Medicinal Plants for the Management of Post Menopausal Osteoporosis: A Review

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Abstract: Osteoporosis, a silent epidemic has become a major health hazard in recent years. Osteoporosis which increases bone fragility and thereby the risk of fractures is associated with high mortality, morbidity and high medical expenses throughout the world. Though ovarian hormone deficiency is a major risk factor for osteoporosis in the postmenopausal women, hormone replacement therapy (HRT), perhaps the most effective treatment, is not preferred as it increases the risk of breast cancer and of cardiovascular diseases. The other available therapeutic agents are also associated with certain adverse effects. In this context, phytoestrogens are believed to play a role in maintaining or improving skeletal health. The present work reviews scientific information on medicinal plants which have already been documented for their antiosteoporotic activity. These plants may differ from each other in their mechanisms of action, they either bind with estrogen receptors which exhibit responses at the cellular and molecular levels, or in some cases they act by improving defense against oxidative stress". The review which covers 18 plants briefly discusses their morphology, family, common name, phytoconstituents and proposed mechanism of action.

Keywords: Antiosteoporotic, hormone replacement therapy (HRT), phytoestrogens, medicinal plants, postmenopausal osteoporosis.

INTRODUCTION

Osteoporosis, a silent epidemic has become a major health hazard in recent years, afflicting over 2000 million people worldwide [1]. It is a major growing health problem for elderly women associated with ovarian hormone deficiency following menopause and is by and far the most common cause of age related bone loss in women. According to the WHO "Osteoporosis is a disease characterized by low bone mass and microarchitectural deterioration of bone tissues, leading to enhanced fragility and consequent increase in fracture risk that results in fractures with minimal trauma".

Osteoporosis is one of the most widespread metabolic bone disorders [2], affecting one in three women and one in twelve men at some point in their lives [3]. The risk of fracture has been reported to increase with age in humans [4]. A sharp decrease in ovarian estrogen production is the predominant cause of rapid, hormone-related bone loss during the first decade after menopause [5], as a result of higher bone turnover, an imbalance between bone formation and bone resorption & net bone loss [6].

The common sites of fracture among postmenopausal women include the vertebrae, forearm and hip. As the population ages, the incidence of hip fractures & cost for treatment will rise dramatically in the future, unless effective prophylactic measures are taken [7]. The projected cost of osteoporotic fractures in white postmenopausal women during the next 10 years in the United States alone is expected to be more than $45 billion [8]. Some epidemiological data suggests that in USA, 10 million individuals already have osteoporosis and 18 million have osteopenia making it to a total of 28 million, American women, however, are four times more likely to develop osteoporosis than men [9]. The incidence in Europe is projected to double in the next 50 years, and the incidence in Latin America is also expected to rise significantly [10]. Globally, osteoporosis is highest in Whites and Asians, and lowest among Blacks. Blacks have more bone density than other racial groups, lowering their risk of osteoporosis. Hispanic-American women have somewhat greater bone density than do non-Hispanic whites [11]. In India, based on 2001 census, approximately 163 million Indians are above the age of 50 and this number is expected to increase to 230 million by 2015 [12]. Even conservative estimates suggest that, of these, 20 per cent of women and about 10-15 per cent of men would be osteoporotic [13].

Several factors such as genetic, nutritional and lack of exercise etc., along with aging have been shown to be risk factors in the aetiology of osteoporosis [13]. With aging, however, an erratic absorption of calcium from gut disturbs the calcium homeostasis leading to an imbalance in the calcium regulating hormones (parathyroid hormone and calcitonin) and thereby increase bone turnover [14]. Osteoblastic activity and calcium absorption from the gut also suffers with the age [15]. In addition to menopause and aging, hereditary factors, lack of exercise or immobilization, lifestyle, prolonged steroid administration, excessive diet,

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alcohol intake, smoking, thyroxin therapy and geographical variations are the major causes of osteoporosis, among which lifestyle changes, diet and oestrogen deficiency are modifiable factors, whereas hereditary factors are non modifiable [16]. Genetic factors responsible for the onset of osteoporosis can be related to family history, small body frame, skin type, low stature, early grey hair and white women [17]. In men, osteoporosis can be linked to decreased testosterone levels or loss of long term remodeling efficiency [18]. Long-term drug therapy with corticosteroids, cyclosporins, cytotoxins or certain anticonvulsants like phenytoin are the prime candidates for osteoporosis [16].

Osteoporosis is a silent disease, reflected only in a low bone density, till a fracture occurs. Much in the manner that asymptomatic conditions such as hypertension and dyslipidaemia predispose to stroke and myocardial infarction, respectively, a low bone density (reflecting poor bone health) predisposes to osteoporotic fractures [13]. It is best diagnosed by the estimation of bone mineral density and bone mineral content by Duel energy X-ray absorptiometry (DXA) or peripheral quantitative computed tomography. The diagnosis of this silent disease, in the underdeveloped and developing countries, however, is largely made only after a fracture occurs due to a low bone density. In India the first DXA became available only in 1997 in Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, subsequently few other hospitals/institutions also acquired the instrument and thus presently only about 200 instruments are available in entire country of 1.14 billion people. Therefore it is inaccessible to a large segment of the Indian population [13].

The earlier treatment regimens for postmenopausal osteoporosis suggested prevention by classical hormone replacement therapy (HRT) which has today become obsolete. As HRT increases the risk of breast cancer and of cardiovascular diseases, compliance is low. Recently, a clinical trial with HRT in healthy postmenopausal women was stopped by the American National Institute of Health as the increase of cardiovascular and mammary cancer risks under HRT far outweighed the benefits, namely a reduction of hip fractures (antiosteoporotic effect) and of colon cancer [19]. Besides HRT, many pharmacological agents, used to manage the osteoporosis act by decreasing the rate of bone resorption, thereby slowing the rate of bone loss or by promoting bone formation. Synthetic agents like calcium carbonate (calcium consumption), vitamin D supplements, Raloxifene and Droloxifene (selective estrogen receptor modulators), calcitonin, bisphosphonates, sodium fluoride (increases trabecular bone mineralization), along with physical activity to strengthen muscles, stimulate osteoblast formation and prevent resorption [18]. They are also however associated with side effects such as hypercalcemia, hypercalciuria, increased risk of endometrial and breast cancer, breast tenderness, menstruation, thromboembolic events, vaginal bleeding, hot flashes, dyspepsia and gastrointestinal ulcers [20-22] further, the lack of direct head to head trials of treatments for osteoporosis, with reduction in fractures as an end point, makes it difficult to determine the relative efficacy of the different treatments [13].

To overcome the wide range of side effects produced by these synthetic drugs, there is an increasing demand for 'green medicines' which are thought to be healthier and safer for the treatment of osteoporosis. The phytoestrogens, which are known to bind to the estrogen receptor sites of the cell and trigger the components and processes of estrogenic activity, have a promising role in the treatment of osteoporosis [23]. The isoflavonoids are among the most active phytoestrogens in the flavonoid class. Ipriflavone, a synthetic flavonoid derivative [24] has been found to be effective in preserving bone mass in several models of experimental osteoporosis [25]. The isoflavones found in soybeans, such as genistein, were found to prevent bone loss in the ovariectomized rat model of osteoporosis [26, 27].

The present work has been undertaken to present scientific information on medicinal plants which have been documented for their antiosteoporotic activity. These plants may differ from each other in their mechanisms of action, they either bind with estrogen receptors which exhibit responses at the cellular and molecular levels, or in some cases they act by improving defense against oxidative stress [28, 29].

(1) HADJOD (*CISSUS QUADRANGULARIS* LINN.)

Cissus quadrangularis (Vitaceae), a rambling shrub, characterized by a thick quadrangular fleshy stem, is an edible plant found in hotter parts of India, Sri Lanka, Malaya, Java and West Africa. Commonly known as the "bone setter," the plant is referred to as "Asthisamdhani" in Sanskrit and "Hadjod" in Hindi because of its ability to join bones [30]. The stem juice is used to treat scurvy and irregular menstruation, the plant juice in otorrhoea and epistaxis while the root is specific for bone fracture. Nadkarni describes the root as most useful for the fractures of bones, with the same effects as plaster externally [31]. In earlier studies the fracture healing property of the plant extract [32] isolation of a phytoestrogenic steroid and its ability in influencing early regeneration and quick mineralization of the callus [33] were reported.

Modern research has shed light on the ability of *C. quadrangularis* to hasten bone healing by acting as a glucocorticoid antagonist [34, 35]. Since anabolic/androgenic compounds are known antagonists to the glucocorticoid receptor as well as promoters of bone growth and fracture healing, it has been postulated that *C. quadrangularis* possesses anabolic and/or androgenic properties [32, 34]. In addition to speedening the remodelling process of the healing bone, it also leads to a much faster increase in bone tensile strength and fracture healing time [33]. Its antiglucocorticoid property has also been reported in another study [32], wherein bones were weakened by treatment with cortisol. Upon administration of *C. quadrangularis* extract, the cortisol induced weakening was halted, and the healing process begun.

The ethanolic extract of *C. quadrangularis* was evaluated for its anti-osteoporotic activity in ovariectomized rat model of osteoporosis at two different dose levels. The findings assessed on the basis of biomechanical, biochemical and histopathological parameters showed that the ethanol extract of the plant has a definite antiosteoporotic effect [36]. The alcoholic extract was shown to enhance the development of cortical bone and trabeculae in the foetal femur [37]. The

increased bone formation in the *C. quadrangularis* plant extract treated pups was attributed to the rich calcium and phosphorous present in the plant [38]. The stem extract of this plant contains a high percentage of calcium ions (4% by weight) and phosphorus, both essential for bone growth [38]. Thus the plant *C. quadrangularis* appears to be very useful in treating diseases involving deficiency in the bone formation and fracture healing. The calcium ions, phosphorous and phytoestrogens present in this plant extract may contribute in the process of ossification and fracture healing.

(2) BLACK COHOSH (*CIMICIFUGA RACEMOSA*) [L.] NUTT.

Cimicifuga racemosa, commonly known as black cohosh (Ranunculaceae), a member of the buttercup family is a perennial plant native to North America and has been traditionally used by American Indians for a variety of "female complaints" including menstrual problems and childbirth. At the turn-of-the-last century, black cohosh was used by women to ease "all those painful complaints and weaknesses so common to our best female population" [39]. Next to soy, black cohosh is the most widely studied botanical for menopausal symptoms.

Black cohosh contains triterpene glycosides, flavonoids, aromatic acids, and other numerous constituents [40] but the exact mechanism of action of this botanical has not been clearly understood. Commission E reported estrogen-like action, luteinizing hormone suppression, and binding to estrogen receptors [41].

Three animal studies conducted using black cohosh extracts found no estrogenic increases in uterine weight, stimulation of vaginal cornification, proliferation of the mammary gland or increases in prolactin, FSH, or LH [42-44].

Interestingly, animal studies using ovariectomized rat model of osteoporosis suggest that extracts of black cohosh exhibit protective effects on estrogen deficiency induced bone loss [45]. However, the mechanism of black cohosh on bone cells and the active components responsible remain unclear. Identification of its active compound, acetylcimigenol xylopyranoside (*ACCX)*, as the main constituent which potentially blocks osteoclastogenesis induced by RANKL and TNFα, may account for the bone protection activity of black cohosh. The blockage of osteoclastogenesis elicited by ACCX results from abrogating activation of the NF-κB and ERK pathway induced by either RANKL or TNF. Importantly, the efficacy of this compound on prevention of TNF induced bone loss has been verified *in vivo* [46].

(3) SOYBEAN (*GLYCINE MAX*) (L.) MERR.

The soybean (U.S.) or soya bean (UK) (*Glycine max*), belonging to the Family Fabaceae, is a species of legume native to East Asia. It is an annual plant that may vary in growth, habit, and height. It may grow prostrate, not growing higher than 20 cm (7.8 inches), or even stiffly erect up to 2 meters (6.5 feet) in height. The pods, stems, and leaves are covered with fine brown or grey pubescence.

The oil and protein content together account for about 60% of dry soybeans by weight with protein at 40% and oil at 20%. The remainder consists of 35% carbohydrate and about 5% ash. Soybean cultivars comprise approximately 8% seed coat or hull, 90% cotyledons and 2% hypocotyls. Soy foods and supplements have been the subject of much interest for the reduction of menopausal symptoms because of their high concentrations of phytoestrogens (formononetin, biochanin A, daidzein, and genistein). The three main classes of phytoestrogens are isoflavones, lignans, and coumestans. The phytoestrogens found in soy i.e. isoflavones are beleived to possess estrogenic properties, although the mechanism of action is not fully understood. In a study [47], 50 women were randomized to consume either soy protein isolates (40 g soy protein and 118 mg isoflavone) or placebo, and then measures of hepatic proteins and gonadotropin concentrations were assessed. At the end of three months, there were no differences between the treatment and control group suggesting that soy isoflavones do not affect *in vivo* biological indicators of estrogenicity and most likely act more like Selective Estrogen Receptor Modulators (SERMs). A recent review of the evidence for the treatment of menopausal symptoms suggests that phytoestrogens available as soy foods and soy extracts do not improve hot flashes or other menopausal symptoms [48] and at best, have only minimal effects on hot flashes.

Animal studies show consistent bone conserving effects or improvement in bone mineral density (BMD). The human studies are mixed showing some modest yet significant gains in BMD and bone mineral content [49-51]. However, a recent study in which 25g of soy protein was substituted for meat in the diet showed no improvement of calcium retention, cardiovascular, or bone health indicators in postmenopausal women [52]. Studies of soy in targeted populations, such as postmenopausal Chinese women with lower bone mass, have shown a greater effect on increasing bone mineral content for women consuming a high dose of soy extract as compared to placebo [51].

(4) MACA (*LEPIDIUM MEYENII* WALP.)

Maca (*Lepidium meyenii* Walp.) from the Brassicaceae Family grows exclusively at altitudes over 4000m at the Peruvian central Andes. The hypocotyl, edible part of the plant, has widely been used as a nutritional supplement and folk medicine to increase fertility and sexual function [53]. Maca hypocotyl has been also used to treat women with menopausal symptoms including hot flushes, tender breasts, vaginal dryness, osteoporosis, etc. The Maca alkaloids, steroids, glucosinolates, isothiocyanates and macamides are probably responsible for its aphrodisiac, adaptogen, anabolic, immunostimulant and hormonal balance properties. A study showing the aphrodisiac effects of Maca in both animals and humans have appeared over the last few years [54-56].

The ethanol extract of Maca was effective in the prevention of estrogen deficient bone loss in postmenopausal osteoporosis in ovariectomized rats. The findings derived on the basis of bone mineral density, biomechanical, biochemical and histopathological parameters indicated that the higher dose of ethanol extract of Maca was effective [57].

(5) PILA BHRINGA (*WEDELIA CALENDULACEA* LESS.)

Wedelia calendulacea (Less.) or *W. chinensis* known also as pila bhangra is a perennial herb with erect stems, 20-40 cm. high, with bright yellow flowers and a light, camphor-like odor. Leaves are opposite, subsessile, coarsely toothed, with coarse hairs on both sides. Inflorescence is axillary and terminal, solitary head on long stalk, flowers yellow with the outer flowers linear and the inner tubular. The fruit is an achene. It grows wild in wet places and is propagated by seeds. The plant is abundantly found in India, and is used traditionally as a cholagogue and deobstruent in hepatic enlargement and jaundice. A decoction of the herb is used in uterine hemorrhage and menorrhagia. The leaves contain as its principal constituent, isoflavanoids and wedelolactone, which is analogous in structure to the clover estrogen coumestrol [58].

Shirwaikar *et al* studied the antiosteoporotic effect of the ethanol extract of *W. calendulacea* in the ovariectomized rat model of osteoporosis, at two different dose levels of 500 and 750 mg/kg body wt. The findings, assessed on the basis of biomechanical and biochemical parameters, showed that the ethanol extract of the plant had a definite protective effect. This was further supported by the histopathological studies. They suggested that the presence of isoflavones and wedelolactone, which are known to act as phytoestrogens may be responsible for the antiosteoporotic activity [59].

(6) FOETID BUGBANE (*CIMICIFUGA FOETIDA*)

Cimicifuga foetida or *Cimicifuga europaea* or *Actaea cimicifuga* (L.) (Ranunculaceae) is commonly known as foetid bugbane. It is found in shrubberies and forest clearings on open humus-rich soils up to 4000 meters in the Himalayas and is frequently found in the fir forests of Kashmir. It is a perennial growing plant which flowers from July to August. The scented flowers are hermaphrodite. The plant prefers woodland, dappled shade and shady edges for favorable growth. Though a very attractive plant, the flowers and the green seed pods have an unusual, slightly unpleasant smell that is reminiscent of decaying fish.

Chemical constituents isolated from *C. foetida* include Cimicifoetisides A and B, two cytotoxic cycloartane triterpenoid glycosides from the rhizomes which inhibit proliferation of cancer cells [60]. Increasing research suggest that Cimicifugae rhizoma might be protective against osteoporosis. Three cycloartane-type triterpenoids isolated from Cimicifugae rhizome includes cimicidol-3-O-β-d-xyloside, cimicidanol-3-O-β-d-xyloside and acetylacteol-3-O-β-d-xyloside have been observed to exhibit effective results on bone resorption *in vitro* and on bone loss in ovariectomized (OVX) mice [61].

The antiosteoporotic effect was also confirmed by studying the biomechanical characteristics of the femur (bone density, mineral content and three point bending test) and uterine index in ovariectomized rats. The *C. foetida* extract exerted estrogenic effects in the bone, particularly on osteoblasts, while it didn't play a role in the uterus of ovariectomized rats. The extract appeared to contain rat organ-specific SERMs, and if these findings can be ascertained in humans, it may be a good alternative to hormone replacement therapy (HRT) [62].

(7) RED CLOVER (*TRIFOLIUM PRATENSE*)

Trifolium pratense (Fabaceae) commonly known as red clover is a species of clover, native to Europe, western Asia and northwest Africa, but planted and naturalized in many temperate areas including the Americas and Australia. Pratense is Latin for "found in meadows". It is a herbaceous perennial plant, very variable in size, growing to about 20-80 cm tall. *T. pratense* is a familiar meadow herb, with a reddish stem, three oval leaflets which grow on alternate sides of the stem and a purple ovoid flower head [63]. It is widely grown as a fodder crop, valued for its nitrogen fixation as it increases soil fertility. For these reasons it is used as a green manure crop.

In the homeopathic Materia Medica, *T. pratense* is said to stimulate the secretion of the salivary glands, to be beneficial in mumps and affections of the pancreas and to have anticancer effects [64]. Modern studies confirm the antitumoral, anti-inflammatory and oestrogenic effects of *T. pratense*. The isoflavones biochanin A and genistein present in the leaves have oestrogenic activity. Methanol extract of *T. pratense* shows significant competitive binding to oestrogen receptors alpha and beta. In cultured Ishikawa (endometrial) cells, *T. pratense* exhibited oestrogenic activity, as indicated by induction of alkaline phosphatase activity and up-regulation of progesterone receptor activity. In S-30 breast cancer cells, pS2 (presenelin-2) another oestrogen-inducible gene, was up-regulated in the presence of *T. pratense* [65].

Total isoflavones isolated from *T. pratense* were fed to ovariectomized rats at an oral dose of 20 and 40 mg/kg body weight for 14 weeks. Treatment with isoflavones significantly increased bone mineral content, mechanical strength of the tibia, femoral weight, femoral density and prevented the rise of serum alkaline phosphatase levels. In addition, the treatment with isoflavones significantly reduced the number of osteoclasts as compared to the ovariectomized control rats. The findings suggested that *T. pratense* isoflavones are effective in reducing bone loss induced by ovariectomy, probably by reduction of the bone turnover via inhibition of bone resorption [66].

(8) JAPANESE PAGODA TREE (*SOPHORA JAPONICA*)

Sophora japonica or *Styphnoloium japonicum* (L.) Schott (Leguminosae), commonly known as the Japanese pagoda tree is one of the 50 fundamental herbs of the Traditional Chinese medicine system [67]. *S. Japonicum* though native to eastern Asia, is a popular ornamental tree in Europe, North America and South Africa. It is grown for its white flowers, borne in late summer after most other flowering trees have long finished flowering. It grows into a lofty tree 10-20 m tall with an equal spread, and produces a fine, dark brown timber.

It has abortifacient, antibacterial, anticholesterolemic, anti-inflammatory, antispasmodic, diuretic, emetic, emol-

lient, febrifuge, hypotensive, purgative, styptic, and tonic properties [67, 68].

Genistein, a most potential and naturally occurring isoflavonoid, isolated from *Sophora japonica* was studied for its anti osteoporotic activity on ovariectomized Sprague-dawley rats with different doses viz. 4.5, 9 and 18 mg/kg body weight for 12 weeks. The animals treated with genistein at a dosage of 4.5 mg/kg or 9 mg/kg had a significantly ($p<0:01$) higher density of femur and tibia than the control group, especially at the 4th week after treatment. The low dosage treated Genistein group had higher bone calcium, while the other treatment groups had higher bone phosphorous and magnesium ($p<0:05$). In addition, the OVX rats treated with low to medium dosages of genistein displayed higher trabecular thickness, trabecular area percentage and trabecular number. Results indicated that, in comparison to the anti-osteoporotic effects of soybean genistein, treatment with a low or medium dosage of genistein from Sophora japonica could also prevent osteoporosis [69].

(9) HERBA EPIMEDII (*EPIMEDIUM BREVICORNIUM* MAXIM)

Epimedium brevicornium Maxim commonly known as Herba epimedii (HEP) is one of the most frequently used herbs in formulae that are prescribed for the treatment of osteoporosis in China. The plant grows mainly on mountainous areas and is distributed throughout China. It is pungent in nature and sweet in flavour [70]. Herba epimedii is traditionally used to relieve stress and fatigue. Chinese folk healers were reported to use 100-200g (10 times the common modern clinical dose) of Herba epimedii decocted with squid and red wine as a treatment for bodily fatigue and lack of strength due to overtaxation [71].

Icarin is the marker compound present in the plant [72]. Besides Icarin, other compounds reported from the plant are linoleic acid, oleic acid, palmitic acid, sterols, tannins, vitamin E, and flavonoids. Icarin and other flavonoids present in the plant demonstrated proliferation and differentiation of osteoblast *in vitro* [73, 74].

Aqueous extract of herba epimedii showed positive osteoprotective effect *in vivo and in vitro* [72]. The lowered serum ALP activity, urinary calcium excretion and urinary DPD levels in ovariectomized rats, suggested that Herba epimedii extract could prevent ovariectomy-induced increase in bone turnover rate in rats. In addition, HEP extract could increase trabecular bone area as well as decrease trabecular separation in rat tibia. An *in vitro* study was found to stimulate osteoblastic cell proliferation and differentiation in UMR-106 cells. In addition, HEP extract stimulated the expression of OPG mRNA but suppressed the expression of RANKL mRNA in the UMR-106 cell line, resulting in a dose-dependent increase in the OPG/RANKL ratio. The results suggested that HEP extract might inhibit osteoclastogenesis *via* the modulation of the OPG/RANKL system in osteoblastic cells [72].

(10) MUSHROOM (*PLEUROTUS ERYNGII*)

Pleurotus eryngii or king trumpet mushroom or king oyster mushroom, belonging to Family Pleurotaceae, is an edible mushroom, growing in the greater Mediterranean area, in close association with several genera of plants in the Family Apiaceae [75]. *P. eryngii* has a protective effect on the liver and kidney, and aids in gastrointestinal disorders. Research on mice has suggested that *P. eryngii* might have anti-tumor properties, as well [76].

Mushrooms are fungi belonging to the class Basidomycetes. Mushrooms have been reported earlier for anticancer, immune enhancer, cholesterol-lowering, anti-inflammatory, antithrombotic, and antiviral effects on hypoglycemic and cardiac function [77]. More recently, mushrooms have been reported to have antioxidant properties, as well [78]. Major chemical constituents isolated from the mushroom are polysaccharides. Other constituents include a small amount of heterogalactan protein, the ganoderan A, B, C protein complex, volvatoxin, flamotoxin, ganoderol, ganoderic acid, eritadenin, and some nucleotides [79].

Oral treatment with aqueous extract of *P. eryngii* at the dose of 0.4 ml/day to bilaterally ovariectomized rats for 4 weeks stimulated the activity of bone forming osteoblasts via increasing ALP activity and OPG gene expresion level, while inhibiting the generation and activity of bone resorbing osteoclasts via decreasing the number of number of TRAP (+) multinucleated cells and resorption areas. In addition, it was demonstrated that *P. eryngii* attenuated the progress of bone loss in rats with ovariectomy-induced osteoporosis. Although the active substances of *P. eryngii* have not yet been identified, it is suggested that *P. eryngii* contains substances that have the potential to enhance bone metabolism [80]. An *in vitro* study also confirmed the proliferating and differentiating effect of *P.eryngii* aqueous extract on osteoblast cells [80].

(11) FRUCTUS LIGUSTRI LUCIDI (*LIGUSTRUM LUCIDUM* AIT)

Fructus Ligustri Lucidi (FLL, Chinese name), is the ripe fruit of *Ligustrum lucidum* Ait, an evergreen arbor tree of Family Oleaceae. It grows up to 30 feet in height. Fruits ripen to a dark purple to black color and persist into winter. It has been used in the traditional Chinese medicine for over 1000 years, mainly to treat ailments such as menopausal problems, blurred vision, rheumatic pains, palpitations, backache, and insomnia as well as to alleviate age-related symptoms [81].

Oleanolic acid is a commonly used marker for the authentication of FLL [70]. Besides oleanolic acid the fruit contains nuzhenide, olenropein, manitol, betulin, lupeol, salidroside, oleic acid, linolenic acid, palmitic acid and other fatty acids. In Traditional Chinese Medicine (TCM), it is a commonly prescribed herbal material in a number of formulae used to tone the kidneys and strengthen bones [82]. Modern research has shown that FLL is useful for the prevention of bone marrow loss in cancer patients receiving chemotherapy [68].

Oral treatment with the aqueous extract of FLL at the dose of 550 mg/kg body weight to bilaterally ovariectomized rats for 14 weeks suggested that FLL extract could prevent ovariectomy induced increase in bone turnover by suppression of both serum osteocalcin and urinary deoxypyri-

dinoline levels. In addition, FLL extract prevented ovariectomy induced loss of calcium in rats by increasing the intestinal calcium absorption rate, suppressing urinary calcium excretion as well as by increasing bone calcium content. FLL also demonstrated selective estrogen-like effects on the bone but without the detrimental stimulatory effects in the uterus [83]. FLL improved calcium balance in aged female rats by increasing serum $1,25(OH)2D_3$ level and vitamin D-dependent CaBPs (Calcium binding proteins) expression [84].

Ethanolic extract of FLL improved bone properties in aged rats possibly via its direct action on osteoblastic cells by enhancement of the mineralization process. Treatment of osteoblast like UMR-106 cells with FLL extracts accelerated the formation of calcified matrix and increased extracellular calcium and P depositions in a time- and dose-dependent manner. This finding was confirmed by measuring bone mineral density, bone mineral content and by biomechanical testing of the tibia and femur. Combination of FLL and high calcium diet significantly improved bone mass of cortical and trabecular bone at appendicular bones and LV-2 and decreased bone loss associated with ovariectomy and low calcium feeding [85].

(12) TEA (*CAMELLIA SINENSIS*)

Tea is the most widely consumed beverage aside from water, with a per capita worldwide consumption of approximately 0.12 liter per day [86]. *Camellia sinensis*, a member of the Family Theaceae is the tea plant, whose leaves and leaf buds are used to produce tea. *Camellia sinensis* is native to mainland South and Southeast Asia, but is today cultivated across the world, in tropical and subtropical regions. It is an evergreen shrub or small tree that is usually trimmed to below two meters (six feet) when cultivated for its leaves.Tea is manufactured in four basic forms viz Green tea, White tea, Black tea and Oolong tea. These are processed in differently to attain different levels of oxidation. Of the approximately 2.5 million metric tons of dried tea manufactured, only 20% is green tea, and less than 2% is oolong tea [86].

The major constituents in tea are polyphenols and flavonoids. The four major flavonoids in green tea are the catechins i.e. epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), and epigallocatechin gallate (EGCG). EGCG is richest in the leaf bud and occurs first in the leaves. The usual concentration of total polyphenols in dried green tea leaves is about 8% to 12%. Other compounds of interest in dried green tea leaves include gallic acid, quercetin, kaempferol, myricetin, caffeic acid, and chlorogenic acid [86, 87].

EGCG and ECG were found to be potent inhibitors of influenza virus replication in cell culture. Both effectively suppressed the viral RNA synthesis and inhibited the neuraminidase enzyme activity. Neuraminidase is an antigenic glycoprotein enzyme found on the surface of the influenza virus, which stimulates the efficiency of virus release from cells [88]. EGCG prevents the binding of HIV to human T cells, the first step in HIV infection. Nance [89] demonstrated that EGCG inhibited the binding of human immunodeficiency virus (HIV) to human CD4 (+) lymphocytes, which is a crucial step in HIV infection. The antioxidant properties of green tea may help in the prevention of atherosclerosis, particularly coronary artery disease [90]. It has been suggested that EGCG and other tea catechins suppress tumor promotion by inhibiting the release of tumor necrosis factor alpha (TNF-α), which is believed to stimulate tumor promotion and progression of initiated cells as well as premalignant cells [91]. Adcocks [92] showed that EGCG protects cartilage destruction in test-tube models of cartilage loss mimicking what happens in the arthritic joint.

Aqueous extract of black tea (*Camellia sinensis*) has shown a positive effect on bilaterally ovariectomized rats in the course of 28 days treatment. Investigation with extract treated animals revealed the significant improvement in levels of ALP, TRAP, calcium, phosphate, creatinine, calcium, creatinine and hydroxyproline as compared with control animals. Body weight study revealed that rats in the treatment group showed significant decrease in the final weight as compared to ovariectomized rats, while uterus weight was higher than control animals indicating the estrogenic activity of the plant. Findings were supported by increase in the bone densities of right femur, eighth thoracic rib, eighth thoracic vertebra, and fourth lumbar vertebra in extract supplemented rats as compared to rats in the sham group [93]. Das [94] studied the phytoestrogenic effect of the aqueous extract of *Camellia sinensis* on the oophorectomized rat model of osteoporosis (2.5%, 1 ml/100 g body weight/ day for 28 days). Estrogenic potential was confirmed by signs of revival of copulation period (estrous stage) from non-receptive diestrous stage, after 21 days treatment with the aqueous extract. Serum estradiol level was also observed to increase significantly. This estrogenic potential helped to revert ovariectomy induced bone damage. It was confirmed by assessing marker parameters of bone resorption and osteoclastic activity (tartrate-resistant acid phosphatase), collagen degradation (urinary hydroxyproline), bone loss (bone ash mineral content) and bone breaking strength (bone density).

(13) CHINESE FOXGLOVE (*REHMANNIA GLUTINOSA* LIBOSCH)

Rehmannia glutinosa Libosch or Chinese foxglove belonging to the Family Scrophulariaceae has been widely used as a herbal medicine in traditional oriental medicine for more than 2000 years. It is a perennial herb with reddish-violet flowers native to China, Japan and Korea. It grows on well-drained stony ground along roadsides, in woods, mountain slopes and trailsides from near sea level to 1100 metres.

The steamed root of *Rehmannia glutinosa* is traditionally prescribed to attenuate the clinical manifestation of inner ear dysfunction and various clinical situations, including inflammatory diseases, high fever, night sweat, headache, and dizziness in Oriental Medicine [95]. The roots are also used as a haemostatic, cardiotonic, and diuretic agent. Recently, it has been reported that the herb possesses antibacterial and anti-inflammatory properties [96-98]. Kim [99] demonstrated that *R. glutinosa* Libosch inhibits the secretion of both interleukin-1 (IL-1) and tumor necrosis factor- α (TNF-α) from mouse astrocytes. These cytokines are well known

regulators of bone metabolism [100]. IL-1 is known as a highly potent bone resorptive cytokine. Stashenko [101] reported that TNF-α appears to synergize with IL-1 in its ability to increase bone resorption.

Many constituents have been isolated from both the Rehmanniae Radix (the root of *R. glutinosa* Libosch) and the fresh plant. The major constituents of the herb are β-sitosterol, mannitol, iridoids, phenethyl alcohol, and norcarotenoids. The other constituents include a small amount of stigmasterol and a trace amount of campesterol, catalpol, rehmannin, and vitamin A. In the course of chemical investigation on the dried roots of *R. glutinosa*, a new eremophilane-type metabolite, named remophilanetriol was encountered, together with aeginetic acid [102].

In vitro and *in vivo* studies of the aqueous extract of *R. glutinosa* demonstrated the antiosteoporotic effect of the plant. *In vitro* findings showed a significant increase in both the proliferation and ALP activity of osteoblasts as well as in the expression of the bone-related genes. OPG (osteoprotegerin) secretion was also increased after the treatment. In addition it decreased the number of TRAP (+) MNCs (multinucleated cells) and the resorption areas. Administration of 220 µl aqueous extract solution/day for 4 or 8 weeks to bilaterally ovariectomized rats alleviated the decrease in the trabecular BMD, and increased the cortical bone thickness and trabeculation of the bone marrow spaces [103].

(14) *SAMBUCUS WILLIAMSII* HANCE (SWH)

Sambucus williamsii Hance (Jie gu mu, Chinese name) belonging to Family Caprifoliaceae is a deciduous herb having hermaphrodite flowers. It grows well in heavy clay soils. It can tolerate some shade but grows best in a sunny position. *S. williamsii* is widely distributed in China. The stem and ramulus of *S. williamsii* has been used for centuries for the treatment of inflammation [104], bone fractures and joint diseases by the Chinese people [105].

The major active chemical constituents of the plant are lignans, steroids, triterpenoids, phenolic acid, tianshic acid, and methyl ester of tianshic acid. The latter two compounds possessed stimulating effects on alkaline phosphatase (ALP) activity of UMR-106 cell (about 1.5 fold at 30 µmol/l) but had no effects on cell proliferation [106]. Three new lignans, sambucunol A, sambucunol B, Buddlenol G, along with seven known ones, including (-)-syringaresinol, (-)-pinoresinol, 1, 2-bis(4-hydroxy-3-methoxy phenyl)-1, 3-propanediol, (-)-erythro-1-(4-hydroxy-3-methoxyphenyl)-2-[4-(3-hydroxy propanyl)-2-methoxy phenoxy]-1, 3-propanediol, (-)-threo-1-(4-hydroxy-3-methoxyphenyl)-2-[4-(3-hydroxypropanyl)-2-methoxy phenoxy]-1, 3-propanediol, (-)-lariciresinol and (-)-dihydrodehydrodiconiferyl alcohol, were isolated from the 60% ethanol extract of stems of *S. williamsii* by chromatographic methods. The effects of the isolated compounds on the osteoblast-like UMR-106 cell proliferation and ALP activities were determined. Com-pounds 3, 5 and 10 showed stimulating effects both on UMR-106 cell proliferation and ALP activity. Compounds 1, 4, 6 and 9 stimulated UMR-106 cell proliferation, while 7 and 8 induced ALP activity in UMR-106 cell [107].

In vitro studies of the chloroform and ethyl acetate fractions of SWH inhibited osteoclastogenesis by modulating the expression of osteoprotegrin (OPG) and receptor activity of NF-kB ligand (RANKL) mRNA in osteoblastic UMR-106 cells. Both of them increased OPG mRNA and decreased RANKL mRNA expression, resulting in a dose-dependent increase in OPG/RANKL mRNA ratio ($p<0.01$, *vs*. vehicle-treated) [108]. Oral administration of 60% alcoholic extracts of SWH at the doses of 300 and 600 mg/Kg body weight/day to four-month-old ovariectomized rats for 3 months significantly increased serum calcium levels as well as decreased urinary calcium excretion. The Up- regulation of serum alkaline phosphatase, serum osteocalcin as well as urinary deoxypyridinoline levels by ovariectomy was suppressed significantly by treatment with SWH extracts in rats. However SWH extracts did not alter weight gain and uterus weight in ovariectomized rats. SWH extract increased the stiffness of femur at both doses studied and increased tibial bone mineral density at 600mg/Kg body weight/d in OVX rats. The entire study indicates that SWH treatment can effectively suppress the OVX-induced increase in bone turnover and its effects might be mediated by a decrease in osteoclastogenesis [108].

(15) *ONOBRYCHIS EBENOIDES*

Onobrychis ebenoides belonging to Family Leguminosae is a perennial herb, distributed in central and southern Greece.

Three novel arylobenzofurans namely ebenfuran I, ebenfuran II and ebenfuran III have been isolated and evaluated for their potential selective estrogen receptor modulator (SERM)-like properties. Studies reveal that ebenfuran II is a highly potent SERM, exhibiting antiestrogenic activity in breast cancer cells via the estrogen receptor, estrogenic effect on osteoblasts and no stimulatory effect on cervix adenocarcinoma cells [109]. A new isoflavone, named ebenosin in addition to the known ones, afrormosin, formononetin and daidzein has been isolated. Receptor binding study suggested that all four compounds have similar relative binding affinities for ERα, with the latter having significantly higher binding affinities for ERβ than the other three compounds. It also evaluated that ebenosin was much less cytotoxic and only weakly estrogenic for ER-positive endometrial adenocarcinoma cells. Other studies suggested that the C-8 isoprenyl substituent of ebenosin rendered it cytotoxic and/or estrogenic in a cell-dependent manner [110].

The methanolic extract of *O. ebenoides* at a dose of 300 mg/kg body weight was given to the bilaterally ovariectomized rats for 3 months and 6 months. Comparison of BMD absolute values of the whole tibia of treated and ovariectomized (Control) animals of both 3 and 6 months post-ovariectomy were highly significant ($p<0.0005$), showing a protective effect on treated animals. Percent changes from baseline measurement of the whole tibia of treated and sham operated on comparison showed no significant difference at 3 or 6 months, demonstrating a highly protective effect. However comparison of BMD of proximal tibia of treated animals and % changes in proximal tibia did not display such protection in the 3 months study, but was statistically significant as compared to the non-treated rats

after 6 months. Body and uterine weights comparisons showed no significant difference between ovariectomized and treated rats favour its selective estrogen receptor modulator-like activity [111].

O. ebenoides extract displayed a significant estrogenic activity on both ERα and ERβ subtypes in addition to estrogenic activity in MCF7 cells. The methanolic extract exhibited direct action on osteoblasts by inducing mineralization. These findings suggest that the beneficial effect of *O. ebenoides* extract on bone loss is mediated through an estrogen-like action *via* activation of ERα-ERE and ERβ-ERE pathways and *via* direct action on the mineralization process of osteoblasts [112].

(16) INDIAN CORAL TREE (*ERYTHRINA VARIEGATA*)

Erythrina variegata, a member of Leguminosae Family is a showy, spreading tree, a legume with brilliant red blossoms. Commonly known as the 'Indian coral tree' in Asia or 'tropical coral' in the Pacific, this highly valued ornamental has been described as one of the gems of the floral world. The plant has also proven valuable for fodder production and as a sturdy component of windbreaks. It is a useful species for soil enrichment because it modulates readily and prolifically in both acid and alkaline soils. Farmers in India appreciate *E. variegata* as fodder, light timber and, more recently, as pulp for the paper industry [113]. *E. variegata* is native to the coast of India and Malaysia. It has been widely introduced in coastal areas of the Old World tropics, extending from East Africa and Madagascar through India, Indochina, Malaysia, northern Australia and Polynesia.

E.variegata is used as a folk medicine in many countries [114]. In india the juice from the leaves is mixed with honey and ingested to kill tapeworm, roundworm and threadworm [113]. In Tonga, the bark of *E. variegata* is used in a formula with other herbs to treat stomachache. In India, China, and Southeast Asia, its bark and leaves are used to treat wind-damp obstruction syndrome manifested as rheumatic joint pain, spasm of the limbs as well as lower back and knee pain, and to stimulate lactation and menstruation for women [114].

Phytochemical studies on the different parts of the *E. variegata* plant have led to the isolation of many isoflavones. Sphaerobioside, genistein and other derivatives have been isolated from the stem bark of *E. variegata* [115]. Three new isoflavonoids, eryvarins M-O, two new 2-arylbenzofurans, eryvarins P and Q, and a new 3-aryl-2, 3-dihydrobenzofuran, eryvarin R, together with three known compounds, were isolated from the roots of *E. variegata*. Eryvarin R is an unusual 3-aryl-2, 3-dihydrobenzofuran derivative with a formyl (CHO) group. Eryvarin Q showed potent antibacterial activity against methicillin-resistant *Staphylococcus aureus* [116, 117]. Two new isoflavonoids, eryvarin A and eryvarin B were isolated from the wood of *E. variegata*. Erycristagallin and Orientanol B have been isolated with other 5 isoflovonoids and the former is a highly potent antibacterial against mutans streptococci [118]. Two 3-phenoxychromones namely eryvarins F and G with an isoprenoid group in its structure were isolated from the roots of *E. variegata* [119].

Wong [115] studied the antiosteoporotic effect of the ethanol extract of *E. variegata* in the ovariectomized rat model of osteoporosis, at two different dose levels of 300 and 600 mg/kg body wt for 14 weeks. Rats treated with the alcoholic extract prevented the ovariectomy induced increase in the serum osteocalcin, ALP, and urinary deoxypyridoline levels. Histomorphometric analysis of the proximal end of the tibia showed that the extract prevented the estrogen deficiency-induced decrease in trabecular thickness and trabecular area, as well as restored the increase in trabecular separation in a dose-dependent manner. Moreover, the extract improved the energy absorption and stiffness of the mid-shaft of the rat femur, thus clearly demonstrating that *E. variegata* could suppress the high rate of bone turnover induced by estrogen deficiency, inhibit bone loss and improve the biomechanical properties of bone in ovariectomized rats.

Phytochemical investigation revealed the presence of isoflavones and sphaerobioside, both of which are known to act as phytoestrogens and may hence be responsible for the antiosteoporotic activity.

(17) SAFFLOWER (*CARTHAMUS TINCTORIUS* L.)

Carthamus tinctorius commonly known as safflower or false saffron, belonging to the Family Asteraceae is a thistle-like, self-compatible, annual, diploid ($2n = 24$) herbaceous crop that thrives in hot, dry climates, and is capable of surviving on minimal surface moisture. It is believed to have been domesticated somewhere in the Fertile Crescent region over 4,000 years ago. Plants are 30 to 150 cm tall with globular flower heads (Capitula) usually with many long sharp spines on the leaves and commonly, brilliant yellow, orange or red flowers which bloom in July. Devoid of essential oil, safflower is widely cultivated for edible oil, which is extracted from the seeds. It contains a high amount of polyunsaturated fatty acid linoleic acid (70%) and monounsaturated oleic acid (10%) with small amounts of stearic acid [120].

The flower of *Carthamus tinctorius* L. is an important medicinal material in prescriptions used for cardiovascular disease. The dye is mainly used as a coloring agent. Safflower is a very good purgative, analgesic, antipyretic and an antidote to poisoning [121]. Safflower is a useful plant in painful menstrual problems, post-partum haemorrhage, whooping cough and chronic bronchitis, rheumatism and sciatica [122]. Treatment demonstrated estrogenic activity by displaying an increase in uterus weight in ovariectomized mice and an increase in seminal vesicle weight in castrated mice [123].

Flavonoid glycosides are the main homologous constituents in the flower of *C. tinctorius* L. Carthamin, a dye of the flavonoid type and safflower yellow are the main constituents. In addition to these, it also contains carthamidin, isocarthamidin, quercetin, kaempferol, 6-hydroxykaempferol, chalcone and safflomin-A [124-126]. Some acetylenic glucosides namely carthamoside A_1 and Carthamoside A_2 are also reported [127]. Two new quinochalcone C-glycosides, hydroxysafflor yellow A and tinctormine, were isolated from *Carthamus tinctorius* L. together with carthamin, safflor yellow B and safflomin C. Tinctoramine

was demonstrated to have potent calcium antagonistic action [128]. Kinobeon A has been isolated from the cell culture of *Carthamus tinctorius* which exhibited stronger effect on the cell viability than lignan or quercetin when menadion or Xanthine oxidase were used as inducing reagents of oxidative stress. The present study demonstrates for the first time that kinobeon A prevents oxidative stresses and could be a useful cytoprotective reagent [129].

Oral administration of safflower seed oil at a dose of 1 ml/kg to ovariectomized rats for 30 days showed positive changes compared to the vehicle treated ovariectomized control rats. Evaluation was on the basis of changes in serum levels of insulin-like growth factor-I (IGF-I), IGF-II, insulin-like growth factor binding protein-3 (IGBP-3), estrogen, total alkaline phosphatase (TALP), bone-specific alkaline phosphatase (BALP), calcium and phosphorous in serum: the histomorphology of the proximal tibia metaphysis and femur/body weight (F/B) ratio were examined in all the groups at every 10 day interval. Thirty days later, IGF-I, IGF-II, IGBP-3 and BALP levels were observed to be significantly increased ($p < 0.05$) in treated group as compared to the sham operated and control groups. No significant differences were seen in serum levels of estrogen, TALP and F/B ratio between control and treated groups, but estrogen levels were higher in sham operated animals. Results, supported by histopathological study suggested that safflower seeds have a possible role in the improvement of ovariectomy induced osteoporosis in rats [130].

The aqueous extract of safflower seed significantly accelerated rates of osteoblast differentiation in the experimental group as compared to the control group in murine osteoblastic cells of the MC3T3-E1 line cultured on modified Eagle's minimum essential medium [131]. Phytoestrogen rich safflower seeds demonstrated a protective effect on bone loss caused by estrogen deficiency, without substantial effect on the uterus examined by scanning electron microscopy and histomorphometric analysis. The beneficial effect of safflower seeds may be mediated, at least in part, by the stimulating effect of polyphenolic compounds on proliferation of osteoblasts [132].

(18) BAWCHI (*PSORALEA CORYLIFOLIA* LINN.)

Bawchi or Babachi is the dried ripe fruit of the annual herbage *Psoralea corylifolia* L. belonging to family Fabaceae. It grows as winter season weed. The plant is widely distributed in India and in Southeast Asian countries. *P. corylifolia* seeds are extensively used in Ayurvedic medicine as stomachic, diuretic, deobstruent, anthelmintic and also against certain skin diseases e.g. leucoderma and leprosy [133]. Its cytotoxic, anticancer and immunomodulatory properties have already been reported [134]. Several furanocoumarins, meroterpenoids and flavonoids have been isolated from seed of *P. corylifolia.* Psoralin, psoralidin, bakuchin, angelicin, psoralenoside, isopsoralenoside, bakuchiol, chalcone, coumestone and coumarin derivatives have been isolated. Some of the above compounds demonstrated antibacterial, antidiabetic, anti-inflammatory, antioxidant, cytotoxic and hepatoprotective activities [135-137].

Psoralea corylifolia (PC) extract ameliorated experimental osteoporosis in ovariectomized (OVX) rats at the dose of 50 mg/kg/day when given for 3 months. *P. coryfolia* extract significantly increased serum calcium levels ($p < 0.05$, *vs*. OVX group) as well as decreased urinary calcium excretion ($p < 0.05$ *vs*. OVX group) in OVX rats. The upregulation of serum osteocalcin level by ovariectomy was suppressed by treatment with PC extract in rats. PC extract also significantly increased bone mineral density at 50 mg/kg body weight/day in OVX rats ($p < 0.05$, *vs*. OVX group). PC extract regulated Calcium levels as well as decreased urinary osteocalcin resulting in positive effects on bone mineral density as well as bone formation and hence could be a potential candidate for treatment of postmenopausal osteoporosis [138]. *P. corylifolia* L. fruit extracts exhibited osteoblastic proliferation stimulating activity in UMR-106 cell line cultured *in vitro*. The flavonoids of corylin and bavachin were isolated and identified as active principles by activity-guided fractionation. The results suggested that *P. corylifolia* L. fruit extracts, corylin and bavachin probably stimulated bone formation and possessed potential activity against osteoporosis [139].

SUMMARY

The silent epidemic osteoporosis has been neglected for years and is often under- diagnosed and under- treated. The awareness of the consequences of the disease, among the public and a majority of health professionals particularly in developing countries, is poor. Adequate infrastructure for proper diagnosis is also lacking in these countries. As the longevity of the world population increases, the risk of low bone mass and osteoporosis is also increasing. In addition to balanced nutrition which is most essential, many dietary supplements such as soy, echinacea, clover, parsley, grapes etc., are readily available for the maintenance of bone health. Treatment modalities for osteoporosis include anabolic agents, antiresorptive agents and estrogenic modulators. Phytoestrogens with established safety may be useful as alternative medicines for osteoporosis especially in the early stages along with exercise, calcium and vitamin D supplementation to slow down the bone loss. However much more work needs to be done to show the preclinical and clinical safety and efficacy of specific phytoconstituents in the treatment of osteoporosis.

ABBREVIATIONS

ACCX	=	Acetylcimigenol xylopyranoside
ALP	=	Serum Alkaline Phosphatase
BALP	=	Bone-specific alkaline phosphatase
BMD	=	Bone mineral density
Ca	=	Calcium
DPD	=	Dihydropyrimidine dehydrogenase
EC	=	Epicatechin
ECG	=	Epicatechin gallate
EGC	=	Epigallocatechin
EGCG	=	Epigallocatechin gallate
ERα	=	Estrogen receptor α
ERβ	=	Estrogen receptor β

ERK = Extracellular signal Regulated Kinases
FLL = Fructus Ligustri Lucidi
FSH = Follicular stimulating hormone
HEP = Herba epimedii
HRT = Hormone replacement therapy
IL-1 = Interlukin-1
IGF-I/II/III = Insulin-like growth factor-I/II/III
IGBP-3 = Insulin-like growth factor binding protein-3
LH = Leutinizing hormone
MNC = Multinucleated cells
mRNA = Messenger Ribose Nucleic Acid
NF-κB = Nuclear factor kappa-light-chain-enhancer of activated B cells
OPG = Osteoprotogerin
OVX = Ovariectomy/Ovariectomized
RANKL = Receptor Activator for Nuclear Factor κ B Ligand
SERM = Selective Estrogen Receptor Modulators
TCM = Traditional Chinese Medicine
TNFα = Tumor necrosis factor α
TALP = Total alkaline phosphatise
TRAP = Tartarate resistant acid phophatase

Appendix I

Table 1. List of Medicinal Plants Reviewed

Sr. No	Common Name	Biological Source	Chemical Constituents	Proposed Mechanism	References
1	Hadjod	*Cissus quadrangularis* (Vitaceae)	Steroids, alkaloids, calcium	Rich in calcium. Osteoclastic inhibition	Shirwaikar *et al.*, 2003
2	Black Cohosh	*Cimicifuga racemosa* (Ranunculaceae)	Flavonoids, triterpine glycoside and aromatic acids	Osteoclastic inhibition by ER binding to	Ping *et al.*, 2006
3	Soybean	*Glycine max* (Fabaceae)	Isoflavonoids; genistein daidzein, etc	Binding with estrogen receptor	Chen *et al.*, 2003
4	Maca	*Lepidium meyenii* (Brassicaceae)	Alkaloids, steroids, glucosinolates, macamides	By osteoclastic inhibition	Yong zhong *et al.*, 2006
5	Pila Bhangara	*Wedelia calendulaceae* (Asteraceae)	Isoflavonoids, wedelolactone	Not clear may act like SERM	Shirwaikar *et al.*, 2006
6	Foetid bugbane	*Cimicifuga foetida* (Ranunculaceae)	Cimicifoetisides A and B, triterpenoides	Acts like SERMs	Li. *et al.*, 2005
7	Red Clover	*Trifolium pratense* (Fabaceae)	Isoflavonoids like biochanin A and genistein	Estrogen Receptor binding	Circosta *et al.*, 2006
8	Japanese pagoda tree	*Sophora japonica* (Leguminosae)	isoflavonoids	Genistein like action	Wang *et al.*, 2006
9	Herba epimeddi	*Epimedium brevicornium* (Berberidaceae)	Icarin, flavonoids, sterols, fatty acids	Exact mechanism unknown.	Wong *et al.*, 2005
10	Mushroom	*Pleurotus eryngii* (Pleurotaceae)	Polysaccharides, volvatoxin, ganoderic acid, etc.	Anti bone resorption and bone forming	Kim *et al.*, 2006
11	Fructus Ligustri Lucidi	*Ligustrum lucidum Ait* (Oleaceae)	Oleanolic acid, lupeol, betulin, fatty acids etc	Direct stimulation of osteoblasts	Wong *et al.*, 2007
12	Tea	*Camellia sinensis* (Theaceae)	Polyphenols and flavonoids	Antiresorptive due to estrogenic	Das *et al.*, 2005
13	Chinese Foxglove	*Rehmannia glutinosa* (Scrophulariaceae)	Steroids, norcarotenoids, remophilanetriol etc.	Anti bone resorption and bone forming	Oh *et al.*, 2003
14	Jie gu mu	*Sambucus williamsii* (Caprifoliaceae)	Steroids, triterpenoids, phenolic acid etc.	By decreasing osteoclastogenesis	Yao *et al.*, 2005
15	Onobrychis	*Onobrychis ebenoides* (Leguminosae)	Arylobenzofuransand isoflavonoid	Binding to ERα-ERE and ERβ-ERE	Moutsatsou *et al.*, 2007
16	Indian coral tree	*Erythrina variegate* (Leguminosae)	Isoflovonoids, Sphaerobioside, Orientanol B	Phytoestrogens like actions	Wong *et al.*, 2007
17	Safflower	*Carthamus tinctorius* L. (Asteraceae)	Flavonoids, Kinobean A, fixed oil etc.	Osteoblastic stimulation	Kim *et al.*, 2002
18	Bawachi	*Psoralea corylifolia* (Fabaceae)	Furano-coumarins, Flavonoids, terpenoids	Osteoblastic stimulation	Wang *et al.*, 2001

Note: The authors have suggested the possible mechanism of action but for the exact mechanism of action and phytoconstituents responsible for antiosteoporotic activity, further detailed study will be necessary.

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