Traditional Chinese Medicine formulas for the treatment of osteoporosis: Implication for antiosteoporotic drug discovery

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Abstract

ETHNOPHARMACOLOGICAL RELEVANCE:

Osteoporosis is a chronic epidemic which can lead to enhanced bone fragility and consequent an increase in fracture risk. Traditional Chinese medicine (TCM) formulas have a long history of use in the prevention and treatment of osteoporosis. Antiosteoporotic TCM formulas have conspicuous advantage over single drugs. Systematic data mining of the existing antiosteoporotic TCM formulas database can certainly help the drug discovery

processes and help the identification of safe candidates with synergistic formulations. In this review, the authors summarize the clinical use and animal experiments of TCM formulas and their mechanism of action, and discuss the potential antiosteoporotic activity and the active constituents of commonly used herbs in TCM formulas for the therapy of osteoporosis.

MATERIALS AND METHODS:

The literature was searched from Medline, Pubmed, ScienceDirect, Spring Link, Web of Science, CNKI and VIP database from 1989 to 2015, and also collected from Chinese traditional books and Chinese Pharmacopoeia with key words such as osteoporosis, osteoblast, osteoclast, traditional Chinese medicine formulas to identify studies on the antiosteoporotic effects of TCM formulas, herbs and chemical constituents, and also their possible mechanisms.

RESULTS:

Thirty-three TCM formulas were commonly used to treat osteoporosis, and showed significant antiosteoporotic effects in human and animal. The herb medicines and their chemical constituents in TCM formulas were summarized, the pharmacological effects and chemical constituents of commonly used herbs in TCM formulas were described in detail. The action mechanisms of TCM formulas and their chemical constituents were described. Finally, the implication for the discovery of antiosteoporotic leads and combinatory ingredients from TCM formulas were prospectively discussed.

CONCLUSIONS:

Clinical practice and animal experiments indicate that TCM formulas provide a definite therapeutic effect on osteoporosis. The active constituents in TCM formulas are diverse in chemical structure, and include flavonoids, lignans, saponins and iridoid glycosides. Antiosteoporotic mechanism of TCM formulas and herbs involves multi regulatory pathways, such as Wnt/β-catenin, BMP/Smad, MAPK pathway and RANKL/OPG system. Phytochemicals from TCM formulas and their compositional herb medicines offer great potential for the development of novel antiosteoporotic drugs. The active ingredients in TCM formulas can be developed in combination as potent drugs, which may exhibit better antiosteoporotic effects compared to the individual compound.

Chemical compounds studied in this article

Aucubin (PubChem CID: 91458); Ecdysterone (PubChem CID: 271605); Catalpol (PubChem CID: 91520); Ferulic acid (PubChem CID: 445858); Sweroside (PubChem CID: 161036); Formononetin (PubChem CID: 5280378); Cinnamaldehyde (PubChem CID: 637511); AsperosaponinⅥ (PubChem CID: 71307450); Emodin (PubChem CID: 3220); Kaempferol (PubChem CID: 5280863)

Key words: osteoporosis; traditional Chinese medicine formulas; clinical use; action mechanism; active ingredients

Contents

1. Introduction

Osteoporosis, a chronic epidemic, is characterized by low bone mass and microarchictectural deterioration of bone tissues, leading to enhanced bone fragility and consequent increase in fracture risk (Appelman-Dijkstra and Papapoulos, 2015). Osteoporosis is a growing problem worldwide, with the greatest burden resulting from fractures. It is

estimated that more than 200 million people worldwide suffer from osteoporosis.(Lewiecki, 2011). These numbers are expected to steadily increase over time, with osteoporosis affecting an estimated 14 million people with over 47 million cases of low bone mass by the year 2020 (Ford et al., 2011). Treatment of osteoporosis consists of pharmacotherapy and lifestyle measures, including dietary changes, mineral supplementation, and exercise programs. Currently, the most commonly used agents for the treatment of osteoporosis include raloxifene; bisphosphonates alendronate, ibandronate, risedronate and zoledronic acid; agents derived from parathyroid hormone (PTH); denosumab and strontium ranelate, and also hormone replacement. However, due to adverse effects of the drugs, the uses of these medications on a long term basis are limited.

Traditional Chinese medicine (TCM) has been used in China and other Asian countries for thousands of years (Jin et al., 2013), either as mono-therapy or in combination with standard Western medical treatment, to manage the entire spectrum of medical disorders. TCM formulas are often composed of more than one herb, and the main principle underlying the use of herbal formulas is that complex interactions between herbs produce synergistic effects that can improve therapeutic efficacy, or reduce possible side-effects of individual herbs (Gao et al., 2013). In addition, TCM is rich in natural compounds and can be considered as a natural chemical library producing synergistic effects, which has been justified by the revealing function and synergistic mechanism of principle active ingredients, such as Fu Fang Qing Dai Pian. It also presents more diversity in chemical structure and bioactivity, and less toxicity. Therefore, TCM represents an attractive source of new active compounds in drug discovery, for example, derivatives of indirubin, a compound isolated from a TCM formulas Dang Gui Long Hui Wan displays antileukemic properties (Kim et al., 2013b). Our previous review has summarized the commonly used individual herbs and compounds used in the treatment of osteoporosis. In this review, we highlight the research on TCM formulas for

osteoporosis from clinical use to their mechanism of action; this may be helpful in the application of TCM formulas in the treatment of osteoporosis and the discovery of antiosteoporotic lead compounds either on their own or in combination with other herbs.

2. Methods

The literature were searched from Medline, Pubmed, ScienceDirect, Spring Link, Web of Science, CNKI and VIP database from 1989 to 2015, and also collected from Chinese traditional books and Chinese Pharmacopoeia to identify studies on the antiosteoporotic effects of TCM formulas, herbs and chemical constituents, and also their possible mechanisms. The following keywords were used for the search: osteoporosis, osteoblast, osteoclast, traditional Chinese medicine formulas. All of these keywords were searched for each plant and its constituents. All published studies in English or Chinese language were included in the review. The literature search was conducted by both authors independently, with no inconsistencies between the two authors. The review included the following steps: (1) the TCM formulas for the treatment of osteoporosis in human and animal were reviewed using the available literature. (2) The herb medicine and their chemical constituents in TCM formulas were summarized. (3) The possible mechanism of action of TCM formulas and their chemical constituents were reviewed.

3. Clinical efficacy of TCM therapy for osteoporosis

Osteoporosis are caused by multiple factors, such as estrogen deficiency, aging, chemical agents and decreased mechanical loading, and afflicted patients should be treated with different TCM formulas according to different syndromes. Thirty-three TCM formulas have been reported in ethnopharmacological studies for their potential benefits in osteoporosis treatment

including 5 classic formulas, 23 empirical formulas, and 5 Chinese patent medicines. Multiple references were consulted for detailed information on research status of major TCM formulas which are discussed below.

The classic formulas which have been recorded in ancient medicine books have definitive efficacy, and often are standardized in preparation using traditional technique according to traditional Chinese medicine theory, and also commercially available. The formulas, such as Qing E Wan, Er Zhi Wan (EZW), Zuo Gui Wan (ZGW), You Gui Wan (YGW) and Liu Wei Di Huang Wan (LW) are used to treat osteoporosis through reinforcing the kidney, and clinicians can usually remove or add one or more herbs, and adjust their dosage in the prescription according to differentiation of the patient's physical condition. The ingredients, recorded literature, efficacy and indication, usage and dosage of classic formulas for the treatment of osteoporosis are shown in Table 1. Examples are as follows. Treatment of postmenopausal osteoporosis of 48 women with Qing E Wan for 24 weeks significantly increased bone mineral density (BMD), activity of bone alkaline phosphates and osteocalcin (OCN); 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 compared with placebo treatment (Zhao and Shen, 2012), indicating that Qing E Wan can increase osteoblastic bone formation, and inhibit osteoclastic bone resorption, and then reduce bone lose in postmenopausal women. However, this investigation lacks the information of Qing E Wan in relieving clinical syndrome, such as pain in back and loin. The effect of EZW on climacteric osteoporosis has been observed by the administration of EZW to 40 patients for 6 months and it was found that this formula significantly increased BMD and level of estradiol in serum compared to patients treated with calcium preparation (Yu, 2009). Due to lack the biochemical parameters of bone metabolism in serum, it is difficult to evaluate the regulatory effects of EZW on bone metabolism. The increase of the levels of estradiol may lead to adverse effects

on uterus and mammary gland. The adverse effects related with estrogen-like activity should be further investigated. The treatment of 30 patients with type 2 diabetic osteoporosis with ZGW significantly increased BMD, and decreased levels of OCN, parathyroid hormone and alkaline phosphatase (ALP) in serum compared with control group (Wang et al., 2014b). ZGW also showed more potential effects in relieving bone pain, therefore indicating significant advantage than calcium preparation. These findings exhibited that ZGW can regulate bone formation, but the effects on bone resorption are not clear in patients with type 2 diabetic osteoporosis. Administration of YGW in 30 patients with kidney-deficiency osteoporosis for 3 months significantly increased BMD and activity of ALP, and decreased urinary calcium level, indicating that YGW can be used to treat osteoporosis (Liang et al., 2011). In this research, the ages of patients were range from 65 to 83 years old; the indications of YGW should be senile osteoporosis. The therapeutic effects of YGW on postmenopausal osteoporosis need to be further investigated. Treatment with LW in 30 primary osteoporotic patients caused by kidney-yin deficiency for 12 months achieved better effectiveness in BMD of lumber, ratio of urinary Ca/Cr and the total effective rate than that of calcium carbonate treatment (Zhang and Li, 2011). The ages of patients in this study were range from 50 to 80 years old, the senile osteoporosis and postmenopausal osteoporosis are not discriminated during the process of results analysis.

Empirical formulas, which are derived from a summary of clinician's experience in long-term medical practice, has definite efficacy leading to a standard formula composition. These formulas are also standardized in preparation by investigator, but are not permitted to sell in market, and only used for treatment disease in the institute of investigator. The commonly used empirical formulas for the treatment of osteoporosis include Gu Song Kang, Er Xian Decoction (EXD), Fu Fang Lu Rong Jian Gu Jiao Nang (LRJG) etc. The empirical formulas for the treatment of osteoporosis are shown in Table 2. For example, Gu Song Kang

treatment for 50 postmenopausal osteoporotic patients for 90 days showed better therapeutic effects than that with calcium lactate in increasing BMD and improving the patients conditions in osteoporotic patients (Wang et al., 2003) . In the formula of Gu Song Kang, Epimedium brevicornu Maxim and Cullen corylifolium (Linnaeus) Medikus and Angelica sinensis (Oliv.) Diels has estrogen-like activity, maybe increases the estrodiol level in serum and produces unwanted effects on patients. However, investigator did not concern this problem. Treatment with EXD in 35 postmenopausal osteoporotic patients for 12 weeks significantly elevated BMD and levels of ALP, estrodiol, OCN and calcitonin in serum, relieve the pain syndrome, and showed more potential efficacy than treatment with salmon calcitonin alone (Zhu and Gu, 2012). The results of biochemical marker in serum exhibited that EXD enhanced BMD through increasing bone formation. But the effects of EXD on bone resorption parameters in serum need to be determined so as to evaluate the regulatory activity on bone resorption. To study the effects of LRJG on primary osteoporosis, 401 patients were selected and randomly divided into LRJG treatment group (n=301) and Gu Shu Kang (GSK) treatment group (n=100), and treatment with LRJG for 6 months significantly improved BMD, and the effective rate of LRJG treatment was significantly higher than observed in the GSK group (Li et al., 2010). LRJG did not produce any effects on calcium, phosphorus and ALP activity, this maybe is related with no discrimination from postmenopausal and senile osteoporosis, and other biochemical markers were not measured in serum. Therefore, this investigation did not clarify the regulatory mechanism on bone metabolism. To objectively evaluate the therapeutic effect and safety of Yi Shen Zhuang Gu He Ji (YSZG) in primary osteoporosis, 96 patients were treated with YSZG, 32 patients were given Ostrea gigas Thunberg and 32 patients were given placebo for 6 months. The results indicated that treatment with YSZG produce more significant therapeutic effects than that of Ostrea gigas Thunberg as evidenced by BMD, serum BGP and PYD (Wang et al., 2005), indicating that YSZG not only regulate bone formation, but also

modulate bone resorption. The change of estrogen levels should be determined so as to predict the action mechanism and safety of YSZG.

Chinese patent medicines, which are approved by the State Food and Drug Administration of China, are standardized in preparation by pharmaceutical company, and are commercially available in market. They are often used alone or in combination with chemical drugs to treat various diseases. Chinese patent medicines for the treatment of osteoporosis include Xian Ling Gu Bao Jiao Nang (XLGB), Gu Shu Kang Jiao Nang (GSK), Gu Song Bao Jiao Nang, Hu Gu Jiao Nang and Jin Tian Ge Jiao Nang (JTG). These TCM formulas have the characteristic of fixed composition and dosage and no herbs can be either added or removed from the formulas. The Chinese patent medicines for the treatment of osteoporosis are shown in Table 3. For example, 180 healthy postmenopausal women more than 60 years old with BMD T-score ≤ -2.0 (lumbar spine or femoral neck) were randomly divided into three groups, and given XLGB at dose of 3 g/day (n=61), XLGB at dose of 6 g/day (n=58) or placebo (n=61), respectively. Treatment with XLGB for 12 months significantly increased lumbar spine BMD, declined bone turnover marker levels and did not produce any adverse effects (Zhu et al., 2012). This was the first multicenter, double-blind, placebo controlled clinical trial to provide evidence showing the safety and efficacy of the oral “bone strengthening” herbal XLGB with phytoestrogenic compounds for the treatment of osteoporosis in postmenopausal women. However, estrogen-dependent tissues were not clinically examined. The future clinical studies shall be designed for safety of estrogen-dependent tissues. To evaluate the curative effects and advantages of combining Chinese and chemical medicine in treating senile osteoporosis, 66 patients of senile osteoporosis were randomly divided into two groups, and treated with GSK (n=35), and simultaneously treated with salcatonin, ossotide for injection, calcium carbonate and Vitamin D3 for 6 months. GSK treatment significantly increased BMD and had a higher effective rate than that of the control group (Feng et al., 2013b), indicating that GSK

combination with chemical medicine enhanced BMD, and improve the clinical syndrome of senile osteoporosis. Due to lack of observation of GSK alone on senile osteoporosis, the therapeutic effectiveness of GSK need to be further investigated. To explore the effectiveness of dynamic hip screw (DHS) combined with JTG Jiao Nang in the treatment of osteoporotic femoral inter-trochanteric fractures, 44 cases were randomly treated with either DHS or DHS combined with JTG Jiao Nang for 6 months. The BMD of the proximal end of the femur of patients treated with combinational therapy was higher than that of treatment of DHS alone (Liao et al., 2011), indicating that JTG can decrease bone loss through increasing BMD and improving bone biomechanical property. However, this study did not determine the alteration of serum biochemical parameters related with bone metabolism.

According to the guidelines for clinical evaluation of agents used in the prevention and treatment of postmenopausal osteoporosis approved by the Food and Drug Administration, the clinical observation for anti-osteoporotic drugs should determine BMD and the serum biochemical parameters, such as vitamin D, urine hydroxyproline, Ca etc, and in double-blind, placebo-controlled trail for 12 months in phase Ⅱ clinical study. Based on the characteristics of TCM formulas, Food and Drug Administration of China suggested that clinical observation of TCM drugs for reducing fracture incidence should last more than for 3 years, and that for relieving or alleviating the clinical symptoms should last for 6 months, while the indexes of effectiveness included bone mineral density and bone turnover markers. The clinical investigations showed that TCM formulas are effectiveness in preventing and treating osteoporosis, especially in alleviation of the pain symptoms. However, there are still many problems in clinical trials. For example, the most of reported clinical investigations are general clinical observations instead of application of RCT designed clinical studies. The method of randomization was often inappropriately described; the sample sizes are 50-100 patients, and are less than 200 in most of studies. Over half did not report and analysis side effects. The

duration of clinical trials often last 3-6 months, did not reach to the requirements of 6 months or 3 years. Most of these trials combined with other interventions, such as calcitonin, calcium preparation; the efficacy of TCM formulas on osteoporosis is not confirmed. Some clinical studies about primarily osteoporosis did not distinguish and assess the effects on postmenopausal and senile osteoporosis, respectively. The indexes of effectiveness focused on bone mineral density, and lack of biochemical parameters in serum and urine. Therefore, in clinical study researchers should adhere to the guidelines of FDA and SFDA of China to design experimental therapeutic efficacy parameters, observational period, sample size, and also including random and double-blinded control to improve and standardize clinical trials of TCM formulas for the treatment of osteoporosis.

4. Antiosteoporotic activity of TCM formulas in several important animal models

The animal models that have been established and used to understand the pathogenesis of osteoporosis and for the preclinical evaluation of drugs include castrated osteoporotic models, osteoporotic models caused by chemical drugs and disuse osteoporotic models. The antiosteoporotic effects of TCM formulas in animal experiments were summarized in Table 4.

4.1 Castrated osteoporotic model

The ovariectomized rat is the most common used animal model in the study of anti-osteoporotic medications. Ovariectomy (OVX) leads to a rapid bone loss up to 100 days. The mechanism of bone loss currently accepted for the estrogen-deficient rat is an imbalance in bone turnover, i.e., bone resorption exceeds bone formation. This model mimics postmenopausal cancellous bone loss when examined over relatively short periods of time (Sharma et al., 2012; Turner et al., 2013). However, observation periods of 12 months or longer have revealed higher values of bone mineral content (BMC), bone area and body weight in ovariectomized rats. Furthermore, the lack of the haversian system in cortical bone, the absence

of impaired osteoblast function during the late stages of estrogen deficiency and the absence of multicellular unitbased remodeling in young rats limits the usefulness of this model (Egermann et al., 2005). Qing E Wan treatments of ovariectomized rats restored the estrus cycle and demonstrated significantly estrogenic activity, as indicated by reversal of uterine atrophy, reduction in rectal temperature (Xu et al., 2010). Administration of EZW could significantly prevent ovariectomy-induced bone loss, biomechanical reduction, deterioration of trabecular microarchitecture and the body weight without affecting the weight of the uterus, and increased Ca and P levels in serum, decreased level of bone turnover markers and Ca and P levels in urine in ovariectomized rats without hyperplastic effect on uterus (Cheng et al., 2011). ZGW improved bone trabecular and decreased OCN and tartrate-resistant acid phosphate (TRAP) in ovariectomized osteoporotic rats (Lv et al., 2010). EXD could significantly improved BMD, the maximum bending stress, and the loading force of the 5th lumbar vertebra, increase serum ALP and superoxide dismutase (SOD) activity, and lower the serum levels of TRAP and malondialdehyde (MDA) in ovariectomized rats (Liu et al., 2014a). XLGB treatment increased bone density and estrogen level (Wang et al., 2010). GSK had obviously ameliorating effects on the bone loss in OVX rats**.** Treatment with LW for 26 weeks could significantly decrease the level of ALP and OCN in serum, increase the BMD of femurs, and improve the biomechanical capability of vertebral body in maximum loading and elastic modulus. Ba Wei Di Huang Wan combined with antiresorptive agent, alendronate (ALN) increased trabecular bone volume and BMD, improved the microstructure of the bone in both proximal tibia and vertebra with no marked effects on bone formation (Chen et al., 2012). Treatment of mice with Si Wu Tang (SWT) extract increased bone formation, and prevented bone loss induced by ovariectomy in vivo (Wu et al., 2013). Dang Gui Bu Xue Tang (DBT) combined with Epimedii Folium was able to attenuate osteoporosis by elevating the BMD levels of total body, and arrest the bone trabeculae degradation, decreased serum levels of MDA and increased endogenous SOD

activity (Xie et al., 2012).

4.2 Osteoporotic model induced by chemical drugs

Glucocoroticoids reduce bone density by diminishing intestinal calcium absorption, increasing renal clearance of calcium, and sex steroid deficiency. Furthermore, previous studies showed that glucocoroticoids exert pro-survival effect on osteoclasts, inbihit recruitment and activity of osteoblasts, and induce apoptosis of osteoblasts and osteocytes, leading to reduction in bone formation and increased bone resorption (Bocker et al., 2014; Piemontese et al., 2015). Therefore, glucocoroticoids-induced model is used to investigate the mechanism that how the drugs treat osteoporosis. It is known that glucocorticoid-induced osteoporosis differs from postmenopausal or senile osteoporosis. Histomorphometric parameters and biochemical markers of bone metabolism only indicate the decrease in bone formation and minimal changes in bone resorption. In order to study the aspects of bone fragility and implant fixation, the animal models using glucocorticoid induced osteoporosis simulate the human situation more closely than other models do (Allen et al., 2002; Egermann et al., 2005). The decrease of BMD and biomechanical competence is more pronounced when steroid medication is given than it is in the case of ovariectomy alone. However, long-term steroid treatment has been shown to have adverse effects, especially for the immune system, and ethical considerations need to be taken into account when using these models (Feng et al., 2013a). ZGW significantly increased trabecular bone volume (TBV%), trabecular formation surface (TFS%), the level of OCN, insulin-like growth factors (IGF) -1 and estrogen, and decreased content of PTH in serum in osteoporotic rats induced by dexamethasone (Li et al., 2011; Liu et al., 2011). Hu Gu Jiao Nang treatment significantly increased the BMDs of the femur and lumbar vertebra 5, and trabecular number (Tb.N), trabecular bone area (Tb.Ar), mineralizing surface/bone surface (MS/BS), mineral apposition rate (MAR), and bone formation rates (BFRs), and decreased trabecular separation (Tb. Sp) and eroded surface (ES/BS) in osteoporotic rats induced with

dexamethasone (Wang et al., 2012b).

Retinoic acids (RA), major oxidative metabolites of vitamin A, play a regulatory role in many key processes, such as inhibition of cell proliferation, differentiation, apoptosis, shaping of the embryo, and organogenesis. With regard to osteoblastic cells, RA induces differentiation in primary cultures of both mouse and human osteoblasts, while increase the activity of osteoclasts (Broulik et al., 2013; Michalik and Wahli, 2007). Treatment with RA of 70 mg/kg body weight for 2 weeks will lead to bone loss and then osteoporosis. Due to the short time for forming models, osteoporotic model induced by RA is widely used to investigate the effects of drugs. GSK decreased bone resorption and increased bone formation by raising levels of estrogen and testosterone in blood of RA-induced osteoporotic rats (Cui et al., 2001). Jian Gu Ke Li significantly increased BMD of femur and tibia, and the level of chorionic thyrotropin in serum, and decreased the levels of PTH and tumor necrosis factor (TNF)-α in RA-induced osteoporotic rats (Lin et al., 2004).

4.3 Disuse osteoporotic model

The disuse osteoporosis refers to bone mass decrements under conditions of decreased mechanical loading, including decreased ground force reaction, muscular contraction, and microgravity-related bone loss in astronauts after space flights. The aetiology, pathophysiology, and resultant pathology of disuse osteoporosis differ from those of primary osteoporosis. The resulting bone loss from diminished weight bearing has been generated in animal models by nerve sectioning, tenotomy, casting of limbs, leg bandaging, internal joint fixation, external joint fixation, chair immobilization and spaceflight. Most studies reported a significant reduction of mineralization and histomorphometric parameters towards osteoporosis. The bone loss due to immobilization is apparent locally but not systemically, and therefore, is not appropriate to simulate human osteoporosis (Damrongrungruang et al., 2004; Jee and Ma, 1999; Jiang et al., 2006). Yi Shen Hu Gu decoction could increased the levels of OCN in serum, bone

mineral density, and decreased levels of ALP, TRAP, hydroxyproline (HYP) / creatinine (Cr), improved the bone biomechanical parameters (maximum loading and elastic modulus) and Tb.N in sciatic neurotomized disused osteoporotic rats (Ju et al., 2013). Wu Jia Bu Gu recipe increased bone density and femur maximum load and elastic load of rats after 3 weeks tail suspension, increased the activity of ALP, and levels of serum Ca and P, increased the deposition of external calcium, the production of collagen type I, increased BMD of femoral bone, maximum load and elastic load, TBV%, TFS%, anterior functional surface (AFS%) and MAR of lumbar (Fu et al., 2010). Bu Shen Zhuang Gu recipe increased BMD, the number of the trabecula, serum OCN, estrogen and P content, decreased serum calcium levels and resorption surface, improved the bone microstructure in weightlessness simulated rats by tail suspension for 4 weeks (Sun et al., 2007).

In summary, the investigations of TCM formulas on osteoporotic model animals are mainly the search for new drugs or further proving the efficacy. However, these animal models are not totally consistent with human osteoporosis, and still need improvement. On the one hand, traditional Chinese medicine thinks that kidney governs bone, and primary osteoporosis is caused by deficiency of kidney and function disturbance of hypothalamic-pituitary-gonadal axis. However, osteoporotic animal models commonly used in the research of TCM formulas does not associate with kidney deficiency, and the pharmacological parameters also does not reflect the alteration in function of hypothalamic-pituitary-gonadal axis. On the other hand, the kidney deficiency in TCM includes two aspects of yin and yang, but there is no corresponding animal model to reflect the characteristic of kidney yin or yang deficiency. There is no discrimination between the reinforcing kidney yin and yang formulas in the treatment of osteoporosis. Therefore, osteoporotic animal model reflecting TCM yin and yang deficiency syndrome should be established, and used to investigate the antiosteoporotic effects of TCM formulas.

5. The herb medicines and their chemical constituents in TCM formulas for the therapy of osteoporosis

A wealth of information indicates that TCM formulas for the therapy of osteoporosis contains a variety of herbal medicine, which can be divided into 3 categories based on their action in the formula：(1) replenishing kidney herb medicine, which commonly produces the marked effect; (2) strengthening spleen herb medicine; (3) activating blood circulation and dissipating blood stasis. There are more than 20 commonly used herbal medicines occurring in the antiosteoporotic formula. Their chemical constituents and pharmacological activities are summarized in Table 5.

Some important antiosteoporotic traditional Chinese medicine, including Cullen corylifolium (Linnaeus) Medikus (Fruit, Leguminosae), Epimedium plant (Leaf, Berberidaceae), Drynaria fortunei (Kunze ex Mett.) J.Sm.(Rhizome, Polypodiaceae) , Salvia miltiorrhiza Bunge (Root, Labiatae), Morinda officinalis How (Root, Rubiaceae) have been reviewed in our previous paper (Jia et al., 2012). Therefore, in this review we have focused on 11 different antiosteoporotic herbal medicines, and their chemical constituents and antiosteoporotic activity are discussed below.

5.1 Eucommia ulmoides Oliv. (Bark, Eucommiaceae)

The bark of Eucommia ulmoides Oliv. has been shown to possess activity against hypertension, hyperglycemia, diabetes, obesity, osteoporosis, Alzheimer’s disease, aging, and sexual dysfunction, and contain various chemical constituents, including lignans, iridoids, phenolics, steroids, terpenoids, and flavonoids. Lignans and iridoids are the two major constituents, such as liriodendrin, (p)-pinoresinol di-O-β-D-glucopyranoside, (p)-syringaresinol, aucubin, genipin, and geniposidic acid. Modern pharmacological and molecular biology studies have suggested that crude extracts and total glycosides of E. ulmoides may yield safe and mild anti-osteoporosis activities. E. ulmoides cortex extract

(EUCE) significantly inhibited OVX-induced decreases in biomechanical quality of the femur and improved bone microarchitecture, and dose dependently inhibited total bone mineral density decreases in the femur caused by OVX and decreased levels of the bone turnover markers OCN, ALP, deoxypyridinoline, and urinary Ca and P excretions (Zhang et al., 2009). EUCE significantly mitigated the decreases in bone volume/tissue volume, connect density, Tb.N, and trabecula thickness associated with OVX in rats and increased Tb.Sp, indicating that E. ulmoides exhibits preventive effects on estrogen deficiency-induced osteoporosis, and may be a potential alternative medicine for treatment of postmenopausal osteoporosis (Zhang et al., 2009). EUCE could effectively prevent the bone loss induced by hind limb suspension, which was indicated by decreased levels of bone turnover markers as well as the changes in urinary calcium and phosphorus, and also enhanced the biomechanical strength of bone and prevented the deterioration of trabecular bone microarchitecture (Pan et al., 2014).

The total lignans extracted from E. ulmoides significantly prevented OVX-induced decrease in biomechanical quality of femur such as maximum stress and Young's modulus, BMD decrease and microarchitecture deterioration. The total lignans increased cell proliferation, ALP activity and formation of bone calcified nodules, enhanced osteoprotegerin (OPG) expression and decreased RANKL expression of primary osteoblast from calvaria of neonatal rat, indicating that total lignans enhanced bone formation and inhibited osteoclastogenesis through OPG/RANKL system (Zhang et al., 2014).

5-(Hydroxymethyl)-2-furaldehyde (5-HMF), isolated from E. ulmoides could increase the mRNA expression of ALP, pro-collagen type 1 α 1, OCN and OPN in RT-PCR analysis of osteoblast derived from bone mesenchymal stem cells, and decrease the mRNA expression of PPARγ, fatty acid binding protein 4, CCAAT/enhancer binding protein α of adipogenic cells from rat bone mesenchymal stem cells (BMSCs), enhanced the mineralized nodule formations of osteoblast, indicating that 5-HMF is a powerful inhibitor of adipogenesis and enhancer of

osteoblastogenesis (Tan et al., 2014).

5.2 Achyranthes bidentata Bl. (Root, Amaranthaceae)

The root of Achyranthes bidentata Bl. is recommended to reinforce the muscles and bones, improve the tone of the liver and kidneys, promote blood circulation and remove blood stasis, and is known to possess expectorant, anti-inflammatory, antipyretic, antirheumatic, and diuretic activities. The major constituents of the roots have been shown to be polysaccharides, saponins, ketosteroids, flavonoids, sterols and alkaloids (He et al., 2014). The root of A. bidentata slowed down the body weight gain, enhanced the bone strength and prevented the deterioration of trabecular microarchitecture and loss of bone mass induced by the OVX through decreasing level of bone turnover markers, such as serum ALP, OCN and urinary deoxypyridinoline (DPD) (Zhang et al., 2012b). Two compounds, ecdysterone and daucosterol isolated from this plant markedly stimulated proliferation of osteoblast-like UMR106 cells (Li et al., 2001). Five new oleanolic acid glycosides could inhibit the formation of osteoclast-like multinucleated cells (OCLs) induced by 1alpha, $25(OH)_2D_3$, and flavonoid quercetin decreased osteoclastic differentiation in a co-culture system of osteoblast and bone marrow mononuclear cells. (Li et al., 2005a). The ketosteroids has been reported to prevent bone loss in ovariectomized animals (He et al., 2014). A. bidentata saponins are effective in preventing and treating retinoic acid-induced osteoporosis, and were found to induce proliferation and differentiation in bone marrow stromal cells (BMSCs) as evidenced by the osteoblastic proliferation and alkaline phosphatase activity. RT-PCR and Western-blot analysis showed that A. bidentata saponins increased mRNA levels of rat bone morphogenetic protein (BMP)-2,

runt-related transcription factor 2 and osterix, and increased the phosphorylation of ERK, indicating that A. bidentata saponins enhanced bone formation via activation of the ERK signaling pathway in osteoblast (He et al., 2014).

5.3 Rehmannia glutinosa Libosch (Root, Scrophulariaceae)

The root of Rehmannia glutinosa Libosch has been widely used to reduce fever, activate blood circulation, tonify the kidney, and has been used in the treatment of Yin deficiency syndrome in Eastern Asia for more than 2000 years. The steamed roots of R. glutinosa, possess anti-tumor, anti-stress, anti-thrombic, and hypoglycemic effects (Lim and Kim, 2013), and has been used as a haemostatic, cardio-tonic and diuretic agent. The major active components of the root of R. glutinosa are iridoid glycosides, such as catalpol and dihydrocatalpol, while other components are phenol glycoside, ionones, flavonoids, amino acids, inorganic ions and microelements (Zhang et al., 2008). The roots of R. glutinosa alleviated the decrease in the trabecular BMD, and increased the cortical bone thickness and trabeculation of the bone marrow spaces in OVX-induced osteoporotic rats, increased the proliferation and ALP activity and the expression of OPG of osteoblastic MG-63 cells, decreased the number of TRAP (+) multinucleated cells and the resorption areas of osteoclast from bone marrow cells (Oh et al., 2003). The catalpol from fresh root of R. glutinosa has been reported to promote the proliferation and differentiation of osteoblasts of MC3T3-E1 cells (Wu et al., 2010). Acteoside, the main active compound of R. glutinosa, reduced OVX-induced bone loss and inflammatory cytokine production, inhibited osteoclast differentiation and maturation from bone marrow macrophages (BMMs) and RAW264.7 macrophages stimulated by the receptor activator of

nuclear factor-kappaB (NF-κB) ligand (RANKL) through suppressing RANKL-induced activation of mitogen-activated protein kinases and transcription factors such as NF-κ, c-Fos, and nuclear factor of activated T-cells cytoplasmic 1 (NFATc1), suggesting that acteoside may act as an anti-resorptive agent to reduce bone loss by blocking osteoclast activation (Lee et al., 2013).

5.4 Angelica sinensis (Oliv.) Diels (Root, Apiaceae)

The root of Angelica sinensis (Oliv.) Diels. is one of the commonly used herbs in China, and is reported to possess hepatoprotective, neuroprotective, anti-oxidant, anti-osteoarthritis, and anti-cancer properties (Lim and Kim, 2014). The major active compounds of A. sinensis include phthalides, organic acids, polysaccharides and flavones (Chen et al., 2013). The extracts of root of A. sinensis significantly increased the bone femur mineral density, and decreased the markers of bone turnover in osteoporosis, serum ALP, collagen type I C-telopeptide and OCN of OVX rats, indicating that A. sinensis extract can prevent the OVX-induced bone loss in rats via estrogen-independent mechanism (Lim and Kim, 2014). Ferulic acid, a major active compound from A. sinensis significantly increased the BMD of tibia, slightly increased the serum levels of estrogen and progesterone and ALP activity, indicating that ferulic acid promotes bone remodeling, leading to a predominantly osteoblastic phase, besides bone resorption by osteoclasts (Sassa et al., 2003).

5.5 Cornus officinalis Siebold & Zucc. (Fruit, Cornaceae)

The fruit of Cornus officinalis Siebold & Zucc. is a folk medicine with a long history of safe use for the treatment of osteoporosis in postmenopausal women or elderly men in Asia.

The effects of cornus officinalis on RANKL-induced osteoclast differentiation from bone marrow-derived macrophages (BMMs) were evaluated by using RT-PCR and Western blot analysis. The results indicated that C. officinalis significantly inhibits RANKL-mediated osteoclast differentiation in a dose-dependent manner in bone marrow-derived macrophages, and inhibits the mRNA expression of TRAP, osteoclast-associated receptor, c-Fos, and nuclear factor of NFATc1 in BMMs, and protein expression of c-Fos and NFATc1, and greatly inhibits RANKL-induced phosphorylation of p38 and JNK, significantly suppresses RANKL-induced degradation of NF-κB (IκB), suggesting that C. officinalis may be useful in the treatment of osteoporosis (Kim et al., 2012). Sweroside is a bioactive ingredient isolated from C. officinalis, and it significantly increases the proliferation, activity of ALP and OCN, and attenuates and inhibits apoptosis of human MG-63 cells and primary osteoblasts from newborn rat calvaria (Sun et al., 2013).

5.6 Astragalus membranaceus var. mongholicus (Bunge) P.K.Hsiao and A. membranaceus (Fisch.) Bunge (Root, Leguminosae)

The dried root of Astragalus membranaceus var. mongholicus (Bunge) P.K.Hsiao and A. membranaceus (Fisch.) Bunge are known to contain cycloartane triterpene glycosides and flavonoids, particularly isoflavones, as its principal constituents. As a tonic used to strengthen muscles and bones, it is one of the most widely used medicinal herbs in Asian traditional medicine. The OVX rats administered extract from A. membranaceus showed a significant increase in Tb. Ar of the tibia, inhibited tibia and lumbar bone loss and did not cause uterine hypertrophy (Kim et al., 2003). A. membranaceus extract combined with supplemental Ca may

be more protective against the Ca loss of bone than A. membranaceus or supplementation of Ca alone in calcium-insufficient postmenopausal women (Kang et al., 2013). Supplementation with formononetin, the major compound of the A. membranaceus root resulted in slightly enhanced bone mechanical properties and bone density improvement, and prevented osteoporosis development in ovariectomized rats (Kaczmarczyk-Sedlak et al., 2013). The isoflavone calycosin-7-O-β-D-glucopyranoside (CG) is a principal constituent of A. membranaceus. Exposure of bone marrow stromal ST2 cells to CG in osteogenic differentiation medium increased ALP activity, OCN mRNA expression and the osteoblastic mineralization process, enhanced the expression of BMP- 2, p-Smad 1/5/8, β-catenin and Runx2, indicating that CG promotes the osteoblastic differentiation of ST2 cells through regulating the BMP/WNT signaling pathways. (Jian et al., 2015)

5.7 Cinnamomum cassia (L.) J.Presl and Cinnamomum verum J. Pres (Bark, Lauraceae)

Cinnamomum cassia (L.) J. Presl is one of the world's oldest spices that has been used in foods, beverages and the cosmetic industry. In traditional oriental medicine, the bark of C. cassia has been used to improve various diseases caused by insufficient blood microcirculation, to treat gastritis, blood circulation disturbances and inflammatory disease. This medicinal plant has also been often administered to patients suffering from women's diseases. The bark of C. cassia is reported to reduce blood pressure in experimental rats and possesses antiallergic, antiulcerogenic, antipyretic and anaesthetic activities. The ethanol extract from the bark of C. cassia significantly induced the growth of MCF-7 cells, showed higher affinity with estrogen receptor (ER)-β compared with ERα, dose-dependently increased the cell survival, ALP

activity, collagen synthesis and OCN secretion in MC3T3-E1 cells, prevented apoptosis induced by TNF-α in osteoblastic cells. In the presence of TNF-α, the extracts inhibited the production of interleukin (IL)-6 and nitric oxide in osteoblastic MC3T3-E1 cells, suggesting that C. cassia has a direct stimulatory effect on bone formation in vitro and may contribute to the prevention of osteoporosis and inflammatory bone diseases (Lee and Choi, 2006). C. verum dose-dependently inhibited formation of osteoclast induced with RANKL from RAW 264.7 cells without affecting cell viability and bone-resorbing activity of mature osteoclasts, inhibited RANKL-induced NFATc1 and c-fos expression, and moderately inhibited phosphorylation of IκB-α as evaluated by western blot, suggesting that C. verum inhibited bone resorption through regulating the c-fos/NFATc1 pathway rather than NF-κB pathway during RANKL-induced osteoclastogenesis. The cinnamaldehyde and 2-methoxycinnamaldehyde as active components reduced formation of osteoclast induced with RANKL from RAW 264.7 cells and inhibited NFATc1 expression whilst 2-methoxycinnamaldehyde exhibited remarkable inhibitory effects on bone resorption of osteoclast (Tsuji-Naito, 2008).

5.8 Dipsacus asper Wall. (Root, Dipsacaceae)

The dried root of Dipsacus asper Wall. is used for the treatment of traumatic ecchymoma and injury of muscles and bones by strengthening bone and curing bone fractures. It has also been used for treatment of back pain, traumatic hematoma, and bone fractures. Several chemical constituents, particularly saponins, iridoid glycosides and sterols, have been identified from D. asper. The crude extract of D. asper (DRE) is reported to increase bone density and alter bone histomorphology in mice (Wong et al., 2007). Treatment with D. asper extract is reported to

have a positive effect on mechanical strength, BMD, BMC, bone turnover markers, and significantly prevented the reduction of the bone volume fraction, connectivity density, trabecular number, thickness, tissue mineral density, and tissue mineral content as well as structure model index in ovariectomized rats through regulating the rate of bone remodeling, which could be inferred from the decreased level of bone turnover markers, such as serum ALP, OCN and urinary DPD (Liu, et al., 2009). DRE was demonstrated to prevent the loss of rat bone mass induced by hind limb unloading with vehicle treatment, which suggests the potential application of DRE in the treatment of microgravity-induced bone loss (Niu et al., 2015). Total saponins from D. asper enhanced the biomechanical strength of bone and attenuated the deterioration of trabecular bone microarchitecture in ovariectomized rats by decreasing levels of bone turnover markers. Total saponins from D. asper induced MC3T3-E1cells and primary osteoblastic cell maturation and differentiation, and increased bone formation by increasing BMP-2 synthesis, and inhibited osteoclastogenesis through an increase in osteoprotegrin and a decrease in RANKL expression in osteoblasts as detected by western blotting (Niu et al., 2012).

5.9 Polygonium multiflora Thunb. (Root, Polygonaceae)

Polygonium multiflora Thunb, which sometimes is named as Fallopia multiflora Thunb. has been widely used to treat age-related diseases. 2, 3, 5, 4'-tetrahydroxystilbene-2-O-β-D-glucoside, one of the major bioactive constituents extracted from *P. multiflorum* has been shown to have various pharmacologic activities such as antioxidant, increased cell survival, ALP activity, calcium deposition, and the mRNA expression of ALP, collagen I and OCN in osteoblastic MC3T3-E1 cells as measured by quantitative real-time PCR, and decreased

production of RANKL, IL-6 as evaluated by enzyme immunoassay system, intracellular reactive oxygen species and MDA of osteoblastic MC3T3-E1 cells injured by H_2O_2 as detected by assay kit, indicating that this compound may be effective in protection against osteoporosis associated with oxidative stress (Zhang et al., 2012a).

5.10 Cuscuta chinensis Lam. and Cuscuta australis R.Br. (Seed, Convolvulaceae)

The dry seed of Cuscuta chinensis Lam. and Cuscuta australis R.Br. is used as a tonic and aphrodisiac to nourish the liver and kidneys and to treat impotence and seminal emission, it is also widely used to improve sexual function, prevent and treat cardiovascular diseases, osteoporosis and senescence. Moreover, it possesses anti-tumoral, hepaprotective and neuropotective effects. The active constituents include flavonoids, lignans, quinic acids and polysaccharides (Yang et al., 2011b). The aqueous extract of Cuscuta chinensis Lam. treatment mildly promoted the proliferation, ALP activity, collagen synthesis, mineralization and levels of BMP-2 in the MG-63 cells, suggesting that Cuscuta chinensis Lam. can play an important role in osteoblastic bone formation (Yang et al., 2009). ALP-guided fractionation led to the isolation of quercetin, kaempferol, isorhamnetin, hyperoside and astragalin from the crude ethanolic extract of Cuscuta chinensis Lam. Further study showed that kaempferol and hyperoside significantly increased the ALP activity in UMR-106 cells, astragalin promoted the proliferation of UMR-106 cells. The isolated compounds showed estrogenic activity, but quercetin, kaempferol and isorhamnetin showed more potent ER agonist activity, demonstrating that kaempferol and hyperoside are the active compounds of Cuscuta chinensis Lam. demonstrating osteogenic effects (Yang et al., 2011b).

5.11 Cnidium monnieri (L.) Cuss. (Fruit, Apiaceae)

The fruit of Cnidium monnieri (L.) Cuss. is used as a traditional remedy, and has been validated to possess a diverse set of pharmacological activities , including antiproliferative, vasorelaxant, antihepatitis, antimicrobacterial, anti-inflammatory, antiallergic functions and antiosteoporosis. The major active compounds of C. monnieri include essential oil, coumarines, chromones and triterpenoids. C. monnieri has been confirmed to be effective in the treatment of osteoporosis. Total coumarins can decrease bone loss in ovariectomized rats and in glucocorticoids induced osteoporosis rats. Total coumarins inhibited bone resorption of osteoclasts from bone marrow cells of neonatal rat, and increased rat calvaria osteoblast proliferation, differentiation and bone mineralized nodule formation (Qin et al., 2003). Osthole, the most important active compound from C. monnieri, can inhibit bone resorption by decreasing TRAP activity through RANKL/TRAF6/JNK signaling pathway in osteoclasts from long limb bones of new born rabbits as detected by quantitative real time PCR and Western blotting analysis, and obviously promoted the osteoblastic activity possibly through the regulation of Wnt/β-catenin signaling in the mice calvarial osteoblasts, promoted osteogenic differentiation through the BMP-2/p38MAPK/Runx2/osterix pathway as evidenced in quantitative real time PCR and Western blotting analysis (Ming et al., 2012;Tang et al., 2010). Bergapten and imperatorin from C. monnieri also induced bone formation and promoted BMP-2 expression through the p38 and ERK-dependent signaling pathway in osteoblasts from calvaria of fetal rats as detected by quantitative real time PCR and Western blotting (Tang et al., 2008).

6. Antiosteoporotic mechanisms of TCM formulas and their chemical constituents

The research conducted over the years implies that the cause of osteoporosis is predominantly driven by the disorders of bone metabolism, of which the imbalance between bone formation and bone resorption play a major role. Bone formation, primarily mediated by osteoblasts, and resorption which is predominantly depended on the function of osteoclasts, is a balanced and continuous process. The activation of bone cells in bone remodeling process is regulated by multiple pathways that are fundamental in the development of therapy for osteoporosis, including the Wnt/β-catenin pathway, the PTH pathway, the BMP signaling pathway, the RANK/RANKL/OPG system and mitogen-activated protein kinase (MAPK) pathway.

6.1 Antiosteoporotic mechanisms of TCM formulas

6.1.1 Wnt/β-catenin pathway

The Wnt/β-catenin pathway is known to be an important modulator of osteoblast function and bone formation. Wnt is the member of highly conserved secreted glycoprotein family, rich in cystein residue and is divided into two classes: canonical Wnts (wnt1, wnt3a) and non-canonical Wnts (wnt5a). This pathway is triggered by binding of canonical with frizzled (FZD) and LDL receptor related proteins (LRPs) which promote the phosphorylation and inactivation of glycogensynthase kinase 3 β (GSK3β) and prevents the degradation of β-catenin as well as subsequent translocation into the nucleus where it forms a complex with a T-cell factor to induce the transcription of osteoblastic genes (Lin and Hankenson., 2011). With the in-depth and numerous study of osteoporosis pathway, quite a few drugs have been found that they treat osteoporosis through the Wnt/β-catenin pathway. In primary osteoblast from neonatal rat calvaria, serum containing LW elicited significant increase in cell proliferation, alkaline phosphatase activity and amount of calcified nodules. The LRP-5, β-catenin, Runx2

and osterix, which are involved in the canonical Wnt/β-catenin signaling pathway, were significantly up-regulated in the presence of LW both in vivo and in vitro experiments as detected by real-time quantitative PCR, indicating that LW could alleviate osteoporosis through involving canonical Wnt/β-catenin signaling pathway (Xia et al., 2014). EZW increased the expression levels of wnt3α, LRP5 and β-catenin, and reduced the expression of dickkop of homolog 1 (DKK1) of alveolar bone in ovariectomized rats, suggesting that EZW may have potential anti-osteoporotic effects on osteoporotic alveolar bone by stimulating Wnt/β-catenin signaling pathway (Sun et al., 2014).

6.1.2 MAPK pathway

The MARK includes the extracellular signal-related kinases (ERKs), p38 and c-Jun N-terminal kinases, and contributes to cell morphogenesis, kinesis, apoptosis, proliferation, differentiation, growth and other physiological process (Chakraborty et al., 2016). It has been found that pharmacological serum of Bu Shen Ning Xin Decoction (BSNXD) enhanced the osteoblastic proliferation and inhibited the apoptosis of the osteoblasts from neonatal mouse calvaria through the activation of MARK signal transduction pathway via phosphoration of ERK as analyzed by Western blotting (Wang et al., 2009). Gu Ling Pian (GLP) can promote the proliferation and differentiation of MG-63 cells and regulate the ratio of OPG/RANKL via p38 MARK pathway (Zhao et al., 2007). Si Wu Tang (SWT) extracts enhances ALP activity and bone mineralization, increase the expression of BMP-2 and OPN in MC3T3-E1 osteoblast cells by involving the regulation of phosphatidylinositol 3-kinase (PI3K), Akt and NF-κB signaling pathways as evaluated by quantitative real time PCR and Western blotting (Wu et al., 2013).

6.2 Antiosteoporotic mechanisms of herbs and their chemical constituents

6.2.1 BMP/SMAD signaling pathway

BMP, which is pleiotropic cytokines belonging to the TGF-β superfamily, display

osteogenic properties. Runx2, as a transcription factor required for osteoblastogenesis. BMP binds heterodimeric receptors to activate Smad proteins, which transactivate osteoblastogenic genes either directly or via Runx2 (Lin and Hankenson, 2011). Recently, Icariin from Epimedium plant have been found to exert their potent osteogenic effect in pre-osteoblastic MC3T3-E1 cells and mouse primary osteoblasts through induction of Runx2 expression, production of BMP-4 and activation of BMP signaling as analyzed by Real-time RT-PCR analysis (Zhao et al., 2008). Maohuoside A induces SMAD4 expression in osteoblast from mouse bone marrow-derived mesenchymal stem cells as revealed by Real-time PCR and Western blot analysis (Cai et al., 2013). Total saponins from Dipsacus asper and Achyranthes bidentata increase bone formation by increasing BMP-2 synthesis in MC3T3-E1 and primary osteoblastic cells as analyzed using western blotting (Niu et al., 2012). Similarly, the aqueous extract of Cuscuta chinensis treatment mildly releases BMP-2, whilst markedly increasing mRNA expression of BMP-2 in the MG-63 osteoblastic cells (Yang et al., 2009).

6.2.2 OPG/RANKL/RANK signaling pathway

One of the most critical pathways in the osteoblast-osteoclast interaction scheme is the RANKL-OPG relationship. Receptor activator of NF-B (nuclear factor-κB) ligand is a key factor stimulating the differentiation and activation of osteoclasts, and therefore, is essential for bone remodeling (Suda et al., 1999). OPG is a soluble peptide originally described as a factor which markedly inhibits bone resorption and osteoclast differentiation in vitro (Rosen, 2000). Some herbs, including Epimedium brevicornu**,** Rehmannia glutinosa**,** Cornus officinalis have been demonstrated to be capable of inhibiting bone resorption, probably via pathways induced

by RANKL. RT-PCR were used to detect IL-6 and TNF-α expression, ELISA analysis was used to measure the levels of OPG and RANKL in osteoclast induced with lipopolysaccharide (LPS) in co-cultures of primary osteoblast and bone marrow cells. The results indicated that icariin from Epimedium brevicornu significantly inhibited LPS-induced osteoclastic bone resorption and IL-6, and TNF-α expression, up-regulated the gene expression of OPG and down-regulated RANKL in osteoclast (Hsieh et al., 2011). Ikarisoside A and icariin from Epimedium koreanum also showed inhibitory effects on osteoclastogenesis in RANKL-stimulated RAW 264.7 cells as well as in murine bone marrow-derived macrophages, and activation of NF-κB, JNK and Akt mediated by RANKL as analyzed by western blotting method (Choi et al., 2010). Similarly, acteoside, the main active compound of Rehmannia glutinosa, inhibited differentiation and formation of osteoclast from bone marrow macrophages (BMMs) and RAW264.7 macrophages stimulated by RANKL, attenuated RANKL-stimulated activation of p38 kinase, ERK, and JNK, and transcription factors such as NF-κB, c-Fos and NFATc1 as analyzed by electrophoretic mobility shift assay and RT-PCR analysis (Lee et al., 2013). Cornus officinalis significantly inhibits RANKL-mediated osteoclast formation and differentiation from bone marrow-derived macrophages (BMMs) through inhibiting the protein expression of c-Fos and NFATc1, RANKL-induced phosphorylation of p38 and JNK, and degradation of I-κB in BMMs as measured by western blotting. (Kim et al., 2012).

6.2.3 MAPK signaling pathway

The mitogen-activated protein kinases, the family of secondary messengers that convey signals from the cell surface to the nucleus (Yang et al., 2013), play important roles in cellular

response to growth factors, cytokines, or environmental stress. There are three major families of MAPKs, ERK, which is involved in cell proliferation/transformation and survival, c-Jun N-terminal kinase is involved in stress responses and p38 MAPKs is involved in many cellular processes (Kim et al., 2013a). Some herbs have been demonstrated to have an effect on osteoporosis via MAPK pathway. Icariin suppresses LPS-induced osteoclastogenesis program and osteoclastic bone resorption, inhibits LPS-mediated activation of the p38 and JNK of osteoclast in co-culture of primary osteoblast and bone marrow cells as measured by western blotting, indicating that icariin has an in vitro inhibitory effect on osteoclasts differentiation through p38 and JNK pathway (Hsieh et al., 2011). Achyranthes bidentata saponins were effective in preventing and treating retinoic acid-induced osteoporosis. RT-PCR and Western-blotting analysis showed that Achyranthes bidentata saponins induce proliferation and differentiation of osteoblast from bone marrow stromal cells (BMSCs), increase mRNA levels of BMP-2, runt-related transcription factor 2 and osterix via activation of the ERK signaling pathway (He et al., 2014).

7 Comparison between the efficacy of TCM formulas and the efficacy of single herbs

Epimedium plants are commonly used antiosteoporotic botanical medicine, and used in most of antiosteoporotic TCM formulas. The antiosteoporotic chemical constituents in Epimedium plant are flavonoids. Therefore, the flavonoids were extracted and purified from Epimedium plant to observe their antiosteoporotic activity on primary osteoporosis, and Gu Song Bao Jiao Nang was used as control. The content of flavonoids in Epimedium extracts were more than 50%, 360 patients were given 0.7g Epimedium extract/time, three times / day for 24weeks. Epimedium plant is major component of Gu Song Bao Jiao Nang, and occupies

58% in this formula. 120 patients were given 1g Gu Song Bao Jiao Nang/time, three times / day for 24 weeks. The results demonstrated that Epimedium extracts can more effectively improve BMD of patients than Gu Song Bao, but effects of Epimedium extracts in relieving pain are weaker, and the incidence of adverse effect of Epimedium extracts (6.67%) are higher than that of Gu Song Bao (5%). Therefore, TCM formulations showed significant advantage in integral regulation of bone metabolism and reduction in adverse effects (Lu et al., 2013).

EXD is composed of six herbal medicines, with Epimedium herbs being its major ingredient. Epimedium herb occupies 20% in EXD. The content of flavonoids and icariin were respectively as 5.0% and 1.7% in Epimedium herb. The content of flavonoids in Epimedium extract is 60%. At the dose of 6g/kg EXD, 100mg/Epimedium extracts, 20mg/kg icariin and 1mg/kg nylstriol, we compared their potentials on osteoporotic bone and reproductive tissues in ovariectomized rats. The results showed that EXD has more potential effects in increasing BMD and regulating bone histomorphometric parmeters and biochemical parameters in OVX rats, EXD is similar to estrogen and exerts a concomitant effect on bone formation and bone resorption at the tissue level, while Epimedium extracts and icariin produced bone-protective effects mainly by inhibiting bone resorption. Nevertheless, EXD, Epimedium extracts, and icariin treatments manifested a fewer adverse effects on the uterus, mammary gland, and vagina compared to estrogen administrations. Among the EXD, Epimedium extracts, and icariin, EXD was found to have superior efficacy and safety profile (Xue et al., 2012).

8. Conclusion

TCM formulas not only reduce bone loss by decreasing bone resorption and increasing bone formation through multi-component and multi-targets, but also regulate the body's function in overall and relieve the pain in back and lumbago. The herbal medicine that possess activity of replenishing kidney are often shown to have estrogen-like, antioxidant activity or regulating the function of hypothalamus-pituitary axis to enhance the estrogen level in serum,

and the herbal medicine that reinforce spleen can intensify the effects of tonifying kidney, and herbal medicine activating blood can help active chemical constituents to arrive at the skeleton site and regulate bone metabolism. Furthermore, TCM formulas modulate bone metabolism networks modestly and then alleviate symptom of osteoporosis at low concentration through exerting synergistic effects of multiple component. Therefore, rationally designed TCM formula also can be considered as an option for multitarget therapeutic and prophylactic applications. Development of standardized, synergistic, safe and effective TCM formula with robust scientific evidence can offer faster and more economical alternatives.

Aging, estrogen deficiency, chemical drugs, and decreased mechanical loading may cause bone loss leading to osteoporosis. The corresponding animal models are respectively castrated osteoporotic models, osteoporotic models caused by drugs and disused osteoporotic models. The investigations of TCM formulas on osteoporotic animals focus on osteoporotic rats induced by ovariectomy, glucocoroticoids and retinoic acid, and lack other osteoporotic animal, such as disuse model. The determined parameters in animal experiments did not reflect the action characteristic of TCM formulas. Therefore, some novel TCM formulas should be developed and studied in animal models, such as disuse osteoporotic rats, some specific parameters that are associated with animal model should be analyzed to highlight the antiosteoporotic characteristic of TCM formulas.

Antiosteoporotic effects of TCM formulas are attributed to active chemical constituents of their herbs. These active constituents are diverse in chemical structure, including flavonoids, saponin, lignans and coumarins, and have the potential to be developed as antiosteoporotic leads. Hence, phytochemicals from TCM formulas and the composition of their herbs is of great potentials for the development of novel antiosteoporotic drugs. There is some accumulated evidence of the value of TCM in the treatment of osteoporosis. EXD, a TCM formula is composed of Curculigo orchioides Gaertn. (Rhizome, Hypoxidaceae), Epimedium

plant (Leaf, Berberidaceae), Phellodendron chinense C.K.Schneid (Bark, Rutaceae), Morinda officinalis F.C.How (Root, Rubiaceae), Angelica sinensis (Oliv.) Diels (Root, Apiaceae), Anemarrhena asphodeloides Bunge (Rhizome, Liliaceae). By investigating the antiosteoporotic chemical constituents and their interaction relationship, we found that icariin and icaritin from Epimedium leaf has estrogen-like activity and regulatory effects on bone metabolism; curculigoside from rhizome of Curculigo orchioides Gaertn. is an antioxidant and protective agent for injured osteoblast; berberine from bark of Phellodendron chinense is inhibitor for bone resorption; Timosaponin from rhizome of Anemarrhena asphodeloides and ferulic acid from root of Angelica sinensis, respectively are bone anabolic agent. In addition, root of Morinda officinalis contains iridoid glycoside and anthraquinone, which have been shown to have anti-inflammation and inhibitory effects on bone resorption (Chen et al., 2008). Taken together, antiosteoporotic TCM formula indeed contains various antiosteoporotic components, which are likely to act synergistically to decrease bone loss. Thus, combining these antiosteoporotic chemical constituents (or candidates) in TCM formula may exhibit better antiosteoporotic effects than the single compounds; this will provide a good starting point for further research.

Compared with chemical drugs, TCM formulas have the advantage of fewer side effects, are relatively cheap, and suitable for long-term use. A mass of clinical practice and animal experiment shows that TCM formulas can not only repair bone microarchitecture, increase bone mass, improve bone biomechanical properties, but also can reduce or eliminate the lumbar debility, back pain and other symptoms (Yang et al., 2011a; Zhu et al., 2012a). The single herb medicine that exists in TCM formulas for therapy of osteoporosis often contains antiosteoporotic compounds, thus screening active compounds from TCM formulas and determining the composition of the herbal medicine will help to find antiosteoporotic leads or candidates. The chemical compounds in TCM formulas produce synergistic effects; this may

lead to the discovery of antiosteoporotic chemical compounds which can be used in combination. Therefore, systematic data mining of the existing antiosteoporotic TCM formulas database can certainly help the drug discovery processes to identify safe candidates and synergistic formulations.

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Declaration of Conflict interest

None of the authors has any conflicts of interest to declare.

References

Allen, S.P., Maden, M., Price, J.S., 2002. A role for retinoic acid in regulating the regeneration of deer antlers. Developmental Biology 251, 409-423.

Amat, N., Amat, R., Abdureyim, S., Hoxur, P., Osman, Z., Mamut, D., Kijjoa, A., 2014. Aqueous extract of dioscorea opposita thunb. normalizes the hypertension in 2K1C hypertensive rats. BMC Complementary And Alternative Medicine 14, 36.

Appelman-Dijkstra, N.M., Papapoulos, S.E., 2015. Modulating Bone Resorption and Bone Formation in Opposite Directions in the Treatment of Postmenopausal Osteoporosis. Drugs 75, 1049-1058.

Bao, L., Qin, L., Liu, L., Wu, Y., Han, T., Xue, L., Zhang, Q., 2011. Anthraquinone compounds from Morinda officinalis inhibit osteoclastic bone resorption in vitro. Chemico-biological Interactions 194, 97-105.

Bian, Q., Liu, S.F., Huang, J.H., Yang, Z., Tang, D.Z., Zhou, Q., Ning, Y., Zhao, Y.J., Lu, S., Shen, Z.Y., Wang, Y.J., 2012. Oleanolic acid exerts an osteoprotective effect in ovariectomy-induced osteoporotic rats and stimulates the osteoblastic differentiation of bone mesenchymal stem cells in vitro. Menopause 19, 225-233.

Bocker, W., El Khassawna, T., Bauer, N., Brodsky, K., Weisweiler, D., Govindarajan, P., Schlewitz, G., Kampschulte,

M., Durselen, L., Thormann, U., Szalay, G., Schnettler, R., Langheinrich, A.C., Heiss, C., 2014. Short-term glucocorticoid treatment causes spinal osteoporosis in ovariectomized rats. European Spine Journal 23, 2437-2448.

Bouabdallah, I., Bouali, I., Martinez-Force, E., Albouchi, A., Perez Camino, M.C., Boukhchina, S., 2014. Composition of fatty acids, triacylglycerols and polar compounds of different walnut varieties (Juglans regia L.) from Tunisia. Natural Product Research 28, 1826-1833.

Broulik, P.D., Raska, I., Broulikova, K., 2013. Prolonged overdose of all-trans retinoic acid enhances bone sensitivity in castrated mice. Nutrition (Burbank, Los Angeles County, Calif.) 29, 1166-1169.

Cai, M., Li, G., Tao, K., Yang, Y., Lou, L., Cai, Z., Yu, Y., 2013. Maohuoside A acts in a BMP-dependent manner during osteogenesis. Phytotherapy Research : PTR 27, 1179-1184.

Chakraborty C, Sharma A.R., Patra B.C., Bhattacharya M., Sharma G., Lee S.S., 2016. Micrornas mediated regulation of mapk signaling pathways in chronic myeloid leukemia.Oncotarget, 1-15.

Chen, H., Wu, M., Kubo, K.Y., 2012. Combined treatment with a traditional Chinese medicine, Hachimi-jio-gan (Ba-Wei-Di-Huang-Wan) and alendronate improves bone microstructure in ovariectomized rats. Journal of Ethnopharmacol 142, 80-85.

Chen H.Y., Cho W.C., Sze S.C., Tong Y., 2008. Treatment of menopausal symptoms with er-xian decoction: a systematic review. American Journal of Chinese Medicine, 36(2), 233-44.

Chen, X., Pei, L., Zhong, Z., Guo, J., Zhang, Q., Wang, Y., 2011. Anti-tumor potential of ethanol extract of Curcuma phaeocaulis Valeton against breast cancer cells. Phytomedicine : International Journal of Phytotherapy And Phytopharmacology 18, 1238-1243.

Chen, X.P., Li, W., Xiao, X.F., Zhang, L.L., Liu, C.X., 2013. Phytochemical and pharmacological studies on Radix Angelica sinensis. Chinese Journal of Natural Medicines 11, 577-587.

Cheng, M., Wang Q Fau - Fan, Y., Fan Y Fau - Liu, X., Liu X Fau - Wang, L., Wang L Fau - Xie, R., Xie R Fau - Ho, C.C., Ho Cc Fau - Sun, W., Sun, W., 2011. A traditional Chinese herbal preparation, Er-Zhi-Wan, prevent ovariectomy-induced osteoporosis in rats. Journal of Ethnopharmacology 138, 279-285.

Choi, E.M., 2011. Dehydrocostus lactone prevents mitochondrial dysfunction in osteoblastic MC3T3-E1 cells. European Journal of Pharmacology 664, 1-7.

Choi, E.M., Lee, Y.S., 2013. Paeoniflorin isolated from Paeonia lactiflora attenuates osteoblast cytotoxicity induced by antimycin A. Food & Function 4, 1332-1338.

Choi, H.J., Park, Y.R., Nepal, M., Choi, B.Y., Cho, N.P., Choi, S.H., Heo, S.R., Kim, H.S., Yang, M.S., Soh, Y., 2010. Inhibition of osteoclastogenic differentiation by Ikarisoside A in RAW 264.7 cells via JNK and NF-kappaB signaling pathways. European Journal of Pharmacology 636, 28-35.

Cui, S.Q., Li, S.Q., Gang, P.H., Pei, Z.G., Liu, Y.M., Jiang, S.Y., Wang, H.Y., Qu, L.R., 2001. Nourishing kidney prescription Gushukang in preventing and treating primary osteoporosis. Journal of Chinese Medicine University 30.

Cuong, N.X., Minh, C.V., Kiem, P.V., Huong, H.T., Ban, N.K., Nhiem, N.X., Tung, N.H., Jung, J.W., Kim, H.J., Kim, S.Y., Kim, J.A., Kim, Y.H., 2009. Inhibitors of osteoclast formation from rhizomes of Cibotium barometz. Journal of Natural Products 72, 1673-1677.

Damrongrungruang, T., Kuroda, S., Kondo, H., Aoki, K., Ohya, K., Kasugai, S., 2004. A simple murine model for immobilization osteopenia. Clinical Orthopaedics And Related Research, 244-251.

Durnova, G.N., Kaplanskii, A.S., 1998. Effect of ephedrine and support loads on development of osteopenia and growth of shin bones in suspended rats. Aviakosmicheskaia Ikologicheskaia Meditsina 32, 27-31.

Egermann, M., Goldhahn, J., Schneider, E., 2005. Animal models for fracture treatment in osteoporosis.

Osteoporos International 16 Suppl 2, S129-138.
Fan, J., Li, J., Fan, Q., 2015. Naringin promotes differentiation of bone marrow stem cells into osteoblasts by upregulating the expression levels of micro RNA-20a and downregulating the expression levels of PPAR gamma. Molecular Medicine Reports 12, 4759-4765.
Fan, X., Du, Y.C., Wei, J.X., 1994. Chemical constituents of roots, rhizomes and stems of Amomum villosum Lour. China journal of Chinese materia medica 19, 734-736, 762.
Feng, R., Feng, L., Yuan, Z., Wang, D., Wang, F., Tan, B., Han, S., Li, T., Li, D., Han, Y., 2013a. Icariin protects against glucocorticoid-induced osteoporosis in vitro and prevents glucocorticoid-induced osteocyte apoptosis in vivo. Cell Biochemistry and Biophysics 67, 189-197.
Feng, W.J., Li, Q.F., Wang, C.M., Chen, J.K., Zhou, J.L., J.L., H., 2013b. Observation on the effect of Using Chinese and western combined treatment in Senile Osteoporosis. Guangming Journal of Chinese Medicine 28, 1481-1483.
Ford, M.A., Bass, M., Zhao, Y., Bai, J.B., Zhao, Y., 2011. Osteoporosis Knowledge, Self-Efficacy, and Beliefs among College Students in the USA and China. Journal of Osteoporosis 2011, 729219.
Fu, Q., Hu, S.M., Yang, J.J., Hao, X.J., Zhu, B., Wang, Q., Wu, Z.R., Li, J., 2010. Comparison of effects of Wujia Bugu decoction) and alendronate sodium on protection the bone loss of hindlimb unloaded rats. China journal of orthopaedics and traumatology 23, 524-528.
Gao, F., Li, Y.Y., Wang, D., Huang, X., Liu, Q., 2012. Diterpenoid alkaloids from the Chinese traditional herbal "Fuzi" and their cytotoxic activity. Molecules (Basel, Switzerland) 17, 5187-5194.
Gao, X.Y., Wang, D.W., Li, F.M., 2000. [Determination of ecdysterone in Achyranthes bidentata Bl. and its activity promoting proliferation of osteoblast-like cells]. Yao Xue Xue Bao 35, 868-870.
Gao, Z., Lu, Y., Halmurat, U., Jing, J., Xu, D., 2013. Study of osteoporosis treatment principles used historically by ancient physicians in Chinese Medicine. Chinese Journal of Integrative Medicine 19, 862-868.
Lin, G. L., Hankenson, K. D., 2011. Integration of bmp, wnt, and notch signaling pathways in osteoblast differentiation.. Journal of Cellular Biochemistry, 112(12), 3491-501.
Guo, A.J., Choi, R.C., Zheng, K.Y., Chen, V.P., Dong, T.T., Wang, Z.T., Vollmer, G., Lau, D.T., Tsim, K.W., 2012. Kaempferol as a flavonoid induces osteoblastic differentiation via estrogen receptor signaling. Chinese Medicine 7, 10.
Guo, Y., Li, Y., Xue, L., Severino, R.P., Gao, S., Niu, J., Qin, L.P., Zhang, D., Bromme, D., 2014. Salvia miltiorrhiza: an ancient Chinese herbal medicine as a source for anti-osteoporotic drugs. Journal of Ethnopharmacol 155, 1401-1416.
He, G., Guo, W., Lou, Z., Zhang, H., 2014. Achyranthes bidentata saponins promote osteogenic differentiation of bone marrow stromal cells through the ERK MAPK signaling pathway. Cell Biochemistry And Biophysics 70, 467-473.
He, J.Y., Ma, N., Zhu, S., Komatsu, K., Li, Z.Y., Fu, W.M., 2015. The genus Codonopsis (Campanulaceae): a review of phytochemistry, bioactivity and quality control. Journal of Natural Medicines 69, 1-21.
Hsieh, T.P., Sheu, S.Y., Sun, J.S., Chen, M.H., 2011. Icariin inhibits osteoclast differentiation and bone resorption by suppression of MAPKs/NF-kappaB regulated HIF-1alpha and PGE(2) synthesis. Phytomedicine : International Journal of Phytotherapy And Phytopharmacology 18, 176-185.
Huang, Y., Chen, L., Feng, L., Guo, F., Li, Y., 2013. Characterization of total phenolic constituents from the stems of Spatholobus suberectus using LC-DAD-MS(n) and their inhibitory effect on human neutrophil elastase activity. Molecules (Basel, Switzerland) 18, 7549-7556.
Huh, J.E., Lee, W.I., Kang, J.W., Nam, D., Choi, D.Y., Park, D.S., Lee, S.H., Lee, J.D., 2014. Formononetin attenuates

osteoclastogenesis via suppressing the RANKL-induced activation of NF-kappaB, c-Fos, and nuclear factor of activated T-cells cytoplasmic 1 signaling pathway. Journal of Natural Products 77, 2423-2431.
Hyun, H., Park, H., Jeong, J., Kim, J., Kim, H., Oh, H.I., Hwang, H.S., Kim, H.H., 2014. Effects of Watercress Containing Rutin and Rutin Alone on the Proliferation and Osteogenic Differentiation of Human Osteoblast-like MG-63 Cells. The Korean Journal of Physiology & Pharmacology : Official Journal of The Korean Physiological Society and the Korean Society of Pharmacology 18, 347-352.
Jee, W.S., Ma, Y., 1999. Animal models of immobilization osteopenia. Morphologie : bulletin de l'Association des anatomistes 83, 25-34.
Jia, M., Nie, Y., Cao, D.P., Xue, Y.Y., Wang, J.S., Zhao, L., Rahman, K., Zhang, Q.Y., Qin, L.P., 2012. Potential antiosteoporotic agents from plants: a comprehensive review. Evidence-based Complementary And Alternative Medicine : eCAM 2012, 364604.
Jian J., Sun L., Cheng X., Hu X., Liang J., Chen Y., 2015. Calycosin-7-o-β-d-glucopyranoside stimulates osteoblast differentiation through regulating the bmp/wnt signaling pathways. Acta Pharmaceutica Sinica B, 5(5), 454-460.
Jiang, S.D., Jiang, L.S., Dai, L.Y., 2006. Spinal cord injury causes more damage to bone mass, bone structure, biomechanical properties and bone metabolism than sciatic neurectomy in young rats. Osteoporos International 17, 1552-1561.
Jin, R., Lin, Z.J., Xue, C.M., Zhang, B., 2013. An improved association-mining research for exploring Chinese herbal property theory: based on data of the Shennong's Classic of Materia Medica. Journal of Integrative Medicine 11, 352-365.
Ju, G., Qian-de, Z., Xi, Z., Ji-hong, H., 2013. Effect of Yishen Hugu Decoction on disused osteoporosis in rats. Chinese Journal of Experimental Traditional Medicine Formulae 19, 179-182.
Kaczmarczyk-Sedlak, I., Wojnar, W., Zych, M., Ozimina-Kaminska, E., Taranowicz, J., Siwek, A., 2013. Effect of formononetin on mechanical properties and chemical composition of bones in rats with ovariectomy-induced osteoporosis. Evidence-based Complementary And Alternative Medicine : eCAM 2013, 457052.
Kang, S.C., Kim, H.J., Kim, M.H., 2013. Effects of Astragalus membranaceus with supplemental calcium on bone mineral density and bone metabolism in calcium-deficient ovariectomized rats. Biological Trace Element Research 151, 68-74.
Kim, C., Ha, H., Lee, J.H., Kim, J.S., Song, K., Park, S.W., 2003. Herbal extract prevents bone loss in ovariectomized rats. Archives of Pharmacal Research 26, 917-924.
Kim, H.K., Kim, M.G., Leem, K.H., 2013a. Osteogenic activity of collagen peptide via ERK/MAPK pathway mediated boosting of collagen synthesis and its therapeutic efficacy in osteoporotic bone by back-scattered electron imaging and microarchitecture analysis. Molecules (Basel, Switzerland) 18, 15474-15489.
Kim, J.Y., Cheon, Y.H., Kwak, S.C., Baek, J.M., Yoon, K.H., Lee, M.S., Oh, J., 2014. Emodin regulates bone remodeling by inhibiting osteoclastogenesis and stimulating osteoblast formation. Journal of Bone and Mineral Research : the official journal of the American Society for Bone and Mineral Research 29, 1541-1553.
Kim, J.Y., Kim, Y.K., Choi, M.K., Oh, J., Kwak, H.B., Kim, J.J., 2012. Effect of Cornus Officinalis on Receptor Activator of Nuclear Factor-kappaB Ligand (RANKL)-induced Osteoclast Differentiation. Journal of Bone Metabolism 19, 121-127.
Kim, W.S., Lee, M.J., Kim, D.H., Lee, J.E., Kim, J.I., Kim, Y.C., Song, M.R., Park, S.G., 2013b. 5'-OH-5-nitro-Indirubin oxime (AGM130), an Indirubin derivative, induces apoptosis of Imatinib-resistant chronic myeloid leukemia cells. Leukemia Research 37, 427-433.
Lee, H.W., Suh, J.H., Kim, H.N., Kim, A.Y., Park, S.Y., Shin, C.S., Choi, J.Y., Kim, J.B., 2008. Berberine promotes osteoblast differentiation by Runx2 activation with p38 MAPK. Journal of Bone and Mineral Research : the

official journal of the American Society for Bone and Mineral Research 23, 1227-1237.
Lee, J.W., Kobayashi, Y., Nakamichi, Y., Udagawa, N., Takahashi, N., Im, N.K., Seo, H.J., Jeon, W.B., Yonezawa, T., Cha, B.Y., Woo, J.T., 2010. Alisol-B, a novel phyto-steroid, suppresses the RANKL-induced osteoclast formation and prevents bone loss in mice. Biochemical Pharmacology 80, 352-361.
Lee, K.H., Choi, E.M., 2006. Stimulatory effects of extract prepared from the bark of Cinnamomum cassia blume on the function of osteoblastic MC3T3-E1 cells. Phytotherapy Research : PTR 20, 952-960.
Lee, S.Y., Lee, K.S., Yi, S.H., Kook, S.H., Lee, J.C., 2013. Acteoside suppresses RANKL-mediated osteoclastogenesis by inhibiting c-Fos induction and NF-kappaB pathway and attenuating ROS production. PloS one 8, e80873.
Lewiecki, E.M., 2011. Safety of long-term bisphosphonate therapy for the management of osteoporosis. Drugs 71, 791-814.
Li, F., Yang, Y., Zhu, P., Chen, W., Qi, D., Shi, X., Zhang, C., Yang, Z., Li, P., 2012. Echinacoside promotes bone regeneration by increasing OPG/RANKL ratio in MC3T3-E1 cells. Fitoterapia 83, 1443-1450.
Li, F.C., Xie, H.Q., Ling, X.Y., 2010. Clinical obseravtion in Phase Ⅲ of Fufang Lurongjiangu Jiao Nang treated for primary osteoporosis. Chinese Traditional and Herbal Drugs 41, 1856-1858.
Li, F.M., Gao, X.Y., Wang, D.W., Zhao, H., 2001. Screening active constituents of Achyranthes bidentata BI. stimulating bone formation. Pharmaceutical and Pharmacological Letters 11, 95-97.
Li, G., Seo, C.S., Lee, K.S., Kim, H.J., Chang, H.W., Jung, J.S., Song, D.K., Son, J.K., 2004. Protective constituents against sepsis in mice from the root cortex of Paeonia suffruticosa. Archives of Pharmacal Research 27, 1123-1126.
Li, H.H., Ju, D.H., Teng, J.R., Li, Y., Li, Y., Liu, H., Wand, S.J., Pan, J.H., Yu, Z., Liu, M.J., 2011. Effects of Zuogui Pill on E2 and PTH in serum of Glucocorticoid-induced Osteoporosis Rats. Chinese Journal of Basic Medicine in Traditional Chinese Medicine 17, 744,745,763.
Li, J.X., Hareyama T Fau - Tezuka, Y., Tezuka Y Fau - Zhang, Y., Zhang Y Fau - Miyahara, T., Miyahara T Fau - Kadota, S., Kadota, S., 2005a. Five new oleanolic acid glycosides from Achyranthes bidentata with inhibitory activity on osteoclast formation. Planta Medica 71, 673-679.
Li, J.X., Hareyama, T., Tezuka, Y., Zhang, Y., Miyahara, T., Kadota, S., 2005b. Five new oleanolic acid glycosides from Achyranthes bidentata with inhibitory activity on osteoclast formation. Planta Medica 71, 673-679.
Li, Y., Wang, J., Chen, G., Feng, S., Wang, P., Zhu, X., Zhang, R., 2015. Quercetin promotes the osteogenic differentiation of rat mesenchymal stem cells via mitogen-activated protein kinase signaling. Experimental and Therapeutic Medicine 9, 2072-2080.
Liang, Q.M., Xu, X.Z., Pan, G.Q., Zeng, W.L., Fang, Y.Z., 2011. Clinical Reaserch To Add and Subtract Kidney Shot Right Treatment of Osteoporosis. Guide of China Medicine 9, 5-7.
Liao, Y.Q., Yang, K., He, M.W., 2011. Clinical observation for the treatment of osteoporotic femoral intertrochanteric fracture with DHS combined with Jintiange Jiao Nang. Chinese Journal of Osteoporosis 17, 523-525.
Lim, D.W., Kim, Y.T., 2013. Dried root of Rehmannia glutinosa prevents bone loss in ovariectomized rats. Molecules (Basel, Switzerland) 18, 5804-5813.
Lim, D.W., Kim, Y.T., 2014. Anti-osteoporotic effects of Angelica sinensis (Oliv.) Diels extract on ovariectomized rats and its oral toxicity in rats. Nutrients 6, 4362-4372.
Lin, Y.P., Feng, E.Y., H.M., W., Huang, M.Y., 2004. Effect of Jiangu granule on Retinoic acids-induced osteoporotic rats. China Journal of Traditional Chinese Medicine and Pharmacy 19, 531-533.
Liu, B., Chen, M., Li, S.S., Liu, Z.L., Wang, L., Xie, Z., Li, M., 2014a. Effect of Er-xian decoction on osteoporosis in ovariectomized rats. Chinese Journal of Osteoporosis 20, 129-132.

Liu, H.J., Wang, X.P., Lin, J., Yu, Y.C., Jiang, X.Q., Zhang, X.L., Zhou, Z.T., Zhang, W.D., 2006. The effect of icariin and astragalosid I on the proliferation and differentiation of bone marrow stromal cells. Journal of Chinese Medicinal Materials 29, 1062-1065.

Liu, J., Li, Q., Yin, Y., Liu, R., Xu, H., Bi, K., 2014b. Ultra-fast LC-ESI-MS/MS method for the simultaneous determination of six highly toxic Aconitum alkaloids from Aconiti kusnezoffii radix in rat plasma and its application to a pharmacokinetic study. Journal of Separation Science 37, 171-178.

Liu, M.J., Pan, J.H., Li, Y., Liu, H., Teng, J.R., Wang, S.J., Zhang, Y., Du, Z.P., Yu, Z., Ju, D.H., 2011. Effects of Zuoguiwan on BGP and IGF-1 in Serum of Glucocorticoid-induced Osteoporosis Rats. Chinese Journal of Experimental Traditional Medicine Formulae 17, 133-136.

Liu, Q.M., Zhao, H.Y., Zhong, X.K., Jiang, J.G., 2012. Eclipta prostrata L. phytochemicals: isolation, structure elucidation, and their antitumor activity. Food and Chemical Toxicology : an international journal published for the British Industrial Biological Research Association 50, 4016-4022.

Liu, Z.G., Zhang,R., Li, C., Ma, X., Liu, L., Wang, J.P., Mei, Q.B., 2009. The osteoprotective effect of radix dipsaci extract in ovariectomized rats. Journal of Ethnopharmacology, 123(1), 74-81.

Lo, Y.C., Chang, Y.H., Wei, B.L., Huang, Y.L., Chiou, W.F., 2010. Betulinic acid stimulates the differentiation and mineralization of osteoblastic MC3T3-E1 cells: involvement of BMP/Runx2 and beta-catenin signals. Journal of Agricultural And Food Chemistry 58, 6643-6649.

Lu, M., Wang, L.H., Luo, Y.W., Ge, J.R., Gao, S.T., Chen, J.Y., Yang, F.Y., Shen, L., 2013. Treatment of primary osteoporosis with epimedium total flavone capsule: a multicenter clinical observation on 360cases. Chinese Journal of Osteoporosis, 3(19), 279-282.

Luo, G., Gu, F., Zhang, Y., Liu, T., Guo, P., Huang, Y., 2015. Icariside II promotes osteogenic differentiation of bone marrow stromal cells in beagle canine. International Journal of Clinical And Experimental Pathology 8, 4367-4377.

Lv, H.B., Ren, Y.L., Wang, Y., Zhao, J.R., Liu, L.P., X.D., M., 2010. Experimental research of the perventive and therapeutic effect of Zuogui Pill on ovariectomized rats. Chinese Journal of Osteoporosis 16.

Majewski, M., 2014. Allium sativum: facts and myths regarding human health. Roczniki Panstwowego Zakladu Higieny 65, 1-8.

Miao, J., Jiang, Y., Wang, D., Zhou, J., Fan, C., Jiao, F., Liu, B., Zhang, J., Wang, Y., Zhang, Q., 2015. Trichosanthin suppresses the proliferation of glioma cells by inhibiting LGR5 expression and the Wnt/beta-catenin signaling pathway. Oncology Reports 34, 2845-2852.

Michalik, L., Wahli, W., 2007. Guiding ligands to nuclear receptors. Cell 129, 649-651.

Ming, L.G., Wang, M.G., Chen, K.M., Zhou, J., Han, G.Q., Zhu, R.Q., 2012. Effect of osthol on apoptosis and bone resorption of osteoclasts cultured in vitro. Acta Pharmaceutica Sinica 47(2),174-179.

Nicolin, V., Dal Piaz, F., Nori, S.L., Narducci, P., De Tommasi, N., 2010. Inhibition of bone resorption by Tanshinone VI isolated from Salvia miltiorrhiza Bunge. European Journal of Histochemistry : EJH 54, e21.

Niu, K., Zhao, Y.J., Zhang, L., Li, C.G., Wang, Y.J., Zheng, W.C., 2014. The synergistic effect of amygdalin and HSYA on the IL-1beta induced endplate chondrocytes of rat intervertebral discs. Yao Xue Xue Bao 49, 1136-1142.

Niu, Y., Li, C., Pan, Y., Li, Y., Kong, X., Wang, S., Zhai, Y., Wu, X., Fan, W., Mei, Q., 2015. Treatment of Radix Dipsaci extract prevents long bone loss induced by modeled microgravity in hindlimb unloading rats. Pharmaceutical Biology 53, 110-116.

Niu, Y., Li, Y., Huang, H., Kong, X., Zhang, R., Liu, L., Sun, Y., Wang, T., Mei, Q., 2011. Asperosaponin VI, a saponin component from Dipsacus asper wall, induces osteoblast differentiation through bone morphogenetic protein-2/p38 and extracellular signal-regulated kinase 1/2 pathway. Phytotherapy Research : PTR 25,

1700-1706.
Niu, Y.B., Li, Y.H., Kong, X.H., Zhang, R., Sun, Y., Li, Q., Li, C., Liu, L., Wang, J., Mei, Q.B., 2012. The beneficial effect of Radix Dipsaci total saponins on bone metabolism in vitro and in vivo and the possible mechanisms of action. Osteoporos International 23, 2649-2660.
Oh, K.O., Kim, S.W., Kim, J.Y., Ko, S.Y., Kim, H.M., Baek, J.H., Ryoo, H.M., Kim, J.K., 2003. Effect of Rehmannia glutinosa Libosch extracts on bone metabolism. Clinica Chimica Acta; International Journal of Clinical Chemistry 334, 185-195.
Oh, S., Han, A.R., Park, H.R., Jang, E.J., Kim, H.K., Jeong, M.G., Song, H., Park, G.H., Seo, E.K., Hwang, E.S., 2014. Suppression of Inflammatory cytokine production by ar-Turmerone isolated from Curcuma phaeocaulis. Chemistry & Biodiversity 11, 1034-1041.
Pan, Y., Niu, Y., Li, C., Zhai, Y., Zhang, R., Guo, X., Mei, Q., 2014. Du-zhong (Eucommia ulmoides) prevents disuse-induced osteoporosis in hind limb suspension rats. The American Journal of Chinese Medicine 42, 143-155.
Park, C.K., Lee, Y., Chang, E.J., Lee, M.H., Yoon, J.H., Ryu, J.H., Kim, H.H., 2008. Bavachalcone inhibits osteoclast differentiation through suppression of NFATc1 induction by RANKL. Biochemical Pharmacology 75, 2175-2182.
Piemontese, M., Onal, M., Xiong, J., Wang, Y., Almeida, M., Thostenson, J.D., Weinstein, R.S., Manolagas, S.C., O'Brien, C.A., 2015. Suppression of autophagy in osteocytes does not modify the adverse effects of glucocorticoids on cortical bone. Bone 75, 18-26.
Qin, L. P., Zhang, Q. Y., Tian, Y. P., Zheng, H. C., Huang, M., Huang B.K., 2003. Total coumarins from fruits of cnidium monnieri inhibit formation and differentiation of multinucleated osteoclasts of rats. Acta Pharmacologica Sinica, 24(2), 181-6.
Ramli, E.S., Suhaimi, F., Asri, S.F., Ahmad, F., Soelaiman, I.N., 2013. Glycyrrhizic acid (GCA) as 11beta-hydroxysteroid dehydrogenase inhibitor exerts protective effect against glucocorticoid-induced osteoporosis. Journal of bone and mineral metabolism 31, 262-273.
Ran, X., Ma, L., Peng, C., Zhang, H., Qin, L.P., 2011. Ligusticum chuanxiong Hort: a review of chemistry and pharmacology. Pharmaceutical Biology 49, 1180-1189.
Rosen, C.J., 2000. The Epidemiology and Pathogenesis of Osteoporosis, in: De Groot, L.J., Beck-Peccoz, P., Chrousos, G., Dungan, K., Grossman, A., Hershman, J.M., Koch, C., McLachlan, R., New, M., Rebar, R., Singer, F., Vinik, A., Weickert, M.O. (Eds.), Endotext. MDText.com, Inc., South Dartmouth (MA).
Sassa, S., Kikuchi, T., Shinoda, H., Suzuki, S., Kudo, H., Sakamoto, S., 2003. Preventive effect of ferulic acid on bone loss in ovariectomized rats. In vivo (Athens, Greece) 17, 277-280.
Sharma, D., Ciani, C., Marin, P.A., Levy, J.D., Doty, S.B., Fritton, S.P., 2012. Alterations in the osteocyte lacunar-canalicular microenvironment due to estrogen deficiency. Bone 51, 488-497.
Shen, Q., Zeng, D., Zhou, Y., Xia, L., Zhao, Y., Qiao, G., Xu, L., Liu, Y., Zhu, Z., Jiang, X., 2013. Curculigoside promotes osteogenic differentiation of bone marrow stromal cells from ovariectomized rats. The Journal of Pharmacy And Pharmacology 65, 1005-1013.
Suda, T., Takahashi, N., Udagawa, N., Jimi, E., Gillespie, M.T., Martin, T.J., 1999. Modulation of osteoclast differentiation and function by the new members of the tumor necrosis factor receptor and ligand families. Endocrine Reviews 20, 345-357.
Sun, H., Li, L., Zhang, A., Zhang, N., Lv, H., Sun, W., Wang, X., 2013. Protective effects of sweroside on human MG-63 cells and rat osteoblasts. Fitoterapia 84, 174-179.
Sun, P., Huang, Z., Cai, D.H., He, L., 2007. Effects of Chinese Bushen Zhuanggu medicine on bone loss in female rats after simulated weightlessness. Journal of Southern Medical University 27, 212-214.

Sun, W., Wang, Y.Q., Yan, Q., Lu, R., Shi, B., 2014. Effects of Er-Zhi-Wan on microarchitecture and regulation of Wnt/beta-catenin signaling pathway in alveolar bone of ovariectomized rats. Journal of Huazhong University of Science and Technology. Medical sciences 34, 114-119.

Tan, X.L., Zhang, Y.H., Cai, J.P., Zhu, L.H., Ge, W.J., Zhang, X., 2014. 5-(Hydroxymethyl)-2-furaldehyde inhibits adipogenic and enhances osteogenic differentiation of rat bone mesenchymal stem cells. Natural Product Communications 9, 529-532.

Tang, C. H., Yang, R. S., Chien, M. Y., Chen, C. C., Fu, W. M., 2008. Enhancement of bone morphogenetic protein-2 expression and bone formation by coumarin derivatives via p38 and erk-dependent pathway in osteoblasts. European Journal of Pharmacology, 579(1-3), 40–49.

Tang, D.Z., Hou, W., Zhou, Q., Zhang, M., Holz, J., Sheu, T.J., Li, T.F., Cheng, S.D., Shi, Q., Harris, S.E., Chen, D., Wang, Y.J., 2010. Osthole stimulates osteoblast differentiation and bone formation by activation of beta-catenin-BMP signaling. Journal of Bone And Mineral Research : The Official Journal of The American Society for Bone and Mineral Research 25, 1234-1245.

Tong, Y., Xu, W., Han, H., Chen, Y., Yang, J., Qiao, H., Hong, D., Wu, Y., Zhou, C., 2011. Tanshinone IIA increases recruitment of bone marrow mesenchymal stem cells to infarct region via up-regulating stromal cell-derived factor-1/CXC chemokine receptor 4 axis in a myocardial ischemia model. Phytomedicine : International Journal of Phytotherapy And Phytopharmacology 18, 443-450.

Tseng, S.H., Sung, C.H., Chen, L.G., Lai, Y.J., Chang, W.S., Sung, H.C., Wang, C.C., 2014. Comparison of chemical compositions and osteoprotective effects of different sections of velvet antler. Journal of Ethnopharmacol 151, 352-360.

Tsuji-Naito, K., 2008. Aldehydic components of cinnamon bark extract suppresses RANKL-induced osteoclastogenesis through NFATc1 downregulation. Bioorganic & Medicinal Chemistry 16, 9176-9183.

Turner, R.T., Iwaniec, U.T., Andrade, J.E., Branscum, A.J., Neese, S.L., Olson, D.A., Wagner, L., Wang, V.C., Schantz, S.L., Helferich, W.G., 2013. Genistein administered as a once-daily oral supplement had no beneficial effect on the tibia in rat models for postmenopausal bone loss. Menopause 20, 677-686.

Wang, C.H., W., W., Li, J.C., Jiang, Y.P., Li, F., Chen, X.F., Su, S.Y., Lin, T.M., Feng, W., Shi, S.P., 2003. Clinical efficacy of GusongKang Jiao Nang in treatment of postmenopausal osteoporosis. Chinese Journal Osteoporosis 9, 165,166,131.

Wang, D., Li, F., Jiang, Z., 2001. Osteoblastic proliferation stimulating activity of Psoralea corylifolia extracts and two of its flavonoids. Planta Medica 67, 748-749.

Wang, D.P., Lou, H.Y., Huang, L., Hao, X.J., Liang, G.Y., Yang, Z.C., Pan, W.D., 2012a. A novel franchetine type norditerpenoid isolated from the roots of Aconitum carmichaeli Debx. with potential analgesic activity and less toxicity. Bioorganic & Medicinal Chemistry Letters 22, 4444-4446.

Wang, J.S., Hu, X.Q., Zhuo, J., Li, Y.L., Wang, L., 2010. Effect of Xianlinggubao on bone density and metabolism of ovariectomized rats. Journal of Shanxi College of Traditional Chinese Medicine 33, 64-65.

Wang, M.Y., Gong, L., Xia, B., Cao, J.Q., Zhou, P.Q., Hu, J., 2005. Clinical Observation on 96 Cases of Primary Osteoporosis Treated with Kidney-Tonifying and Bone-Strengthening Mixture. Journal of Traditional Chinese Medicine 25, 132-136.

Wang, P., Wei, X., Zhang, F., Yang, K., Qu, C., Luo, H., He, L., 2014a. Ginsenoside Rg1 of Panax ginseng stimulates the proliferation, odontogenic/osteogenic differentiation and gene expression profiles of human dental pulp stem cells. Phytomedicine : international journal of phytotherapy and phytopharmacology 21, 177-183.

Wang, R.R., Ju, D.H., S.N., H., H.Y., Z., Ma, L., Fu, X.W., Li, H.Y., Wang, S.J., Wang, B.J., Liu, H., Zhang, S.Y., Zhao, S., 2014b. Zuozui Pills treat Type 2 Diabetes accompany with osteoporosis and Kidney Yin deficency: A Clinical

Observation of 30 cases. Chinese Journal of Basic Medicine in Traditional Chinese Medicine 20, 259-261.
Wang, W., Dong, H., Yan, R., Li, H., Li, P., Chen, P., Yang, B., Wang, Z., 2015. Comparative study of lanostane-type triterpene acids in different parts of Poria cocos (Schw.) Wolf by UHPLC-Fourier transform MS and UHPLC-triple quadruple MS. Journal of Pharmaceutical And Biomedical Analysis 102, 203-214.
Wang, X.D., Dong, Q.W., Hong, M.J., Sun, P., Hu, L.P., Liu, Z.H., 2012b. Effect of Hugu Jiao Nang on BMD and histomorphometry in glucocorticoid-induced osteoporotic rats. Chinese Journal of Osteoporosis 18, 880-882.
Wang, Y., Cui, K., Zhao, H., Li, D., Wang, W., Zhu, Y., 2009. Bushen Ningxin Decoction pharmacological serum promotes the proliferation and suppresses the apoptosis of murine osteoblasts through MAPK pathway. Jounal of Ethnopharmacol 122, 221-226.
Wang, Y., Dan, Y., Yang, D., Hu, Y., Zhang, L., Zhang, C., Zhu, H., Cui, Z., Li, M., Liu, Y., 2014c. The genus Anemarrhena Bunge: A review on ethnopharmacology, phytochemistry and pharmacology. Journal of Ethnopharmacol 153, 42-60.
Wong, R.W., Rabie, A.B., Hagg, E.U., 2007. The effect of crude extract from Radix Dipsaci on bone in mice. Phytotherapy Research : PTR 21, 596-598.
Wu, C.M., Chen, P.C., Li, T.M., Fong, Y.C., Tang, C.H., 2013. Si-Wu-tang extract stimulates bone formation through PI3K/Akt/NF-kappaB signaling pathways in osteoblasts. BMC complementary and alternative medicine 13, 277.
Wu, M.S., Zhao, S.Z., Li, E., Bai, X., 2010. Effects of catalpol from Radix rehmanniae on proliferation, differentiation and matrix mineration of MC3T3-E1 cells. Chinese Pharmacological Bulletin 26, 509-513.
Xia, B., Xu, B., Sun, Y., Xiao, L., Pan, J., Jin, H., Tong, P., 2014. The effects of Liuwei Dihuang on canonical Wnt/beta-catenin signaling pathway in osteoporosis. Journal of Ethnopharmacol 153, 133-141.
Xie, Q.F., Xie, J.H., Dong, T.T., Su, J.Y., Cai, D.K., Chen, J.P., Liu, L.F., Li, Y.C., Lai, X.P., Tsim, K.W., Su, Z.R., 2012. Effect of a derived herbal recipe from an ancient Chinese formula, Danggui Buxue Tang, on ovariectomized rats. Journal of Ethnopharmacol 144, 567-575.
Xu XH, L.T., Wang YT, Lu JJ, 2015. Research progress in Persicae Semen. Chinese Traditional and Herbal Drugs 46, 2649-2655.
Xu, Y., Zhang Zj Fau - Geng, F., Geng F Fau - Su, S.-b., Su Sb Fau - White, K.N., White Kn Fau - Bligh, S.W.A., Bligh Sw Fau - Branford-White, C.J., Branford-White Cj Fau - Wang, Z.-t., Wang, Z.T., 2010. Treatment with Qing'E, a kidney-invigorating Chinese herbal formula, antagonizes the estrogen decline in ovariectomized mice. Rejuvenation Research 13.
Xue, L., Wang, Y., Jiang, Y., Han, T., Nie, Y., Zhao, L., Zhang,Q.Y., Qin, L.P., 2012. Comparative effects of er-xian decoction, epimedium herbs, and icariin with estrogen on bone and reproductive tissue in ovariectomized rats..Evidence-based complementary and alternative medicine, eCAM, 2012:241416. doi:10.1155/2012/241416.
Yang, F., Tang, D.Z., Cui, X.J., Holz, J.D., Bian, Q., Shi, Q., Wang, Y.J., 2011a. Classic yin and yang tonic formula for osteopenia: study protocol for a randomized controlled trial. Trials 12, 187.
Yang GQ, Z.X., 2003. Research advances on chemical compositions, pharmacological effect and clinic application of placenta and its extract from human and animals. Journal of Shenyang Agricultural University 34, 150-154.
Yang, H.M., Shin, H.K., Kang, Y.H., Kim, J.K., 2009. Cuscuta chinensis extract promotes osteoblast differentiation and mineralization in human osteoblast-like MG-63 cells. Journal of Medicinal Food 12, 85-92.
Yang, L., Chen, Q., Wang, F., Zhang, G., 2011b. Antiosteoporotic compounds from seeds of Cuscuta chinensis. Journal of Ethnopharmacol 135, 553-560.
Yang, S.H., Sharrocks, A.D., Whitmarsh, A.J., 2013. MAP kinase signalling cascades and transcriptional regulation. Gene 513, 1-13.
Yang, Z., Huang, J.H., Liu, S.F., Zhao, Y.J., Shen, Z.Y., Wang, Y.J., Bian, Q., 2012. The osteoprotective effect of

psoralen in ovariectomy-induced osteoporotic rats via stimulating the osteoblastic differentiation from bone mesenchymal stem cells. Menopause 19, 1156-1164.
Yu, Q.Y., 2009. Clinical effect of Er-Zhi pills on postmenopausal osteporosis. Strait Pharmaceutical Journal 21, 169-170.
Zhang, H., Zhang, L., Liu, Y., 2011. Studies on chemical components and pharmacological activities of Os Draconis (Longgu) and Ostreae Concha. China Journal Of Chinese Materia Medica 36, 1839-1840.
Zhang, H.B., Li, C.Y., 2011. Effect of Liuwei Dihuang Pill on Bone Mineral Density of the Primary Osteoporosis(kidney yin deficiency). Chinese Journal of Traditional Medicine Traumatology & Orthopedics 19, 18-20.
Zhang, J.K., Yang, L., Meng, G.L., Fan, J., Chen, J.Z., He, Q.Z., Chen, S., Fan, J.Z., Luo, Z.J., Liu, J., 2012a. Protective effect of tetrahydroxystilbene glucoside against hydrogen peroxide-induced dysfunction and oxidative stress in osteoblastic MC3T3-E1 cells. European Journal of Pharmacology 689, 31-37.
Zhang, R., Hu, S.J., Li, C., Zhang, F., Gan, H.Q., Mei, Q.B., 2012b. Achyranthes bidentata root extract prevent OVX-induced osteoporosis in rats. Journal of Ethnopharmacol 139, 12-18.
Zhang, R., Liu, Z.G., Li, C., Hu, S.J., Liu, L., Wang, J.P., Mei, Q.B., 2009. Du-Zhong (Eucommia ulmoides Oliv.) cortex extract prevent OVX-induced osteoporosis in rats. Bone 45, 553-559.
Zhang, R., Pan, Y.L., Hu, S.J., Kong, X.H., Juan, W., Mei, Q.B., 2014. Effects of total lignans from Eucommia ulmoides barks prevent bone loss in vivo and in vitro. Journal of Ethnopharmacol 155, 104-112.
Zhang, R.X., Li, M.X., Jia, Z.P., 2008. Rehmannia glutinosa: review of botany, chemistry and pharmacology. Journal of Ethnopharmacol 117, 199-214.
Zhao, G., Shen, L., 2012. Effects of Qing'e Pills on Bone Mineral Density,Serum MMP-2 Level and Bone Metabolic Markers in Women with Postmenopausal Osteoporosis. Research of Integrated Traditional Chinese and Western Medicine 4, 113-117.
Zhao, J., Ohba, S., Shinkai, M., Chung, U.I., Nagamune, T., 2008. Icariin induces osteogenic differentiation in vitro in a BMP- and Runx2-dependent manner. Biochemical and biophysical research communications 369, 444-448.
Zhao, L., Ye, J., Wu, G.T., Peng, X.J., Xia, P.F., Ren, Y., 2015. Gentiopicroside prevents interleukin-1 beta induced inflammation response in rat articular chondrocyte. Journal of Ethnopharmacol 172, 100-107.
Zhao SX, Z.L., Li J, 2012. Identification and research progress on oysters. China Modern Medicine 19, 18-19.
Zhao, Y., Li, J., Liu, Y., Yu, K.Q., Zhang, J., Chen, X.G., 2007. Gu Ling Pian, a traditional Chinese medicine, regulates function and OPG/RANKL synthesis of osteoblasts via the p38 MAPK pathway. The Journal of pharmacy and pharmacology 59, 1167-1173.
Zhong, S., Yang, D.P., Cui, Z., 2008. Studies on anticoagulant constituents in dried Whitmania pigra. China journal of Chinese materia medica 33, 2781-2784.
Zhou, R., Li, B.G., Zhang, G.L., 2005. Chemical study on Cyathula officinalis Kuan. Journal of Asian natural products research 7, 245-252.
Zhu, H.M., Qin, L., Garnero, P., Genant, H.K., Zhang, G., Dai, K., Yao, X., Gu, G., Hao, Y., Li, Z., Zhao, Y., Li, W., Yang, J., Zhao, X., Shi, D., Fuerst, T., Lu, Y., Li, H., Zhang, X., Li, C., Zhao, J., Wu, Q., Zhao, S.J., 2012. The first multicenter and randomized clinical trial of herbal Fufang for treatment of postmenopausal osteoporosis. Osteoporos International 23, 1317-1327.
Zhu, Q.A., Gu, M.Q., 2012. Efficacy of Salmon Calcitionin plus Erxian Decoction (EXD) for Osteoporosis in Postmenopausal Women: A Clinical Observation. Chinses Archives of Traditonal Chinese Medicine, 30(12), 2806-2809.