Botanicals in Postmenopausal Osteoporosis

Subjects: Chemistry, Medicinal

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Osteoporosis is a systemic bone disease characterized by reduced bone mass and the deterioration of bone microarchitecture leading to bone fragility and an increased risk of fractures. Conventional anti-osteoporotic pharmaceutics are effective in the treatment and prophylaxis of osteoporosis, however they are associated with various side effects that push many women into seeking botanicals as an alternative therapy.

Keywords: osteoporosis ; menopause ; botanicals ; herbs

1. Introduction

Women's health and quality of life is modulated and affected strongly by hormone status. An oestrogen level that changes dramatically throughout life determines the development of women's age-associated diseases. Age-associated hormonal imbalance and oestrogen deficiency are involved in the pathogenesis of various diseases, e.g., obesity, autoimmune disease and osteoporosis. Many female patients look for natural biological products deeply rooted in folk medicine as an alternative to conventional pharmaceutics used as the prophylaxis of perimenopausal health disturbances. This review will focus on botanicals and plant derived substances that may be used to maintain bone health in perimenopausal and postmenopausal females.

Osteoporosis is a systemic bone disease characterized by the reduced bone mass and deterioration of bone microarchitecture leading to bone fragility and the increased risk of fractures [1]. Osteoporosis-associated fragility fractures constitute a major health problem all over the world. It is estimated that more than 40 million American citizens over 50 years of age are at risk of osteoporotic fractures, and that due to the demographic changes, this number will at least double until the year 2040 [2]. It is also predicted that 25% of people over 50 who have experienced osteoporotic hip fracture will die within a year [2]. Hypogonadism, mainly associated with menopause, is the main cause of osteoporosis. High social and individual costs of osteoporosis and its complications remain a challenge for health systems, especially because most of the patients with osteoporosis remain untreated. The data indicate that almost 60% patients at high risk of osteoporotic fractures are not receiving osteoprotective treatment [3]. Additionally, a decrease in the usage of antiosteoporotic drugs, especially bisphosphonates, has been observed in recent years [3]. Oral bisphosphonates, that bind to hydroxyapatite and inhibit osteoclastic bone resorption, are the drug of choice for the treatment of primary osteoporosis. However, they are associated with side effects including oesophagitis and oesophageal ulcers, jaw osteonecrosis, and atypical femoral fractions. In case of intolerance or lack of efficacy, they might be switched to intravenous bisphosphonates, strontium ranelate, denosumab, teriparatide, abaloparatide or romosozumab. As additional options in postmenopausal women, raloxifene and hormonal replacement therapy may be used [4]. However, as those pharmaceutics are associated with various side effects, many women seek for botanicals as an alternative therapy.

Bones undergo continuous remodelling, osteoblasts synthetize the bone matrix and, at the same time, osteoclasts degrade bone tissue. In physiological conditions, we observe the balance between the resorption and formation of bone tissue. This balance depends on the activity, differentiation, and apoptosis of bone forming osteoblasts and bone-resorbing osteoclasts. Multiple factors and signalling pathways modulate bone homeostasis (Figure 1). Bone cells' activity is controlled, among others, by growth factors (IGF—insulin-like growth factor, TGFβ—tumour growth factor β, PDGF—platelet-derived growth factor), bone morphogenic proteins (BMPs), hormones (parathormone, thyroid hormones, sex hormones, insulin, prolactin, growth hormone) and vitamins (vitamin D). Wnt, BMPs and TGFβ pathways interact with other signalling molecules such as basic fibroblast growth factor (bFGF), Hedgehog (Hh) and IGF-1, and regulate the differentiation and activity of osteoclasts [5]. Runx2 (Runt-related transcription factor 2) and OSX (Osterix) are the main transcription factors involved in the modulation of osteoblast differentiation. Osteoclastogenesis is regulated by two main pathways: RANK/RANKL (Receptor Activator for Nuclear Factor κB/Receptor Activator for Nuclear Factor κB Ligand) and M-CSF/c-FMS (the macrophage colony-stimulating factor/colony-stimulating factor-1 receptor) system. Parathyroid hormone (PTH) and calcitriol induce RANKL synthesis in osteoblasts and afterwards promote osteoclastogenesis through

RANK activation. RANK activation is counteracted by OPG (osteoprotegerin), which is a decoy receptor of free RANKL. M-CSF/c-FMS interaction leads to mitogen-activated protein kinase (MAPK) activation that induces RANKL production and activates AKT/mTOR (protein kinase B/mechanistic target of rapamycin) pathway engaged in the survival of osteoclasts [5].

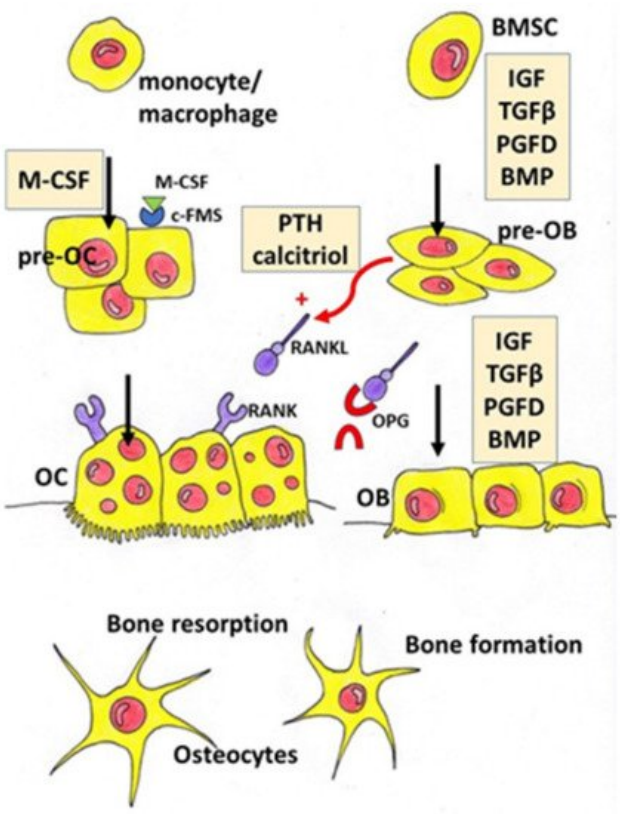


Figure 1. Schematic diagram representing regulation of osteoblast and osteoclast differentiation. BMP—bone morphogenic protein, BMSC—bone marrow-derived mesenchymal stem cells, c-FMS—colony-stimulating factor-1 receptor, IGF—insulin-like growth factor, M-CSF—macrophage colony-stimulating factor, OB—osteoblast, OC—osteoclast, OPG—osteoprotegerin, PGFD—platelet-derived growth factor, pre-OB—pre-osteoblasts, pre-OC—pre-osteoclasts, PTH—parathyroid hormone, RANK—Receptor Activator for Nuclear Factor κB, RANKL—Receptor Activator for Nuclear Factor κB Ligand, TGFβ—tumour growth factor β.

Oestrogen plays an important role in maintaining bone mineral density in both rodents and humans (Figure 2). A decrease in the oestrogen level associated with menopause leads to a decrease in bone mineral density (BMD) that increases the risk of fractures [6][7]. The protective effect of oestrogen in bone is due to many mechanisms. Oestrogen, among other things, inhibits bone resorption by the suppression of the synthesis of proinflammatory cytokines in osteoblasts via the inhibition of nuclear factor-kappa B (NFκB) signalling pathway [8]. They also activate the transcription of a gene encoding Fas Ligand (FasL) in osteoblasts. Soluble FasL (sFasL) released from the osteoblast binds to the transmembrane Fas receptor (FasR) on the osteoclast's surface and induces the apoptosis of osteoclasts [9]. Additionally, oestrogen decreases the RANKL/OPG ratio and prevents bone resorption [10].

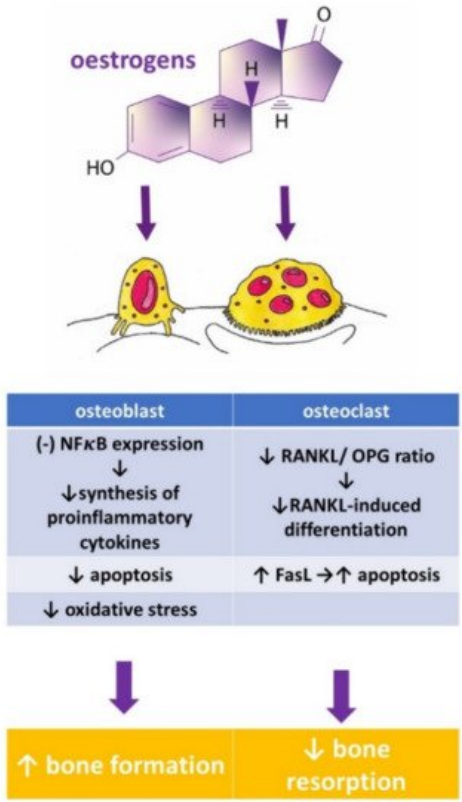
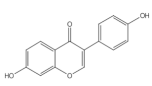
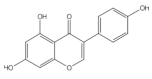
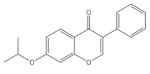
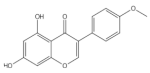
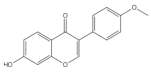
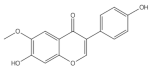
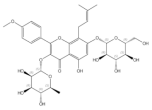
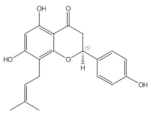
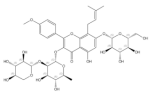
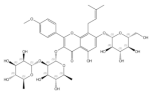
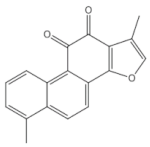
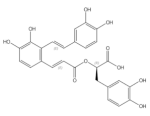
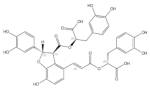
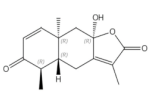


Figure 2. Influence of oestrogens on osteoblast and osteoclast function, and bone turnover. FasL—Fas Ligand, NFκB—Nuclear Factor κB, OPG—osteoprotegerin, RANKL—Receptor Activator for Nuclear Factor κB Ligand, ↑—increased, ↓—decreased

Women's health and quality of life are modulated and affected strongly by hormone status. An oestrogen level that changes dramatically determines the development of women's age-associated diseases. Age-associated hormonal imbalance and oestrogen deficiency are involved in the pathogenesis of various diseases, e.g., obesity, autoimmune diseases, and osteoporosis. As postmenopausal osteoporosis is characterised by bone resorption that exceeds bone formation, antiresorptive drugs are one of the therapeutic options and most current therapies exert mainly antiresorptive effects. Another therapeutic solution may be the use of anabolic drugs that would enhance bone formation. Bone morphogenic protein (BMP), Wnt, and insulin-like growth factor 1 (IGF1) are the key molecules involved in the regulation of osteoblast formation and activation [11][12][13]. Oestrogens, SERMs (selective oestrogen receptor modulators), bisphosphonates, strontium ranelate, denosumab, teriparatide, abaloparatide or romosozumab are clinically used as effective therapies against postmenopausal osteoporosis [4]; however, their usage is associated with the established risk of the side effect. Therefore, many female patients look for natural biological products deeply rooted in folk medicine as an alternative to conventional pharmaceutics used as the prophylaxis of perimenopausal health disturbances. Table 1 summarizes the information about the main active ingredients discussed in the article, and Table 2 clinical studies and their main findings.

Table 1. Herbal compounds with antiosteoporotic properties investigated in vitro and in animal models.

Herbal Compounds	Subgroup	Chemical Structure	Proposed Mechanism of Action
Daidzein	isoflavones		ER mediated signalling pathway, activation of intracellular pathways: AKT, phospholipase C (PLC), mitogen-activated protein kinase (MAPK) [14]
Genistein	isoflavones		ER-mediated signalling pathway, activation of intracellular pathways: AKT, PLC, MAPK [14]

Herbal Compounds	Subgroup	Chemical Structure	Proposed Mechanism of Action
Ipriflavone	isoflavones		Modulation of key signalling pathways to regulate bone resorption (e.g., ↓urinary DPD, NTX) and bone formation (e.g., ↑BALP and osteocalcin [15]
Biochanin A	O-methylated isoflavones		ER mediated signalling pathway, activation of intracellular pathways: AKT, PLC, MAPK [14]
Formononetin	O-methylated isoflavones		ER mediated signalling pathway, activation of intracellular pathways: AKT, PLC, MAPK [14]
Glycitein	O-methylated isoflavones		ER mediated signalling pathway, activation of intracellular pathways: AKT, PLC, MAPK [14]
Icariin	prenylated flavonol glycoside		Stimulation of bone formation by promotion of osteoblasts differentiation and enhancement of their activity [16]; activation of BMP-2/Smad4, Wnt and IGF-1 signal transduction pathways [5][17], induction of ERK, JNK and p38 kinase activation [18]; decreasing of RANKL-induced osteoclastogenesis via inhibition of NFκB and MAPK expression [19]
8-prenylnaringenin	prenylflavonoids		Promotion of osteoblast differentiation and induction of osteoclast apoptosis [20]
Epimedin B	prenylflavonoids		Inhibition of bone resorption, bone formation promotion and urinary calcium excretion blocking [21]
Epimedin C	prenylflavonoids		Inhibition of bone resorption, bone formation promotion and urinary calcium excretion blocking [21]
Tanshinones (dihydrotanshinone, tanshinone I, or tanshinone IIA)	diterpenes	 Tanshinon 1	Inhibition of the TRAP5b-expressing osteoclasts formation by suppressing RANKL-induced expression of c-fos and NFATc1 [22][23]
Salvianolic acid A	phenolic acids		osteoblast differentiation modulation and osteoblast activity upregulation [24][25]
Salvianolic acid B	phenolic acids		osteoblast differentiation modulation and osteoblast activity upregulation [24][25]
Eudebeiolide B	eudesmane-type sesquiterpenoid		Osteoclastogenesis inhibition and ovariectomy-induced bone loss prevention by regulating RANKL-Induced NF-κB, c-Fos and Calcium Signaling [26]

AKT—protein kinase B, BALP—bone-specific alkaline phosphatase, CTX—type I collagen cross-linked C-telopeptide, DPD—deoxypyridinoline, ER—oestrogen receptor, ERK—extracellular signal-regulated kinase, JNK—c-Jun N-terminal kinase, MAPK—mitogen-activated protein kinase, NFκB—nuclear factor-kappa B, NTX—type I collagen cross-linked N-telopeptide, PLC—phospholipase C, RANKL—Receptor Activator for Nuclear Factor κB Ligand, TRAP 5b—Tartrate-resistant acid phosphatase 5b.

Table 2. Summary of potential anti-osteoporotic properties of botanicals in clinical trials.

Botanicals	Population and Design	Intervention	Outcome	Authors and References
Soy isoflavones	single open-group prospective clinical intervention; 42 postmenopausal women,	three daily servings for 12 consecutive weeks of whole soy foods containing approximately 60 mg/day of isoflavones	↓ NTX, ↑ osteocalcin	Scheiber 2001 [27]
Soy isoflavones	RCT with 3 groups: soy rich diet, HRT, control; 187 healthy asymptomatic postmenopausal women aged 39–60,	approximately 47 mg/day of isoflavones in diet group; duration: 6 moths	↑ bone osteoblastic activity but not as effective as HRT in reducing the postmenopausal turnover, ↑ osteocalcin	Chiechi 2002 [28]
Soy isoflavones	RCT with 3 groups: placebo, mid-dose, and high-dose, in pill form; 203 postmenopausal Chinese women aged 48 to 62,	placebo (daily dose of 0 mg isoflavones + 500 mg calcium, *n* = 67) mid-dose (40 mg isoflavones + 500 mg calcium, *n* = 68) and high-dose (80 mg isoflavones + 500 mg calcium, *n* = 68); duration: 12months	favourable effect on rates of change in BMC at the total hip and trochanter among later postmenopausal women (>4 y), in women with lower body weight (≤median, 55.5 kg), or among women with lower level of calcium intake (≤median, 1095 mg/day)	Chen 2004 [29]
Soy isoflavones	RCT with 3 groups: placebo, mid-dose, and high-dose; 90 Chinese postmenopausal women aged 45–60	placebo (daily dose of 0 mg isoflavones) mid-dose (84 mg) and high dose (126 mg), 30 subjects/group; duration: 6months	Retardation of lumbar and femoral bone loss at the lumbar spine (L1–L4) and bone resorption	Ye 2006 [30]
Soy isoflavones	double-blind RCT with 2 groups: placebo, isoflavone conjugates in capsule form, 68 postmenopausal Japanese women	Isoflavone group (75 mg of isoflavone conjugates/day), 34 subjects/group; duration: 12 months	↑ serum equol in the equol producers but not in the nonproducers, preventive effects of isoflavones on hip BMD	Wu 2007 [31]

Botanicals	Population and Design	Intervention	Outcome	Authors and References
Soy isoflavones	double-blind RCT with 3 groups: placebo, mid-dose, and high-dose in tablet form; 255 postmenopausal women aged 46–63	placebo (daily dose of 0 mg isoflavones) mid-dose (80 mg) and high dose (120 mg); duration: 3 years	mild beneficial femoral BMD—and SSI	Shedd-Wise 2011 [32]
Soy isoflavones	double-blind RCT with 2 groups: placebo, isoflavones in tablet form; 87 Korean postmenopausal women aged 45–60	Isoflavone group = 70 mg in 2 tablet per day (8.0 mg glycitin, 20 mg daidzein, and 12.4 mg genistin); duration: 12 weeks	↑ serum BALP and osteocalcin	Lee 2017 [33]
Soy isoflavones	RCT with 3 groups; placebo, HRT, phytoestrogens; 325 postmenopausal women	HRT group (1 mg oestradiol and 0.5 mg norethisterone acetate p.o. daily, phytoestrogens group (40% standardized extract with 20 mg soy isoflavones (genistein and daidzein), two capsules = 40 mg p.o. daily; duration: 12 months	no significant differences between the effectiveness of the HRT and phytoestrogen in terms of effects on BMD and bone resorption	Tit 2018 [34]
Soy isoflavones	double-blind RCT with 3 groups: placebo, soy protein, soy protein + isoflavone in snack bar; 200 women within 2 years of the onset of their menopause	placebo (isoflavone of less than 300 parts per billion) PI (15 g soy protein with 66 mg of isoflavones), SP (15 g soy protein alone, isoflavone free) daily, 100 women/group; duration: 6 months	↓CTX with SPI supplementation compared to SP, ↓ P1NP with SPI supplementation	Sathyapalan 2017 [35]
Soy isoflavones	double-blind RCT with 2 groups: placebo, isoflavones in form of tablet	placebo (0 mg of isoflavones), isoflavones extracted from soy protein (200 mg daily = 4tablets) 248 multi-ethnic menopausal women aged 45 to 60; duration: 2 years	not superior to placebo in preventing bone loss or in reducing bone turnover or menopausal symptoms in women in the first 5 years of menopause	Levis 2011 [36]
Soy isoflavones	double-blind RCT with 2 groups: placebo, phytoestrogens; 202 postmenopausal women aged 60–75	placebo (milk protein), phytoestrogens (25.6 g soy protein containing 52 mg genistein, 41 mg daidzein and 6 mg glycetein (aglycone weights; duration: 12 months	no significant differences for BALP, calcium, and phosphorus measurements.	Kreijkamp-Kaspers 2004 [37]

Botanicals	Population and Design	Intervention	Outcome	Authors and References
Soy isoflavones	double-blind, multicentre RCT with 2 groups: isoflavone-enriched biscuits and bars and control biscuits and bars; 237 early postmenopausal women aged 53 ± 3y	placebo group (biscuits and cereal bar), isoflavone- enriched foods (soy isoflavone concentrate containing 40–50% of isoflavones) providing a mean daily intake of 110 mg isoflavone aglycones/day; duration: 12 months	isoflavone-enriched products did not alter lumbar and total body BMD or markers of bone formation and bone resorption	Brink 2008 [38]
Genistein	double-blind RCT with 2 groups: placebo, genistein; 389 postmenopausal women	placebo group (calcium and vitamin D, *n* = 191), genistein aglycone group (54 mg/day + calcium and vitamin D, *n* = 198) duration: 36 months	↑lumbar and femoral BMD, ↓bone resorption markers (DPD, CTX, RANKL), ↑ bone formation markers (BALP, IGF-1 and OPG)	Marini 2007 [39]; Marini 2008 [40]
Genistein	double-blind RCT with 2 groups: placebo, genistein; 138 postmenopausal women (age 49–67 years)	placebo (0mg of isoflavones, *n* = 67), genistein (54 mg/day, *n* = 71), duration: 24 months	↑ femoral and lumbar BMD, improvement of the quantitative ultrasound parameters (stiffness index, amplitude-dependent speed of sound, and bone transmission time)	Atteritano 2009 [41]
Genistein	double-blind RCT with 2 groups: placebo, geniVida™ bone blend group; 70 postmenopausal women	placebo (calcium only, *n* = 28), genistein group = 30 mg/daygenistein + vitamin D3 (800 IU/days) + vitamin K1 (150 µg/days) + polyunsaturated fatty acids (1 g polyunsaturated fatty acids as ethyl ester: eicosapentaenoic acid/docosahexaenoic acid ratio = ~2/1, *n* = 30); duration: 6 months	↑ BMD, ↑ BALP and NTX	Lappe 2013 [42]
Genistein	double-blind RCT with 2 groups: placebo, genistein, 121 postmenopausal women	placebo (1000 mg of calcium and 800 IU vitamin D3; *n* = 59), genistein aglycone group (54 mg/day + calcium, vitamin D3; *n* = 62, duration: 24 months	↑ femoral and lumbar BMD, ↑ BALP	Arcoraci 2017 [43]

Botanicals	Population and Design	Intervention	Outcome	Authors and References
Red clover isoflavones (genistein, daidzein, formononetin, biochanin A)	double-blind RCT with 4 groups: placebo, red clover isoflavone preparation (Rimostil) in 3 doses, 46 postmenopausal women	placebo, Rimostil (phytoestrogens)—28.5 mg, 57 mg, or 85.5mg/day, duration: 6 months,	↑ BMD after 57 mg and 85.5 mg/day	Clifton-Bligh 2001 [44]
Red clover isoflavones	double-blind RCT with 2 groups: placebo, isoflavone supplement Promensil®; 205 pre-, peri-, and postmenopausal women aged 49–65	placebo, isoflavone supplement (providing 26 mg biochanin A, 16 mg formononetin, 1 mg genistein, 0.5 mg daidzein/daily); duration: 12 months	↑ bone formation markers (BALP, P1NP), ↓ lumbar spine BMC and BMD	Akinson 2004 [45]
Red clover isoflavones	double-blind, parallel RCT with 2 groups: placebo, red clover extract; 78 postmenopausal osteopenic women supplemented with calcium 1200 mg/day, magnesium 550 mg/day, calcitriol 25 µg/day	placebo, red clover extract (60 mg isoflavone aglycones/day + probiotics); duration: 12 months	↓ lumbar and femoral BMD loss, ↓ CTX	Lambert 2017 [46]
Red clover isoflavones	double-blind RCT with 2 groups: placebo, red clover extract; 60 menopausal women	placebo, red clover extract (daily dose of 150 mL containing 37.1 mg isoflavones = 33.8 mg as aglycones); duration: 12 weeks	↑ spinal BMD	Thorup 2015 [47]
Red clover isoflavones	double-blind RCT with 2 groups: placebo, standardized red clover isoflavone dietary supplement (Promensil®); 401 healthy women aged 35–70 years	Placebo, red clover isoflavones (40 mg/day); duration: 36 months	safe and well tolerated but no effect on BMD	Powles 2008 [48]

Botanicals	Population and Design	Intervention	Outcome	Authors and References
Red clover isoflavones	double-blind RCT with 3 groups: placebo and 2 dietary supplements derived from red clover, 252 menopausal women ages 45–60 years	placebo, Promensil® (82 mg total isoflavones), Rimostil® (57.2 mg total isoflavones), duration: 12 weeks	no effect on bone turnover markers.	Knudson Schult 2004 [49]
Kudzu root (*Pueraria candollei var. mirifica*)	double-blind RCT with 4 groups: placebo, 3 dose of Pueraria; 71 postmenopausal women aged 45 to 60 years	placebo (*n* = 20), Pueraria mirifica in capsules (20, 30, or 50 mg once daily, *n* = 51); duration: 24 weeks	↓ BALP	Manonai 2008 [50]
Kudzu root (*Pueraria candollei* var. *mirifica*)	double-blind RCT with 2 groups 19 postmenopausal women	placebo tablet, tablet containing 25 mg dried PM root powder, 4 tablets/day; duration: 2 months	↓ ALP	Okamura 2008 [51]
Epimedium	double-blind RCT with 2 groups: placebo, Epimedium-derived phytoestrogen flavonoids (EPF), 100 healthy late postmenopausal women	placebo (*n* = 50), EPF group (*n* = 50; a daily dose of 60 mg Icariin, 15 mg daidzein, and 3 mg genistein), +300 mg calcium daily for both group; duration: 24 months	↑ lumbar and femoral BMD, ↓ DPD,	Zang 2007 [52]
Dried plums	RCT with 2 groups: placebo (dried apples), dried plums; 58 postmenopausal women	placebo (dried apples 75 g daily), dried plums (100 g daily); duration: 3 months	↑IGF-1, ↑ ALP, ↑ BALP	Ajamandi 2002 [53]
Dried plums	RCT with 2 groups: placebo, dried plums, 160 postmenopausal women with osteopenia	placebo (dried apples 75 g daily), dried plums (100 g daily) + 500 mg Calcium, 400 IU (10 µg) vitamin D daily for both group; duration: 12 months	↑ ulnar and lumbar BMD, ↓ BALP	Hooshmand 2011 [54]
Dried plums	RCT with 3 groups: placebo, 2 dose of dried plums, 48 older postmenopausal women	control (0 g/day dried plum), dried plum (50 or 100 g/day dried plum), duration: 6 months	↑ BMD, ↓ TRAP-5b, ↑ BALP/TRAP-5b ratio	Hooshmand 2016 [55]

Botanicals	Population and Design	Intervention	Outcome	Authors and References
Dried plums	RCT with 3 groups: placebo, 2 dose of dried plums; 35 men between the ages of 55 and 80 with moderate bone loss	control group (0g prunes), 100 g prunes daily, 50 g prunes daily, + multivitamin containing 450 mg calcium and 800 IU vitamin D for all group, duration: 3 months	↓ osteocalcin, ↑ OPG/RANKL ratio	Ajmandi 2020 [56]
Horsetail (*Equisetum arvense*)	Double blind RCT with 4 groups: control, placebo + horsetail extract, horsetail extract, calcium, 122 women in menopause for at least two years	no treatment/control group (*n* = 29), placebo for 40 days and titrated horsetail extract for a further 40 days (*n* = 31), titrated dry horsetail extract for 80 days (*n* = 30); Calcium (Osteosil®) for 80 days (*n* = 32), After the 80-day initial study period, patients treated with titrated horsetail extract and with calcium continued treatment for one year	↑ in the average densitometric values for the vertebra	Corletto 1999 [57]
Black cohosh (*Cimcifuga racemosa*)	double-blind RCT with 3 groups: placebo, black cohosh, oestrogens; 62 postmenopausal women	placebo, black cohosh (40 mg of herbal drug/day), conjugated oestrogens (0.6 mg/day); duration: 12 weeks.	↑ osteoblast activity, weak estrogen-like activity, no significant effects on coagulation markers and liver enzymes	Wuttke 2006 [58]
Black cohosh (*Cimcifuga racemosa*)	prospective clinical trial with 2 groups: untreated control, isopropanolic extract of *Cimicifuga racemosa*, 82 postmenopausal women	control group (*n* = 37), isopropanolic extract of *Cimicifuga racemosa* (Remifemin®, 40 mg/day, *n* = 45), duration: 3 months	↓NTX (marker of bone resorption), ↑ ALP (marker of bone formation)	Garcia-Pérez 2009 [59]
Black cohosh (*Cimcifuga racemosa*)	RCT with 3 groups: control (CG), exercise group (EG), exercise and *Cimicifuga racemosa* (CR) supplementation group (EGCR), 128 early postmenopausal women	CG (wellness control, *n* = 42), EG (*n* = 43), EGCR (40 mg/day of CR BNO 1055; *n* = 43), Calcium (1500 mg/d) + vitamin D (500 IE/d) supplementation for all participant duration:12 months	CR (CR BNO 1055) did not enhance positive effects of exercise on BMD at the lumbar spine	Bebenek 2010 [60]

Botanicals	Population and Design	Intervention	Outcome	Authors and References
Labisia pumila and *Eurycoma longifolia*	double-blind RCT with 2 groups: placebo, Nu-femme™, 119 healthy women aged 41–55 years experiencing peri-menopausal or menopausal symptoms	placebo (*n* = 59), herbal formulation (Nu-femme™, *n* = 60) = 200mg Labisia pumila (SLP+®) + 50mg Eurycoma longifolia (Physta®); duration: 24 weeks	No significant effect on bone formation (BALP) and resorption (NTX) markers	Chinnappan 2020 [61]

ALP—alkaline phosphatase, BALP—bone-specific alkaline phosphatase, BMC—bone mineral content, BMD—bone mineral density, CTX—type I collagen crosslinked beta C-telopeptide, DPD—deoxypyridinoline, HRT—hormonal replacement therapy, IGF-1– insulin-like growth factor 1, NTX—type I collagen crosslinked N- telopeptide, OPG—osteoprotegerin, P1NP—type I procollagen-N-propeptide, RANKL—Receptor Activator for Nuclear Factor κB Ligand, SSI—strength strain index, ↑—increased, ↓—decreased

2. Phytoestrogens

Phytoestrogens are naturally occurring nonsteroidal plant compounds that resemble oestrogens and have oestrogenic and/or antiestrogenic activity. They can be divided into two main groups: flavonoids and non-flavonoids. Isoflavones, coumestans, and prenylflavonoids belong to flavonoids, and lignans belong to non-flavonoids [62].

2.1. Isoflavones

Isoflavones are phenolic compounds that belong to the most estrogenic plant-derived substances. Their chemical structure is similar to that of oestradiol. They include, among others, genistein, daidzein, glycitein, biochanin A, and formononetin (Table 3). The main source of isoflavones are legumes belonging to *Fabaceae*: soybean (*Glycine max*) as a source of genistein, daidzein, and glycitein, and red clover (*Trifolium pratense*) as a source of biochanin A and formononetin [62]. In the group of plants containing isoflavones, there are also alfalfa (*Medicago saltiva* L.), beans (green bean, mung bean), psoralea (*Psoralea corylifolia*) and *kudzu* root (*Pueraria lobata* L.) [14]. In the human gastrointestinal tract formononetin, contained in dietary supplements based on red clover, is transformed to daidzein [63]. The amount of isoflavones in soybeans ranges from 1.2 to 4.2 mg per g of dry weight, whereas in red clover, it ranges from 10 to 25 mg per g of dry weight [14]. Isoflavones exert the biologic effect due to two different mechanisms. On the one hand, they act through the classical oestrogen receptor (ER)-mediated signalling pathway, but additionally, it has been described that they may activate intracellular pathways such as protein tyrosine kinase, phospholipase C and MAPK [14]. As most isoflavones are ERβ-selective ligands, it can be supposed that they selectively target bone cells without having an undesired influence on other oestrogen-sensitive tissues, such as the breast and the uterus.

Table 3. Four chemical forms of main isoflavones.

Aglycones	Glycosides	Acetylglycosides	Malonyl Isoflavone Glycosides
Daidzein	Daidzin	Acetyldaidzin	Malonyldaidzin
Genistein	Genistin	Acetylgenistin	Malonylgenistin
Glycitein	Glycitin	Acetylglycitin	Malonylglycitin
Biochanin A	Sissostrin		Malonylsissostrin
Formononetin	Ononin		Malonylononin

Aglycones	Glycosides	Acetylglycosides	Malonyl Isoflavone Glycosides
Daidzein	Daidzin	Acetyldaidzin	Malonyldaidzin

2.2. Other Plants Containing Phytoestrogens Investigated in Osteoporosis Treatment

2.2.1. Epimedium (Berberidaceae)

Epimedium in Clinical Trials

Epimedium is a genus of about 52 species in the family *Berberidaceae*, which is also known as Rowdy Lamb Herb, Xianlinpi, Barrenwort, Bishop's Hat, Fairy Wings, Horny Goat Weed, and Yangheye or Yin Yang Huo). The traditional Chinese medicinal herb Epimedii has been utilized for centuries to treat bone fractures, bone loss, and menopause-associated disorders [64]. The results of recent clinical trials have reported suggest that compounds or extracts of Epimedium may prevent or delay the onset of osteoporosis and reduce the risk of hip fractures [21]. Icariin is a prenylated flavonol glycoside isolated from Epimedium herbs, and has been shown to be the main bioactive component [16]. In clinics, Epimedium is used to treat osteoporosis, climacteric period syndrome, breast lumps, hyperpiesia, and coronary heart disease [65].

In a 24-month double-blind RCT in healthy, late postmenopausal women, the intervention group (n = 50, a daily dose of 60 mg icariin, 15 mg daidzein, and 3 mg genistein) had a significantly reduced bone loss compared to the placebo group (n = 50). Treatment with icariin maintained BMD at 12 months. A long-term (up to 12–24 months) administration of icariin improved BMD in the lumbar spine and femoral neck in a time-dependent manner. Although the effect of icariin is less effective in the improvement in BMD than oestrogen replacement or treatment with bisphosphonates, it seems to be an attractive alternative therapy due to its low risk of severe side effects. It exerted no oestrogenic effect on the uterus and did not change the serum estradiol level, proving its safety when it comes to the endometrium. A 2-year-long treatment with icariin was also not associated with the incidence of breast cancer or cardiovascular events [52]. Further clinical trials encompassing a larger population are needed to investigate the influence of icariin and its derivatives on bone formation and regeneration in humans, as well as its safety profile [16].

Epimedium in Animal Models and In Vitro Studies

Epimedium flavonoids (icariin, epimedin B, and epimedin C), that possess oestrogenic activity, have been identified as the main constituents of Epimedium plants that exert antiosteoporotic activity, as they inhibit bone resorption, promote bone formation and block urinary calcium excretion [21]. The flavonoids from *Epimedium* promote osteoblast activity through the regulation of the expression of IL-6 (interleukin 6), OPG, RANKL, M-CSF, BMP-2, and Smad4. They modulate the BMP/Smad4 and Wnt/β-catenin signalling pathways, inducing osteoblast differentiation [66]. Icariin is the most abundant flavonoid in *Herba Epimedii* and has a better antiresorptive effect than other components isolated from *Epimedium* plants. It stimulates bone formation by the promotion of osteoblasts differentiation and the enhancement of their activity [16][67]. Icariin activates BMP-2/Smad4, Wnt, and IGF-1 signal transduction pathways [5][17], induces ERK (extracellular signal-regulated kinase), JNK (c-Jun N terminal kinase) and p38 kinase activation [18]. Icariin not only promotes bone formation, but also inhibits osteoclast differentiation and bone resorption. It decreases RANKL-induced osteoclastogenesis via the modulation of NFκB and MAPK expression and downregulation of main regulators of osteoclastogenesis (c-fos and NFAT-c1—nuclear factor of activated T-cells, cytoplasmic 1) [19]. Micro-CT results suggest that icariin improves the bone parameters (BMD, bone volume/tissue volume—BV/TV, connectivity density—Conn.D) and restores bone structure in ovariectomized animals [68]. Ikarisoside A, a flavonoid isolated from *Epimedium koreanum*, also inhibits RANKL-induced osteoclastogenesis [66].

2.2.2. Hop (*Humulus lupulus* L.)

Hop (*Humulus lupulus* L.), which belongs to the Cannabaceae family, has been used worldwide in the brewing industry as a source of bitterness in beer. Apart from this, hop extract is known for containing phytoestrogen components and exerting oestrogenic effects. In general, compounds of the oestrogenically active fraction of lupulin gland secretion belong in the following prenylflavonoids: xanthohumol, being the most abundant prenylflavonoid in hops, izoxanthohumol, 6-prenylnaringenin and 8-prenylnaringenin [69]. Moreover, 8-prenylnaringenin has stronger oestrogenic properties than soy phytoestrogens [70]. Ban et al. reported that hop extract Lifenol® prevented osteoporosis development in ovariectomized rats [71]. Hop extract ameliorated the ovariectomy-induced decreased of BMD, femur weight, and BMC (bone mineral content). Additionally, it restored the trabecular structure of calcaneus bone and inhibited ovariectomy-induced osteoclast activation. A mild osteoprotective effect of hop extract was also reported by other authors [72]. Li et al. reported that

xanthohumol blocks RANKL-induced osteoblast differentiation and bone resorption, in vitro and in vivo, in ovariectomized mice [73]. At the molecular level, it blocks the RANKL/TRAF6 (tumour necrosis factor receptor associated factor 6) signalling pathway involved in osteoclastogenesis. Additionally, xanthohumol stimulates osteogenic marker gene expression in mesenchymal and pre-osteoblastic cells [74]. Furthermore, 8-prenylnaringenin, that is, the strongest phytoestrogen known, similarly to soy phytoestrogen, exerts its osteoprotective effect through ERs. It inhibits RANKL expression and induces the expression of ostoprotegerin (OPG), which is an inhibitor of osteoclast activity [75].

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