr.2020.01.004>
112. Zhou X, Liu H, Zhang M, Li C, Li G. Spectrum-effect relationship between UPLC fingerprints and anti-lung cancer effect of Panax ginseng. *Phytochem Anal* 2020;32(3):339–346. Available at: <http://doi.org/10.1002/pca.2980>
113. Li X, Liu J, Zuo T, et al. Advances and challenges in ginseng research from 2011 to 2020: the phytochemistry, quality control, metabolism, and biosynthesis. *Nat Prod Rep* 2022;39(4):875–909. Available at: <http://doi.org/10.1039/D1NP00071C>
114. Siddiqi MH, Siddiqi MZ, Kang S, et al. Inhibition of Osteoclast Differentiation by Ginsenoside Rg3 in RAW264.7 Cells via RANKL, JNK and p38 MAPK Pathways Through a Modulation of Cathepsin K: An In Silico and In Vitro Study. *Phytother Res* 2015;29(9):1286–1294. Available at: <http://doi.org/10.1002/ptr.5374>
115. Siddiqi MZ, Siddiqi MH, Kim YJ, Jin Y, Huq MdA, Yang DC. Effect of Fermented Red Ginseng Extract Enriched in Ginsenoside Rg3 on the Differentiation and Mineralization of Preosteoblastic MC3T3-E1 Cells. *J Med Food* 2015;18(5):542–548. Available at: <http://doi.org/10.1089/jmf.2014.3251>
116. Zhang X, Huang F, Chen X, Wu X, Zhu J. Ginsenoside Rg3 attenuates ovariectomy-induced osteoporosis via AMPK mTOR signaling pathway. *Drug Dev Res* 2020;81(7):875–884. Available at: <http://doi.org/10.1002/ddr.21705>
117. Chen Z, Zhang C, Gao F, et al. A systematic review on the rhizome of Ligusticum chuanxiong Hort. (Chuanxiong) *Food Chem Toxicol* 2018;119:309–325. Available at: <http://doi.org/10.1016/j.fct.2018.02.050>
118. Pu ZH, Dai M, Xiong L, Peng C. Total alkaloids from the rhizomes of Ligusticum striatum: a review of chemical analysis and pharmacological activities. *Nat Prod Res* 2020;36(13):3489–3506. Available at: <http://doi.org/10.1080/14786419.2020.1830398>
119. Yan H, Zhou Y, Tang F, et al. A comprehensive investigation on the chemical diversity and efficacy of different parts of Ligusticum chuanxiong. *Food Funct* 2022;13(3):1092–1107. Available at: <http://doi.org/10.1039/D1FO02811A>
120. Li W, Tang Y, Chen Y, Duan JA. Advances in the Chemical Analysis and Biological Activities of Chuanxiong. *Mol* 2012;17(9):10614–10651. Available at: <http://doi.org/10.3390/molecules170910614>
121. Wang M, Yao M, Liu J, et al. Ligusticum chuanxiong exerts neuroprotection by promoting adult neurogenesis and inhibiting inflammation in the hippocampus of ME cerebral ischemia rats. *J Ethnopharmacol* 2020;249:112385. Available at: <http://doi.org/10.1016/j.jep.2019.112385>
122. Li D, Long Y, Yu S, et al. Research Advances in Cardio-Cerebrovascular Diseases of Ligusticum chuanxiong Hort. *Front Pharmacol* 2022;12. Available at: <http://doi.org/10.3389/fphar.2021.832673>
123. Yuan X, Han B, Feng ZM, Jiang JS, Yang YN, Zhang PC. Chemical constituents of Ligusticum chuanxiong and their anti-inflammation and hepatoprotective activities. *Bioorg Chem* 2020;101:104016. Available at: <http://doi.org/10.1016/j.bioorg.2020.104016>
124. Zhang K, Fang KL, Wang T, et al. Chemical Constituents from the Rhizome of Ligusticum chuanxiong Hort. and Their Nrf2 Inducing Activity. *Chem Biodivers* 2021;18(11):e2100302. Available at: <http://doi.org/10.1002/cbdv.202100302>
125. Li H, Zhuo H, Yin D, et al. Intra-Articular Injection of a Nanosuspension of Tetramethylpyrazine Dihydroxynaphthalenolate for Stronger and Longer-Lasting Effects Against Osteoarthritis. *J Biomed Nanotechnol* 2021;17(6):1199–1207. Available at: <http://doi.org/10.1166/jbn.2021.3094>
126. Hu J, Jia X, Fang X, Li P, He C, Chen M. Ultrasonic extraction, antioxidant and anticancer activities of novel polysaccharides from Chuanxiong rhizome. *Int J Biol Macromol* 2016;85:277–284. Available at: <http://doi.org/10.1016/j.ijbiomac.2015.12.046>
127. Zhang Q, Yang YX, Li SY, et al. An ultrafiltration and high performance liquid chromatography coupled with diode array detector and mass spectrometry approach for screening and characterizing thrombin inhibitors from Rhizoma Chuanxiong. *J Chromatogr B* 2017;1061–1062:421–429. Available at: <http://doi.org/10.1016/j.jchromb.2017.07.050>
128. Wang L, Zhang HY, Gao B, et al. Tetramethylpyrazine Protects Against Glucocorticoid-Induced Apoptosis by Promoting Autophagy in Mesenchymal Stem Cells and Improves Bone Mass

- in Glucocorticoid-Induced Osteoporosis Rats. *Stem Cells Dev* 2017;26(6):419–430. Available at: <http://doi.org/10.1089/scd.2016.0233>
129. Wang L, Fan J, Lin YS, et al. Glucocorticoids induce autophagy in rat bone marrow mesenchymal stem cells. *Mol Med Rep* 2014;11(4):2711–2716. Available at: <http://doi.org/10.3892/mmr.2014.3099>
  130. Alers S, Löffler AS, Wesselborg S, Stork B. Role of AMPK-mTOR-Ulk1/2 in the Regulation of Autophagy: Cross Talk, Shortcuts, and Feedbacks. *Mol Cell Biol* 2012;32(1):2–11. Available at: <http://doi.org/10.1128/MCB.06159-11>
  131. Noh BK, Lee JK, Jun H, et al. Restoration of autophagy by puerarin in ethanol-treated hepatocytes via the activation of AMP-activated protein kinase. *Biochem Biophys Res Commun* 2011;414(2):361–366. Available at: <http://doi.org/10.1016/j.bbrc.2011.09.077>
  132. He X, Wang J, Li M, et al. *Eucommia ulmoides* Oliv.: Ethnopharmacology, phytochemistry and pharmacology of an important traditional Chinese medicine. *J Ethnopharmacol* 2014;151(1):78–92. Available at: <http://doi.org/10.1016/j.jep.2013.11.023>
  133. Huang L, Lyu Q, Zheng W, Yang Q, Cao G. Traditional application and modern pharmacological research of *Eucommia ulmoides* Oliv. *Chin Med* 2021;16(1). Available at: <http://doi.org/10.1186/s13020-021-00482-7>
  134. Wong SK, Chin KY, Ima-Nirwana S. The Osteoprotective Effects Of Kaempferol: The Evidence From In Vivo And In Vitro Studies. *Drug Des Devel Ther* 2019;13:3497–3514. Available at: <http://doi.org/10.2147/DDDT.S227738>
  135. Imran M, Salehi B, Sharifi-Rad J, et al. Kaempferol: A Key Emphasis to Its Anticancer Potential. *Mol* 2019;24(12):2277. Available at: <http://doi.org/10.3390/molecules24122277>
  136. Prieto-Domínguez N, Garcia-Mediavilla MV, Sanchez-Campos S, Mauriz JL, Gonzalez-Gallego J. Autophagy as a Molecular Target of Flavonoids Underlying their Protective Effects in Human Disease. *Curr Med Chem* 2018;25(7):814–838. Available at: <http://doi.org/10.2174/0929867324666170918125155>
  137. Kim CJ, Shin SH, Kim BJ, et al. The Effects of Kaempferol-Inhibited Autophagy on Osteoclast Formation. *Int J Mol Sci* 2018;19(1):125. Available at: <http://doi.org/10.3390/ijms19010125>
  138. Wattel A, Kamel S, Mentaverri R, et al. Potent inhibitory effect of naturally occurring flavonoids quercetin and kaempferol on in vitro osteoclastic bone resorption. *Biochem Pharmacol* 2003;65(1):35–42. Available at: [http://doi.org/10.1016/S0006-2952\(02\)01445-4](http://doi.org/10.1016/S0006-2952(02)01445-4)
  139. Hao Q, Zhou J, Zhou L, et al. Prediction the contents of fructose, glucose, sucrose, fructo-oligosaccharides and iridoid glycosides in *Morinda officinalis* radix using near-infrared spectroscopy. *Spectrochim Acta A Mol Biomol Spectrosc* 2020;234:118275. Available at: <http://doi.org/10.1016/j.saa.2020.118275>
  140. Luo H, Wang Y, Qin Q, Wang Y, Xu J, He X. Anti-inflammatory naphthoates and anthraquinones from the roots of *Morinda officinalis*. *Bioorg Chem* 2021;110:104800. Available at: <http://doi.org/10.1016/j.bioorg.2021.104800>
  141. Wang M, Wang Q, Yang Q, Yan X, Feng S, Wang Z. Comparison of Anthraquinones, Iridoid Glycosides and Triterpenoids in *Morinda officinalis* and *Morinda citrifolia* Using UPLC/Q-TOF-MS and Multivariate Statistical Analysis. *Mol* 2019;25(1):160. Available at: <http://doi.org/10.3390/molecules25010160>
  142. Zhao X, Wei J, Shu X, Kong W, Yang M. Multi-elements determination in medical and edible *Alpinia oxyphylla* and *Morinda officinalis* and their decoctions by ICP-MS. *Chemosphere* 2016;164:430–435. Available at: <http://doi.org/10.1016/j.chemosphere.2016.08.122>
  143. Chen DL, Zhang P, Lin L, et al. Protective effects of bajijiasu in a rat model of A $\beta$ 25-35-induced neurotoxicity. *J Ethnopharmacol* 2014;154(1):206–217. Available at: <http://doi.org/10.1016/j.jep.2014.04.004>
  144. Zhang Z, Zhang Q, Yang H, et al. Monotropein isolated from the roots of *Morinda officinalis* increases osteoblastic bone formation and prevents bone loss in ovariectomized mice. *Fitoterapia* 2016;110:166–172. Available at: <http://doi.org/10.1016/j.fitote.2016.03.013>
  145. Shen Y, Zhang Q, Wu Y, et al. Pharmacokinetics and tissue distribution of monotropein and deacetyl asperulosidic acid after oral administration of extracts from *Morinda officinalis* root in rats. *BMC Complement Altern Med* 2018;18(1). Available at: <http://doi.org/10.1186/s12906-018-2351-1>
  146. Shi Y, Liu XY, Jiang YP, et al. Monotropein attenuates oxidative stress via Akt/mTOR-mediated autophagy in osteoblast cells. *Biomed Pharmacother* 2020;121:109566. Available at: <http://doi.org/10.1016/j.biopha.2019.109566>
  147. Xu ZS, Wang XY, Xiao DM, et al. Hydrogen sulfide protects MC3T3-E1 osteoblastic cells against H<sub>2</sub>O<sub>2</sub>-induced oxidative damage—implications for the treatment of osteoporosis. *Free Radical Biol Med* 2011;50(10):1314–1323. Available at: <http://doi.org/10.1016/j.freeradbiomed.2011.02.016>
  148. Liu Y, Porta A, Peng X, et al. Prevention of Glucocorticoid-Induced Apoptosis in Osteocytes and Osteoblasts by Calbindin-D28k. *J Bone Miner Res* 2003;19(3):479–490. Available at: <http://doi.org/10.1359/JBMR.0301242>
  149. Ayati Z, Ramezani M, Amiri MS, et al. Ethnobotany, Phytochemistry and Traditional Uses of *Curcuma* spp. and Pharmacological Profile of Two Important Species (*C. longa* and *C. zedoaria*): A Review. *Curr Pharm Des* 2019;25 (8):871–935. Available at: <http://doi.org/10.2174/1381612825666190402163940>
  150. Lu Y, Wang J, Shen G, et al. Rapid Determination and Quality Control of Pharmacological Volatiles of Turmeric (*Curcuma longa* L.) by Fast Gas Chromatography–Surface Acoustic Wave Sensor. *Mol* 2021;26(19):5797. Available at: <http://doi.org/10.3390/molecules26195797>
  151. Nurani LH, Rohman A, Windarsih A, et al. Metabolite Fingerprinting Using 1H-NMR Spectroscopy and Chemometrics for Classification of Three Curcuma Species from Different Origins. *Mol* 2021;26(24):7626. Available at: <http://doi.org/10.3390/molecules26247626>
  152. Chen Z, Quan L, Zhou H, et al. Screening of active fractions from *Curcuma Longa* Radix isolated by HPLC and GC-MS for promotion of blood circulation and relief of pain. *J Ethnopharmacol* 2019;234:68–75. Available at: <http://doi.org/10.1016/j.jep.2018.09.035>
  153. Memarzai A, Khazdair MR, Behrouz S, et al. Experimental and clinical reports on anti-inflammatory, antioxidant, and immunomodulatory effects of *Curcuma longa* and curcumin, an updated and comprehensive review. *Biofactors* 2021;47(3):311–350. Available at: <http://doi.org/10.1002/biof.1716>
  154. Dai W, Yan W, Leng X, Chen J, Hu X, Ao Y. Effectiveness of *Curcuma longa* extract versus placebo for the treatment of knee osteoarthritis: A systematic review and meta-analysis of randomized controlled trials. *Phytother Res* 2021;35(11):5921–5935. Available at: <http://doi.org/10.1002/ptr.7204>
  155. Razavi BM, Ghasemzadeh Rahbardar M, Hosseinzadeh H. A review of therapeutic potentials of turmeric (*Curcuma longa*) and its active constituent, curcumin, on inflammatory disorders, pain, and their related patents. *Phytother Res* 2021;35(12):6489–6513. Available at: <http://doi.org/10.1002/ptr.7224>

156. Sharma M, Monika, Thakur P, Saini RV, Kumar R, Torino E. Unveiling antimicrobial and anticancerous behavior of AuNPs and AgNPs moderated by rhizome extracts of *Curcuma longa* from diverse altitudes of Himalaya. *Sci Rep* 2020;10(1). Available at: <http://doi.org/10.1038/s41598-020-67673-4>
157. Kocaadam B, Şanlıer N. Curcumin, an active component of turmeric (*Curcuma longa*), and its effects on health. *Crit Rev Food Sci Nutr* 2015;57(13):2889–2895. Available at: <http://doi.org/10.1080/10408398.2015.1077195>
158. Soleimani V, Sahebkar A, Hosseinzadeh H. Turmeric (*Curcuma longa*) and its major constituent (curcumin) as nontoxic and safe substances: Review. *Phytother Res* 2018;32(6):985–995. Available at: <http://doi.org/10.1002/ptr.6054>
159. Hu P, Ke C, Guo X, et al. Both glypican-3/Wnt/ $\beta$ -catenin signaling pathway and autophagy contributed to the inhibitory effect of curcumin on hepatocellular carcinoma. *Dig Liver Dis* 2019;51(1):120–26. Available at: <http://doi.org/10.1016/j.dld.2018.06.012>
160. Zhou T, Ye L, Bai Y, et al. Autophagy and Apoptosis in Hepatocellular Carcinoma Induced by EF25-(GSH)2: A Novel Curcumin Analog. *PLoS ONE* 2014;9(9):e107876. Available at: <http://doi.org/10.1371/journal.pone.0107876>
161. Han J, Pan XY, Xu Y, et al. Curcumin induces autophagy to protect vascular endothelial cell survival from oxidative stress damage. *Autophagy* 2012;8(5):812–825. Available at: <http://doi.org/10.4161/auto.19471>
162. Ke D, Wang Y, Yu Y, et al. Curcumin-activated autophagy plays a negative role in its anti-osteoclastogenic effect. *Mol Cell Endocrinol* 2020;500:110637. Available at: <http://doi.org/10.1016/j.mce.2019.110637>
163. Xiu Y, Xu H, Zhao C, et al. Chloroquine reduces osteoclastogenesis in murine osteoporosis by preventing TRAF3 degradation. *J Clin Invest* 2013;124(1):297–310. Available at: <http://doi.org/10.1172/JCI66947>
164. Peng W, Qin R, Li X, Zhou H. Botany, phytochemistry, pharmacology, and potential application of *Polygonum cuspidatum* Sieb. et Zucc.: A review. *J Ethnopharmacol* 2013;148(3):729–745. Available at: <http://doi.org/10.1016/j.jep.2013.05.007>
165. Wang X, Qin Y, Li G, et al. Study on Chemical Constituents in *Polygoni Cuspidati Folium* and its Preparation by UPLC-ESI-Q-TOF-MS/MS. *J Chromatogr Sci* 2018;56(5):425–435. Available at: <http://doi.org/10.1093/chromsci/bmy017>
166. Yi T, Zhang H, Cai Z. Analysis of Rhizoma *Polygoni Cuspidati* by HPLC and HPLC-ESI/MS. *Phytochem Anal* 2007;18(5):387–392. Available at: <http://doi.org/10.1002/pca.993>
167. Nawrot-Hadzik I, Ślusarczyk S, Granica S, Hadzik J, Matkowski A. Phytochemical Diversity in Rhizomes of Three Reynoutria Species and their Antioxidant Activity Correlations Elucidated by LC-ESI-MS/MS Analysis. *Mol* 2019;24(6):1136. Available at: <http://doi.org/10.3390/molecules24061136>
168. Kim J, Kim CS, Jo K, Lee IS, Kim JH, Kim JS. POCU1b, the n-Butanol Soluble Fraction of *Polygoni Cuspidati* Rhizoma et Radix, Attenuates Obesity, Non-Alcoholic Fatty Liver, and Insulin Resistance via Inhibitions of Pancreatic Lipase, cAMP-Dependent PDE Activity, AMPK Activation, and SOCS-3 Suppression. *Nutrients* 2020;12(12):3612. Available at: <http://doi.org/10.3390/nu12123612>
169. Guo W, Wang H, Liu H, et al. Inhibition of inflammatory mediator release from microglia can treat ischemic/hypoxic brain injury. *Neural Regen Res* 2013;8(13):1157–1168. Available at: <http://doi.org/10.4103/1673-5374.112844>
170. Liu L, Guo G, Wu M, Zhang W. The progress of the research on cardio-vascular effects and acting mechanism of polydatin. *Chin J Integr Med* 2012;18(9):714–719. Available at: <http://doi.org/10.1007/s11655-012-1060-8>
171. Yang X, Jiang T, Wang Y, Guo L. The Role and Mechanism of SIRT1 in Resveratrol-regulated Osteoblast Autophagy in Osteoporosis Rats. *Sci Rep* 2019;9(1). Available at: <http://doi.org/10.1038/s41598-019-44766-3>
172. Herbein G. Sirtuin 1 Activity in Peripheral Blood Mononuclear Cells of Patients with Osteoporosis. *Med Sci Monit Basic Res* 2014;20:142–145. Available at: <http://doi.org/10.12659/MSMBR.891372>
173. Liu X, Tao J, Yao Y, et al. Resveratrol induces proliferation in preosteoblast cell MC3T3-E1 via GATA-1 activating autophagy. *Acta Biochim Biophys Sin (Shanghai)* 2021;53(11):1495–1504. Available at: <http://doi.org/10.1093/abbs/gmab135>
174. Wang Y, Dan Y, Yang D, et al. The genus *Anemarrhena* Bunge: A review on ethnopharmacology, phytochemistry and pharmacology. *J Ethnopharmacol* 2014;153(1):42–60. Available at: <http://doi.org/10.1016/j.jep.2014.02.013>
175. Wang Z, Cai J, Fu Q, et al. Anti-Inflammatory Activities of Compounds Isolated from the Rhizome of *Anemarrhena asphodeloides*. *Mol* 2018;23(10):2631. Available at: <http://doi.org/10.3390/molecules23102631>
176. Ji D, Huang Z, Fei C, Xue W, Lu T. Comprehensive profiling and characterization of chemical constituents of rhizome of *Anemarrhena asphodeloides* Bge. *J Chromatogr B* 2017;1060:355–366. Available at: <http://doi.org/10.1016/j.jchromb.2017.06.032>
177. Zhang J, Meng Z, Zhang M, Ma D, Xu S, Kodama H. Effect of six steroidal saponins isolated from *Anemarrhena rhizoma* on platelet aggregation and hemolysis in human blood. *Clin Chim Acta* 1999;289(1–2):79–88. Available at: [http://doi.org/10.1016/S0009-8981\(99\)00160-6](http://doi.org/10.1016/S0009-8981(99)00160-6)
178. Wang HQ, Liu M, Wang L, et al. Identification of a novel BACE1 inhibitor, timosaponin A-III, for treatment of Alzheimer's disease by a cell extraction and chemogenomics target knowledgebase-guided method. *Phytomedicine* 2020;75:153244. Available at: <http://doi.org/10.1016/j.phymed.2020.153244>
179. Shen XB, Ding DL, Yu LZ, et al. Total extract of *Anemarrhena rhizoma* attenuates bleomycin-induced pulmonary fibrosis in rats. *Bioorg Chem* 2022;119:105546. Available at: <http://doi.org/10.1016/j.bioorg.2021.105546>
180. Cao X, Shang Y, Kong W, Jiang S, Liao J, Dai R. Flavonoids derived from *Anemarrhena rhizoma* ameliorate inflammation of benign prostatic hyperplasia via modulating COX/LOX pathways. *J Ethnopharmacol* 2022;284:114740. Available at: <http://doi.org/10.1016/j.jep.2021.114740>
181. Piwowar A, Rembiakowska N, Rorbach-Dolata A, et al. *Anemarrhena asphodeloides* rhizoma Extract Enriched in Mangiferin Protects PC12 Cells against a Neurotoxic Agent-3-Nitropropionic Acid. *Int J Mol Sci* 2020;21(7):2510. Available at: <http://doi.org/10.3390/ijms21072510>
182. Wang N, Xu P, Wu R, et al. Timosaponin BII improved osteoporosis caused by hyperglycemia through promoting autophagy of osteoblasts via suppressing the mTOR/NF $\kappa$ B signaling pathway. *Free Radical Biol Med* 2021;171:112–123. Available at: <http://doi.org/10.1016/j.freeradbiomed.2021.05.014>
183. MEIm XD, CAO YF, CHE YY, et al. Danshen: a phytochemical and pharmacological overview. *Chin J Nat Med* 2019;17(1):59–80. Available at: [http://doi.org/10.1016/S1875-5364\(19\)30010-X](http://doi.org/10.1016/S1875-5364(19)30010-X)
184. Tang H, Qin N, Rao C, Zhu J, Wang H, Hu G. Screening of Potential Anti-Thrombotic Ingredients from *Salvia miltiorrhiza* in Zebrafish and by Molecular Docking. *Mol* 2021;26(22):6807. Available at:

- <http://doi.org/10.3390/molecules26226807>
185. Zhou Y, Wang X, Ying W, Wu D, Zhong P. Cryptotanshinone Attenuates Inflammatory Response of Microglial Cells via the Nrf2/HO-1 Pathway. *Front Neurosci* 2019;13. Available at: <http://doi.org/10.3389/fnins.2019.00852>
  186. Zhang J, Cai Z, Yang M, Tong L, Zhang Y. Inhibition of tanshinone IIA on renin activity protected against osteoporosis in diabetic mice. *Pharm Biol* 2020;58(1):219–224. Available at: <http://doi.org/10.1080/13880209.2020.1738502>
  187. Jiang G, Liu J, Ren B, et al. Anti-tumor and chemosensitization effects of Cryptotanshinone extracted from *Salvia miltiorrhiza* Bge. on ovarian cancer cells in vitro. *J Ethnopharmacol* 2017;205:33–40. Available at: <http://doi.org/10.1016/j.jep.2017.04.026>
  188. Luo H, Kong W, Hu Y, et al. Quality evaluation of *Salvia miltiorrhiza* Bge. by ultra high performance liquid chromatography with photodiode array detection and chemical fingerprinting coupled with chemometric analysis. *J Sep Sci* 2015;38(9):1544–1551. Available at: <http://doi.org/10.1002/jssc.201401430>
  189. Liang W, Chen W, Wu L, et al. Quality Evaluation and Chemical Markers Screening of *Salvia miltiorrhiza* Bge. (Danshen) Based on HPLC Fingerprints and HPLC-MSn Coupled with Chemometrics. *Mol* 2017;22(3):478. Available at: <http://doi.org/10.3390/molecules22030478>
  190. Wang S, Zhang S, Wang S, Gao P, Dai L. A comprehensive review on *Pueraria*: Insights on its chemistry and medicinal value. *Biomed Pharmacother* 2020;131:110734. Available at: <http://doi.org/10.1016/j.biopha.2020.110734>
  191. Shang X, Huang D, Wang Y, et al. Identification of Nutritional Ingredients and Medicinal Components of *Pueraria lobata* and Its Varieties Using UPLC-MS/MS-Based Metabolomics. *Mol* 2021;26(21):6587. Available at: <http://doi.org/10.3390/molecules26216587>
  192. Jin SE, Son YK, Min BS, Jung HA, Choi JS. Anti-inflammatory and antioxidant activities of constituents isolated from *Pueraria lobata* roots. *Arch Pharm Res* 2012;35(5):823–837. Available at: <http://doi.org/10.1007/s12272-012-0508-x>
  193. Jiang Z, Cui X, Qu P, Shang C, Xiang M, Wang J. Roles and mechanisms of puerarin on cardiovascular disease: A review. *Biomed Pharmacother* 2022;147:112655. Available at: <http://doi.org/10.1016/j.biopha.2022.112655>
  194. Yang L, Chen J, Lu H, et al. *Pueraria lobata* for Diabetes Mellitus: Past, Present and Future. *Am J Chin Med* 2019;47(07):1419–1444. Available at: <http://doi.org/10.1142/S0192415X19500733>
  195. Lim D, Lee C, Kim IH, Kim Y. Anti-Inflammatory Effects of Total Isoflavones from *Pueraria lobata* on Cerebral Ischemia in Rats. *Mol* 2013;18(9):10404–10412. Available at: <http://doi.org/10.3390/molecules180910404>
  196. Dong Z, Zhang M, Li H, Zhan Q, Lai F, Wu H. Structural characterization and immunomodulatory activity of a novel polysaccharide from *Pueraria lobata* (Willd.) Ohwi root. *Int J Biol Macromol* 2020;154:1556–1564. Available at: <http://doi.org/10.1016/j.ijbiomac.2019.11.040>
  197. Qin H, Zhao W, Jiao Y, et al. Aqueous Extract of *Salvia miltiorrhiza* Bunge-Radix *Puerariae* Herb Pair Attenuates Osteoporosis in Ovariectomized Rats Through Suppressing Osteoclast Differentiation. *Front Pharmacol* 2021;11. Available at: <http://doi.org/10.3389/fphar.2020.581049>
  198. Amjadi-Moheb F, Akhavan-Niaki H. Wnt signaling pathway in osteoporosis: Epigenetic regulation, interaction with other signaling pathways, and therapeutic promises. *J Cell Physiol* 2019;234(9):14641–14650. Available at: <http://doi.org/10.1002/jcp.28207>
  199. Uehara S, Udagawa N, Kobayashi Y. Non-canonical Wnt signals regulate cytoskeletal remodeling in osteoclasts. *Cell Mol Life Sci* 2018;75(20):3683–3692. Available at: <http://doi.org/10.1007/s00018-018-2881-1>
  200. Nakashima K, Zhou X, Kunkel G, et al. The Novel Zinc Finger-Containing Transcription Factor Osterix Is Required for Osteoblast Differentiation and Bone Formation. *Cell* 2002;108(1):17–29. Available at: [http://doi.org/10.1016/S0092-8674\(01\)00622-5](http://doi.org/10.1016/S0092-8674(01)00622-5)
  201. Witcher PC, Miner SE, Horan DJ, et al. Sclerostin neutralization unleashes the osteoanabolic effects of Dkk1 inhibition. *JCI Insight* 2018;3(11). Available at: <http://doi.org/10.1172/jci.insight.98673>
  202. Takayanagi H. Two-faced immunology—from osteogenesis to bone resorption. *Nat Rev Rheumatol* 2015;11(2):74–76. Available at: <http://doi.org/10.1038/nrrheum.2014.219>
  203. Xiong J, Onal M, Jilka RL, Weinstein RS, Manolagas SC, O'Brien CA. Matrix-embedded cells control osteoclast formation. *Nat Med* 2011;17(10):1235–1241. Available at: <http://doi.org/10.1038/nm.2448>
  204. Kong YY, Yoshida H, Sarosi I, et al. OPGL is a key regulator of osteoclastogenesis, lymphocyte development and lymph-node organogenesis. *Nature* 1999;397(6717):315–323. Available at: <http://doi.org/10.1038/16852>
  205. Lacey DL, Timms E, Tan HL, et al. Osteoprotegerin Ligand Is a Cytokine that Regulates Osteoclast Differentiation and Activation. *Cell* 1998;93(2):165–176. Available at: [http://doi.org/10.1016/S0092-8674\(00\)81569-X](http://doi.org/10.1016/S0092-8674(00)81569-X)
  206. Srivastava RK, Dar HY, Mishra PK. Immunoporosis: Immunology of Osteoporosis—Role of T Cells. *Front Immunol* 2018;9. Available at: <http://doi.org/10.3389/fimmu.2018.00657>
  207. Kamiya Y, Kikuchi T, Goto H, et al. IL-35 and RANKL Synergistically Induce Osteoclastogenesis in RAW264 Mouse Monocytic Cells. *Int J Mol Sci* 2020;21(6):2069. Available at: <http://doi.org/10.3390/ijms21062069>
  208. De Martinis M, Sirufo MM, Suppa M, Ginaldi L. IL-33/IL-31 Axis in Osteoporosis. *Int J Mol Sci* 2020;21(4):1239. Available at: <http://doi.org/10.3390/ijms21041239>
  209. Ginaldi L, De Martinis M. Osteoimmunology and Beyond. *Curr Med Chem* 2016;23(33):3754–3774. Available at: <http://doi.org/10.2174/0929867323666160907162546>
  210. An J, Yang H, Zhang Q, et al. Natural products for treatment of osteoporosis: The effects and mechanisms on promoting osteoblast-mediated bone formation. *Life Sci* 2016;147:46–58. Available at: <http://doi.org/10.1016/j.lfs.2016.01.024>
  211. Farr JN, Khosla S. Cellular senescence in bone. *Bone* 2019;121:121–133. Available at: <http://doi.org/10.1016/j.bone.2019.01.015>
  212. Tao Z, Wang J, Wen K, et al. Pyroptosis in Osteoblasts: A Novel Hypothesis Underlying the Pathogenesis of Osteoporosis. *Front Endocrinol* 2021;11. Available at: <http://doi.org/10.3389/fendo.2020.548812>
  213. Chen Y, Azad MB, Gibson SB. Superoxide is the major reactive oxygen species regulating autophagy. *Cell Death Differ* 2009;16(7):1040–1052. Available at: <http://doi.org/10.1038/cdd.2009.49>
  214. Song S, Tan J, Miao Y, Li M, Zhang Q. Crosstalk of autophagy and apoptosis: Involvement of the dual role of autophagy under ER stress. *J Cell Physiol* 2017;232(11):2977–2984. Available at: <http://doi.org/10.1002/jcp.25785>
  215. Xu Z, Yang L, Xu S, Zhang Z, Cao Y. The receptor proteins: pivotal roles in selective autophagy. *Acta Biochim Biophys Sin (Shanghai)* 2015;47(8):571–580. Available at: <http://doi.org/10.1093/abbs/gmv055>
  216. Hocking LJ, Whitehouse C, Helfrich MH. Autophagy: A new player in skeletal maintenance? *J Bone Miner Res* 2012;27(7):1439–1447. Available at: <http://doi.org/10.1002/jbmr.1668>
  217. Li W, Zhao J, Sun W, et al. Osteocytes promote

- osteoclastogenesis via autophagy-mediated RANKL secretion under mechanical compressive force. *Arch Biochem Biophys* 2020;694:108594. Available at: <http://doi.org/10.1016/j.abb.2020.108594>
218. Chen L, Mo S, Hua Y. Compressive force-induced autophagy in periodontal ligament cells downregulates osteoclastogenesis during tooth movement. *J Periodontol* 2019;90(10):1170–1181. Available at: <http://doi.org/10.1002/JPER.19-0049>
  219. Zheng L, Wang W, Ni J, et al. Role of Autophagy in Tumor Necrosis Factor- $\alpha$ -Induced Apoptosis of Osteoblast Cells. *J Investig Med* 2017;65(6):1014–1020. Available at: <http://doi.org/10.1136/jim-2017-000426>
  220. Shapiro IM, Layfield R, Lotz M, Settembre C, Whitehouse C. Boning up on autophagy. *Autophagy* 2013;10(1):7–19. Available at: <http://doi.org/10.4161/auto.26679>
  221. Fu R, Li J, Yu H, Zhang Y, Xu Z, Martin C. The Yin and Yang of traditional Chinese and Western medicine. *Med Res Rev* 2021;41(6):3182–3200. Available at: <http://doi.org/10.1002/med.21793>
  222. Yao B, Liu J, Zhang M, Leng X, Zhao D. Deciphering the potential pharmaceutical mechanism of Guzhi Zengsheng Zhitongwan on rat bone and kidney based on the “kidney governing bone” theory. *J Orthop Surg Res* 2020;15(1). Available at: <http://doi.org/10.1186/s13018-020-01677-8>
  223. Yang S, Wang T, Zhang J, Leng X, Yao B. Integrated RNA-Seq Analysis Uncovers the Potential Mechanism of the “Kidney Governing Bones” Theory of TCM. *Evid Based Complement Alternat Med* 2022;2022:1–20. Available at: <http://doi.org/10.1155/2022/7044775>
  224. Ju D, Liu M, Zhao H, Wang J. Mechanisms of “kidney governing bones” theory in traditional Chinese medicine. *Front Med* 2014;8(3):389–393. Available at: <http://doi.org/10.1007/s11684-014-0362-y>
  225. Yao Q, Chang B, Chen R, et al. Research Advances in Pharmacology, Safety, and Clinical Applications of Yunnan Baiyao, a Traditional Chinese Medicine Formula. *Front Pharmacol* 2021;12. Available at: <http://doi.org/10.3389/fphar.2021.773185>
  226. Liao L, Gong L, Zhou M, Xue X, Li Y, Peng C. Leonurine Ameliorates Oxidative Stress and Insufficient Angiogenesis by Regulating the PI3K/Akt-eNOS Signaling Pathway in H<sub>2</sub>O<sub>2</sub>-Induced HUVECs. *Oxid Med Cell Longev* 2021;2021:1–12. Available at: <http://doi.org/10.1155/2021/9919466>
  227. Zhao Y, Wang HL, Li TT, Yang F, Tzeng CM. Baicalin Ameliorates Dexamethasone-Induced Osteoporosis by Regulation of the RANK/RANKL/OPG Signaling Pathway. *Drug Des Devel Ther* 2020;14:195–206. Available at: <http://doi.org/10.2147/DDDT.S225516>
  228. KOSTENUIK P. Osteoprotegerin and RANKL regulate bone resorption, density, geometry and strength. *Curr Opin Pharmacol* 2005;5(6):618–625. Available at: <http://doi.org/10.1016/j.coph.2005.06.005>
  229. Ding Z, Shi H, Yang W. Osteoprotective Effect of Cimracemate in Glucocorticoid-Induced Osteoporosis by Osteoprotegerin/Receptor Activator of Nuclear Factor  $\kappa$  B/Receptor Activator of Nuclear Factor Kappa- $\beta$  Ligand Signaling. *Pharmacol* 2019;103(3–4):163–172. Available at: <http://doi.org/10.1159/000495509>
  230. Dischereit G, Lange U. Rheuma und Knochenstoffwechsel. *Orthopade* 2019;48(11):911–916. Available at: <http://doi.org/10.1007/s00132-019-03809-3>
  231. Kitaura H, Marahleh A, Ohori F, et al. Osteocyte-Related Cytokines Regulate Osteoclast Formation and Bone Resorption. *Int J Mol Sci* 2020;21(14):5169. Available at: <http://doi.org/10.3390/ijms21145169>
  232. Bharti AC, Takada Y, Shishodia S, Aggarwal BB. Evidence That Receptor Activator of Nuclear Factor (NF)- $\kappa$ B Ligand Can Suppress Cell Proliferation and Induce Apoptosis through Activation of a NF- $\kappa$ B-independent and TRAF6-dependent Mechanism. *J Biol Chem* 2004;279(7):6065–6076. Available at: <http://doi.org/10.1074/jbc.M308062200>
  233. Ponzetti M, Rucci N. Updates on Osteoimmunology: What’s New on the Cross-Talk Between Bone and Immune System. *Front Endocrinol* 2019;10. Available at: <http://doi.org/10.3389/fendo.2019.00236>
  234. Meng T, Xiao D, Muhammed A, Deng J, Chen L, He J. Anti-Inflammatory Action and Mechanisms of Resveratrol. *Mol* 2021;26(1):229. Available at: <http://doi.org/10.3390/molecules26010229>