1 INTRODUCTION

1.1 Background and Significance

For centuries, herbal medicines have been widely utilized as effective remedies for the prevention and treatment of multiple health conditions. Nowadays, approximately 80% of the world population primarily in developing countries extensively relies on traditional medicine based largely on plants and animals for their primary health care. The increased popularity and demand of herbal medicines is due to their effectiveness, fewer side effects and relatively low cost compared to their counter synthetic drugs related to adverse side-effects or lack of efficacy. It has been estimated that the market of Ayurvedic medicines is expanding at 20% annually with expected export of Rs. 1200 million annually from India, with constant growth of 15% in all major herbal-based pharmaceutical companies.

Bone is a highly dynamic and specialized form of connective tissue, serves both as a tissue and an organ system within higher vertebrates. Whilst bone provides supporting skeleton framework of the body necessary for locomotion, characterized by its rigidity, hardness and power of regeneration and repair. It also protects vital visceral organs and provides microenvironment in marrow for blood formation and fat storage. Besides these, bone also acts as a mineral reservoir for calcium homeostasis and repository of growth factors and cytokines, and participates in acid–base balance (Taichman, 2005).

Throughout the life, bone undergoes continual adaption to attain and preserve skeleton size, shape and structural integrity with the changing biomechanical forces. Bone remodeling and modeling are the two processes that underpin development and maintenance of the skeletal system. Bone modeling is responsible for the growth and mechanically induced adaption of bone, and requires that the processes of bone formation and bone removal (resorption), although globally coordinated, occur independently at distinct anatomical locations (Raggatt & Partridge, 2010). It is also responsible for removal and repair of old, microdamaged bone with new, mechanically stronger bone to restore bone strength and mineral homeostasis. Though bone exhibits significant mechanical strength at a minimum weight, its biomechanical properties allow for significant flexibility without compromising its mechanical strength. Bone remodelling is a regulated balance between bone resorption by osteoclasts and bone deposition by osteoblasts.
However, the correct balance between these two functions is crucial not only quantitatively, but also in time and space for the proper maintenance of the bone mass. When the coupling is lost, the correct bone mass could be compromised, leading to several skeletal pathologies. Osteoporosis and bone loss are indeed the result of an increased osteoclast function and/or a reduced osteoblast activity. In contrast, other pathologies are related to osteoclast failure to resorb bone, such as osteopetrosis, a rare genetic disorder characterized by an increased bone mass and also linked to an impairment of bone marrow functions (Rucci, 2008).

Postmenopausal osteoporosis majorly affects elderly people and women within 10-15 years after menopause. Various biological, endocrinological, genetic, nutritional and environmental factors predispose osteoporosis in both male and female (An et al., 2016). The available treatments such as hormone replacement therapy, bisphosphonates, selective estrogen receptor modulators such as raloxifen and droloxifen, strontium ranelate, denosumab, calcitonin, synthetic parathyroid hormone and other anabolic therapies for osteoporosis, reduces the fracture rates, but each exhibits its own potential adverse effects leading to atraumatic fracture (F. Li et al., 2013).

Hence, natural extracts and its constituents derived from medicinal plants with high efficacy but few side effects are urgently required as alternative approach for the prevention and treatment of osteoporosis. Phytoestrogens such as isoflavones (genistein, daidzein, glycitein, equol and biochanin A), lignins (enterolactone, enterodiol), flavonoids (quercetin, kaempferol) and coumestans, share structural and functional similarities with naturally occurring or synthetic estrogens (Bacciottini et al., 2007). Moreover, their estrogenic activity is mediated via estrogen receptor binding, hence preventing postmenopausal osteoporosis and cardiovascular risks, by improving the defensive system (Thomson, Mundy, & Chambers, 1987).

*Cissus quadrangularis* (CQ), Soy and flaxseeds are rich source of phytoestrogens which are utilized for preventing and treating menopausal related disorders and have been proven by non clinical (Di Pompo et al., 2014) and clinical evaluation (Brahmkshatriya, Shah, Ananthkumar, & Brahmkshatriya, 2015; Lemay, Dodin, Kadri, Jacques, & Forest, 2002; Messina, 2014).
1.2 Osteoporosis – an overview

Osteoporosis is a systemic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue with a consequent increase in bone fragility and susceptibility to fracture (John A Kanis et al., 2008).

It is a very common condition that will affect one in three women and one in twelve men at some point of their lifetime (Ligett & Reid, 2000). The deterioration of one’s bones due to a reduction in bone mineral density (BMD) has a significant impact on one’s quality of life and indeed, morbidity and mortality. Osteoporosis is a silent disease, reflected only in a low bone density, till a fracture occurs and 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 (Malhotra & Mithal, 2008).

In humans, actual bone loss is seen only after third decade of their life at the rate of 0.6 to 1% every year, both in cortical and trabecular bone (Banu et al., 2012) whereas there is drastic bone reduction in women during menopausal age. From the current scenario, it has been emerged as silent epidemic and a major health hazard with serious socioeconomic consequences. It is mainly associated with postmenopausal women and elderly people, and the pathophysiological symptoms of the disease affecting millions of people worldwide irrespective of racial or ethnic group, at any age. The prevalence of the disease was estimated to effect approximately 200 million people worldwide with attendant costs exceeding 10 billion dollars per annum (F. Li et al., 2013). Postmenopausal osteoporosis affects women within 10-15 years after menopause, leading to fractures occurring at sites that contain relatively large amounts of cancellous bone and also by an increase in osteoclastic bone resorption compared with osteoblastic bone formation (Lau, 2009).

According to the World Health Organisation (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”. The prevalence of the disease is best diagnosed by the estimation of bone mineral density and bone mineral content by Dual energy X-ray absorptiometry (DEXA) or peripheral quantitative computed tomography (pQCT). But clinically the condition is not generally recognized until a fracture has occurred, so DXA is used as a surrogate for bone
mass. As such the WHO has developed a BMD cut off 2.5 standard deviations below the average peak adult BMD to define osteoporosis (John A Kanis, Melton, Johnston, & Khatlaev, 1994). A T-score based scale was proposed which expresses a patient’s BMD in terms of the number of standard deviations above or below the average peak young adult reference standard BMD (based on data for 20-29 year olds from the NHANES III database) (Kanis JA., 2002) Table 1. Osteoporosis is defined as a T score of -2.5 or less, identifies approximately 30% of the postmenopausal female population as osteoporotic which is approximately equivalent to the life time risk of osteoporotic fracture (John A Kanis et al., 1994). It is also comparison based scale of a patient’s BMD with that of a healthy young adult and previous history of a fracture (Ferguson N., 2004). Decreased BMD imparts increased risk for bone fracture. Every 1 SD decrease in BMD of the spine below the mean increases risk for new vertebral fracture by factor of 2.0-2.4 (Wasnich, 1993).

### Table 1 World Health Organization Diagnostic Parameters for Low Bone

<table>
<thead>
<tr>
<th>Classification</th>
<th>Bone Mineral Density T-score via Dual-energy X-ray Absorptiometry</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>BMD and BMC value within 1 SD of the young adult reference mean (T-score &gt;-1)</td>
</tr>
<tr>
<td>Osteopenia</td>
<td>BMD and BMC value more than 1 SD below the young adult mean but less than 2 SDs below this value (T-score &lt;-1 and &gt;-2.5)</td>
</tr>
<tr>
<td>Osteoporosis</td>
<td>BMD and BMC value 2.5 SDs or more below the young adult mean (T-score &lt;2.5)</td>
</tr>
<tr>
<td>Established Osteoporosis</td>
<td>BMD and BMC value 2.5 SDs or more below the young adult mean in the presence of one or more fragility fractures</td>
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### 1.3 Epidemiology

#### 1.3.1 Osteoporosis worldwide

According to the WHO, Osteoporosis is the second disease to cardiovascular as a leading health care problem (Archives of Internal Medicine, 2004). It has been calculated that there are approximately more than 200 million osteoporotic subjects in the world, of them one in three women and one in eight men over 50 years of age have osteoporosis. Although it is
worldwide, only one quarter of cases are treated, and a significant percentage is not even diagnosed (Tarantino et al., 2007). It has been estimated that osteoporosis causes more than 8.9 million fractures annually in the world, resulting in an osteoporotic fracture every 3 seconds, with 61% of osteoporotic fractural rate in women, with a female to male ratio of 1.6. Vertebrae, femur and radius are the most common sites of osteoporotic fractures and therefore can cause substantial morbidity and mortality. Overall fractures in women, 80% are forearm, descending to 75%, 70% and 58% of humerus, hip and spine fractures, respectively (Johnell & Kanis, 2006). Even if age-adjusted incidence rates remain stable, estimated number of hip fractures worldwide will rise from 1.66 million in 1990 to 6.26 million in 2050, which may rise by 240% in women and 310% in men (Sambrook & Cooper, 2006). Moreover, the women over 45 years of age, osteoporosis accounts for more days spent in hospital than many other diseases, including diabetes, myocardial infarction and breast cancer (J A Kanis, Delmas, Burckhardt, Cooper, & Torgerson, 1997).

According to the International Osteoporosis Foundation (IOF) survey, conducted in 11 countries, showed denial of personal risk by postmenopausal women, lack of dialogue about osteoporosis with their doctor, and restricted access to diagnosis and treatment before the first fracture result in underdiagnosis and undertreatment of the disease (International Osteoporosis Foundation, 2000). Majority of the Individuals at high risk (possibly 80%), who have already had at least one osteoporotic fracture, are neither identified nor treated. A intial fracture is associated with an 86% increased risk of any fracture. Hip fractures are invariably associated with chronic pain, reduced mobility, disability, and an increasing degree of dependence (Keene, Parker, & Pryor, 1993).
It has been reported that women who have sustained a hip fracture have a 10-20% higher mortality than would be expected for their age (Cummings & Melton, 2002). In 2010, the cost of long-term disability from osteoporosis in the European Union was 10.7 billion Euros. There is a marked variation in hip fracture rates between populations. Although after age adjustment, hip fracture rates are more common in Scandinavian and North America than these observed in southern European, Asian and Latin American countries. There are wide discrepancies between the incidence rate in women and men (75% in women and 25% in men) and 90% occurrence are found above 50 years (Jordan & Cooper, 2002; Sambrook & Cooper, 2006). In the United States, the estimated cases of osteoporosis increase from ~10 million to >14 million people in 2020 (based on 2000 census data) and over 47 million cases of low bone mass. It was reported that vertebral fractures can lead to back pain, loss of height, deformity, immobility, increased number of bed days, and even reduced pulmonary function (Lips et al., 1999; Nevitt et al., 1998; Pluijm et al., 2002). According to the International Osteoporosis Foundation, it is estimated that only one-third of vertebral fractures are reported by physicians and most of the time they remain undiagnosed.

The proportion of vertebral fractures that go unrecognized, during the local assessment of a thora-columbar lateral radiograph, is as high as 46% in Latin America, 45% in North America, and 29% in Europe/South Africa/Australia (Delmas et al., 2005). Osteoporosis and osteoporotic fractures are associated with sustained disability, loss of self-esteem, physical limitations, psychosocial impairment, and reduced quality of life (DT, 2001;
Lyles, 2001). From the data available on the economic burden of osteoporosis, it shows that currently cost of osteoporosis is 37 billion Euros per year in the EU, and 19 billion USD per year in the USA and that may rise dramatically in the upcoming years. Around, 40% of osteoporotic fractures occur in people of working age worldwide, accounting an annual cost of 48 billion USD in Canada, Europe and USA alone, not taking into account indirect costs such as disability and loss of productivity (International Osteoporosis Foundation, 2014).

1.3.2 Osteoporosis Asia and India

Around 60% of the world population resides in Asia where the population is rapidly increasing and ageing. From the Asian audit 2009, it is projected that more than about 50% of all osteoporotic hip fractures will occur in Asia by the year 2050 (International Osteoporosis Foundation., 2009). Osteoporosis is greatly underdiagnosed and undertreated in Asia, even in the most high risk patients who have already fractured, particularly in rural areas. In the countries like China and India, the majority of the population lives in rural areas (60% in China), where hip fractures are often treated conservatively at home instead of by surgical treatment in hospitals. Currently 69.4 million Chinese above 50 age suffer from osteoporosis with 687,000 cases of hip fractures each year, further, 19-26% of post-menopausal women have a vertebral deformity. The projected number of Chinese population with osteoporosis and osteopenia will increase to 286.6 million in 2020 and 533.3 million in 2050, which have serious socio-economic burden and treatment cost rise to 12.5 billion USD in 2020 and more than 264.7 billion USD by 2050 (Q. Shen, Xie, & Xia, 2006). In Japan, the forecasted number of hip fractures was to be 153,000 per year in 2010 and 238,000 in 2030 (Hagino, Katagiri, Okano, Yamamoto, & Teshima, 2005).

With recent trend in increasing longevity of the Indian population, it is now being realized that, as in the West, osteoporotic fractures are a major cause of morbidity and mortality in the elderly. Based on the results of 2001 National Census reported that approximately 163 million Indians are above the age of 50; the number was expected to increase to 230 million by 2015, of them 20% are women and 10-15 per cent men would be osteoporotic (Malhotra & Mithal, 2008). In a reported study among Indian women aged 30-60 years from low income groups, BMD at all the skeletal sites were much lower than values reported from developed countries, with a high prevalence of osteopenia (52%) and osteoporosis (29%) thought to be due to inadequate nutrition (Shatrugna, Kulkarni, Kumar, Rani, & Balakrishna, 2005).
In the 2009 IOF Asian Audit, expert groups made a highly conservative suggestion that approximately 26 million Indians suffer from osteoporosis, and this number would rise to 36 million by 2013. From the sources it is estimated that, in 2013, 50 million people in India would be either osteoporotic (T-score lower than -2.5) or have low bone mass (T-score between -1.0 and -2.5) (Mithal, Bansal, Kyer, & Ebeling, 2014). As the community based epidemiological data is lacking, hospital based studies suggest that hip fractures are common in India. From the data available it has been extrapolated that the number of hip fractures in India would be more than 440,000 every year, with a female: male ratio of about 3:1. The projected number of hip fractures may rise to 600,000 in 2020 and to more than 1 million in 2050 (International Osteoporosis Foundation., 2009).

In India, Osteoporosis is not a National Health Priority (NHP), but the closely related NHPs to the osteoporosis, is the nutritional program aimed at school children to provide vitamins and minerals including vitamin D and calcium. As osteoporosis is not been formally recognized in health programs, but a current proposal has been made under review for including musculoskeletal diseases in a NHP, as vitamin D deficiency has become an increasingly public health issue (Mithal et al., 2014). The major contributing factors of low BMD and high prevalence of osteoporosis in Indian women are low calcium intakes with extensive prevalence of vitamin D deficiency, increasing longevity, sex inequality, early menopause, genetic predisposition, lack of diagnostic facilities and poor knowledge of bone health (Khadilkar & Mandlik, 2015).
1.4 Classification of Osteoporosis

1.4.1 Primary Osteoporosis

a) Type I osteoporosis is generally found in hypogonadal women or men. They are mainly associated with postmenopausal women, who have premature oligo or amenorrhea (due to anorexia nervosa or obsessive exercise programs), and in men after castration or with testosterone deficiencies, leading to net bone loss directly related to the loss of gonadal function (Simon, 2007). As estrogen production falls, it leads to increased serum levels of cytokines, which is thought to lead to increased recruitment and responsiveness of osteoclast precursors in trabecular bone, resulting in increased bone resorption and slow bone formation (Manolagas & Jilka, 1995). These mainly occur in elderly persons above age 50 years, is six times more common in women, and is associated with hip fractures, occasional incidence of upper arm, shin and pelvic fractures, as well as dorsal kyphosis (“dowager’s hump”), vertebral and Colles’ (distal radius) fractures.

b) Type II is age-related or senile osteoporosis, associated with men and women typically after the age of 60 and older. Normally aging is associated with a progressive decline in the osteoblasts number and their activity, but not primarily with an increase in osteoclast activity. However, there is also an age-related decline in renal production of 1,25-dihydroxy-vitamin D, subsequently causing secondary hyperparathyroidism responsible for the excess cortical bone loss (Women’s Health, 2015). Commonly associated fractures of this group are of cortical bone, such as in the femur, femoral neck, proximal tibia, and pelvis (Simon, 2007) and hip fracture.

1.4.2 Secondary Osteoporosis (Drug-induced Osteoporosis)

Certain medical conditions or treatments causes secondary osteoporosis by increasing bone remodeling rate and further, causes an overall increase in the rate of bone loss. Certain disorders results in the bone marrow cavity expansion at the expense of the trabecular bone, which jeopardize bone integrity, causing reduced bone strength, loss of bone mass and increased susceptibility to fracture. These medical conditions and chemicals may include: Serious kidney failure, Cushing's disease, Liver impairment, Anorexia nervosa and bulimia, Early oophorectomy, Rheumatoid arthritis, Malabsorption syndromes such as celiac disease, Multiple sclerosis, Chronic obstructive pulmonary disease, Scurvy, Hyperparathyroidism, Hyperthyroidism, Diabetes, Hypercortisolism, Thalassemia, Multiple myeloma, Leukemia,
Metastatic bone diseases; chemicals - Cigarette smoking, Corticosteroid therapy, Alcohol abuse, Lithium (used to treat many psychiatric disorders), Aluminum, Antacids containing aluminum, long term use of Anticonvulsants, Diuretics, Heparin and Barbiturates (International Osteoporosis Foundation, n.d.).

1.4.3 Idiopathic Osteoporosis

It is a type of rare osteoporosis that occurs in children and young adults under 50 years old who are seemingly healthy: they have normal hormone and vitamin levels and there are no obvious reasons for weak bones or bone loss (Osteoporosis Prevention Education Program, 2004).

1.5 Risk factors in the Etiology of Osteoporosis

Certain risk factors are involved in the development of osteoporosis and contribute the likelihood of developing disease to an individual. After attaining peak bone mass during the third decade of life, both sexes start to lose bone that is the key predictor of osteoporotic fractures at older age. There are several risk factors related to the etiology of the disease, some risk factors cannot be changed, but others can. National Osteoporosis Foundation has categorized risk factors for osteoporosis into nonmodifiable and potentially modifiable risk factors.

1.5.1 Non-Modifiable Risk Factors

a) Gender

Rapid decline in estrogen at menopause is associated with an increase in bone resorption without a corresponding increase in bone formation. This imbalance leads to an accelerated net loss of bone that results in decreased bone strength and ultimately may lead to fractures and osteoporosis. Functionally, Estrogen inhibits IL-2; IL-2 promotes osteoclast activity and therefore, bone resorption. Loss of bone mass during reproductive years, particularly with prolonged lactation may also contribute to reduce BMD in women. Another reason for female predominance is that women live longer than men and men have 30 percent more bone mass than women, and they lose bone more slowly as they age. Lifetime risk of any fracture ranges between 40-50% in women, whereas 13-22% in men (Lin & Lane, 2004).
b) **Age**

Majority of hip fractures (90%) occur in people above aged 50, this may be partially due to reduced bone mineral density and bone mass in old age. Smaller periosteal diameter of bones in women also increases skeletal fragility that provides lighter and thinner bone structure than men. In other words, even older adults with normal bone mineral density are more likely to suffer a fracture than younger people.

c) **Body Size**

Bone mineral density is related to weight, as having a higher body mass means that more weight is borne by the bones, which strengthen in response to the stress. Underweight people tend to have significant lower bone mineral density, which may lead to increased risk of osteoporosis and fractures (Khadilkar & Mandlik, 2015).

e) **Ethnicity**

Caucasian and Asian populations tend to have a lower average bone mass than black or Hispanic groups, so are at highest risk to develop fracture (Cumming, Nevitt, & Cummings, 1997). As evidenced, Indian women have been shown to have lower BMD than their Caucasian and Black counterparts (Khadilkar & Mandlik, 2015).

f) **Family History**

People with a family history of osteoporosis or minimal trauma fracture are also at increased fracture risk. Daughters of women with osteoporosis of the spine tend to have decreased bone mass. The maternal history of hip fracture doubles the risk of hip fracture in women and increases the risk of spinal deformities in men. Gene Polymorphisms in vitamin D receptor and estrogen receptor alpha in different races may be responsible for the ethnic differences in BMD and may influence determinants of bone metabolism (Malhotra & Mithal, 2008).

1.5.2 **Modifiable Risk Factors**

a) **Nutritional Factors**

Calcium and vitamin D are the key players to maintain good bone health and have major role in influencing the risk of osteoporosis. In the bone matrix, calcium has been deposited in the
form of hydroxyapatite crystals and is responsible for the hardness of bone. Insufficient calcium uptake leads to the production of more parathyroid hormone, which boosts bone remodeling, mobilizes osteoclasts in the bone to break down and sacrifice bone calcium to supply the nerves and muscles with the mineral. Dairy products are good source of calcium and easily available. Several studies have reported that Indian diets do not meet the recommended daily intake. Among the rural Indian population, a higher ratio of phytates to calcium have been reported. Phytates hinders calcium absorption which may lower BMD, increase the risk of osteoporosis in calcium-deficient diets (Khadilkar & Mandlik, 2015). Vitamin D is an essential component required in the calcium absorption from the intestines into the blood. It is synthesized in the human skin upon exposure to sunlight. It is reported that Indians suffer from vitamin D deficiency due to low sun exposure, traditional clothing (saris, salwar kameezes), inadequate dietary intake, poor vitamin D fortification of food, and highly pigmented skin. It is recommended that at least 800 International Units of vitamin D and 1,000 to 1,200 mg of daily calcium can protect against osteoporosis (Dawson-Hughes et al., 2005; Indian Council of Medical Research, 2009; Vupputuri et al., 2006).

b) Low Body Mass Index

Leanness (body mass index (BMI) <20 kg/m2) regardless of age, sex and weight loss, is associated with greater bone loss and increased risk of fracture. People with a BMI of 20 kg/m2 have a two-fold increased risk of fracture compared to people with a BMI of 25 kg/m2.

c) Anorexia Nervosa

Osteoporosis can also be compounded by eating disorders such as anorexia nervosa and bulimia, characterized by an irrational fear of weight gain.

d) Lifestyle

Increased sedentary lifestyle, decreased sun exposure, and lesser physical activity, are likely to have a more hip fracture than those who are more active. Physical exercise, especially weight-bearing exercise, improves the body balance and maintains muscle and bone strength (Mithal et al., 2014). Excessive alcohol consumption and cigarette smoking increased the risk of sustaining any osteoporotic fracture by 40%, compared to people with moderate or no alcohol intake and non-smokers. It leads to secondary osteoporosis due to direct adverse
effects on bone-forming cells, on the hormone that regulates calcium metabolism and poor nutritional status (calcium, protein and vitamin D deficiency) (Lin & Lane, 2004).

e) Menopause or Hysterectomy

Onset of early menopause, naturally or surgically, increases the risk of osteoporosis as reduced levels of estrogen for a longer period than with normal menopause. Abrupt cessation of estrogen production due to surgical menopause, women whose ovaries are removed (69 percent in one study) tend to show signs of osteoporosis within 2 years after surgery, if no hormone replacement therapy is instituted. Even after hysterectomy (removal of the uterus), it is medically recommended to keep ovaries intact for proper maintainence of estrogen production.

f) Medications

Long-term glucocorticoid use of corticosteroid medications in certain treatments like of asthma and rheumatoid arthritis increases the risk of fractures. Hypogonadism, thyrotoxicosis, Cushing syndrome, malabsorption syndromes, chronic liver and renal disease may lead to secondary osteoporosis (Malhotra & Mithal, 2008).

Glucocorticoid Induced Osteoporosis

Over the past 50 years, glucocorticoids have been vastly used as a potent therapeutic agents for their anti-inflammatory and immunosuppressive properties for the treatment of various immune-mediated diseases, such as rheumatic diseases, pulmonary disease, asthma and post transplantation immunotherapy (René Rizzoli & Biver, 2015; Shi, Chutkan, Hamrick, & Isales, 2012). According to a study conducted in the UK, approximately 0.9% of the total adult population were using oral steroids at any given time, at a daily dose of 2.5–7.5 mg prednisolone or equivalent and this proportion increases with age to 2.5% by age 70-79 years. The proportionate number of 22% of these individuals received higher doses (>7.5 mg per day) for >6 months as long term therapy (René Rizzoli & Biver, 2015; Van Staa, Leufkens, Abenhaim, Begaud, et al., 2000). In an observational study across 10 countries by Global Longitudinal Study of Osteoporosis in Women (GLOW), reported greater use of glucocorticoids (2.7–4.6%) by women aged >55 years (Diez-Péreza et al., 2011). However, long-term glucocorticoid therapy (>3 months) causes bone loss resulting in osteoporotic fractures in about 30–50% of treated adult patients (E. Canalis, Mazziotti, Giustina, &
It has been shown that daily doses of <5 mg prednisolone increase fracture risk by ~20%, rising to 60% for doses of >20 mg per day, even as low as 2.5 mg/day prednisolone doses have been associated with increased fracture risk (Van Staa, Leufkens, Abenhaim, Zhang, & Cooper, 2000). A meta-analysis study conducted on more than 42,000 patients, compared with the patients on oral corticosteroids use whether present or past with individuals with the same BMD but never treated with glucocorticoids, demonstrated that the relative risk for osteoporotic fracture was 2.63 at the age of 50 and 1.71 at 85 years and for hip fractures ranged from 4.42 to 2.48 (J. Kanis et al., 2004). Although, the fracture risk increases rapidly (within 3–6 months of initial oral glucocorticoid therapy), it is independent of underlying disease, age, race and sex, (Van Staa, Leufkens, & Cooper, 2002). Increased risk of various fractures have also been reported in users of inhaled glucocorticoids, although this effect was less pronounced than with systemic glucocorticoid therapy.

### 1.6 Relevance of the Study

Fortunately, there are available treatments that can reduce osteoporosis by suppressing the bone resorption, bone turnover and increasing the BMD but each has its limitations. Hormone replacement therapy (HRT) is widely used effective therapy for the prevention of osteoporosis, however, evidences have shown that long-term HRT increases the high risk of breast cancer, endometrial cancer, thromboembolic events and vaginal bleeding. Bisphosphonates such as alendronate, ibandronate, risedronate, and zoledronic acid, inhibits the bone resorption and increase the remodeling rates generally by 2-fold within 12 months of menopause, 3-fold by age 60, and remain elevated in untreated osteoporosis patients, but also exhibit potential adverse effects leading to atraumatic fracture (Khosla et al., 2012). Selective estrogen receptor modulators such as raloxifene and droloxifene, strontium ranelate, denosumab, calcitonin and synthetic parathyroid hormone are other anabolic therapies for osteoporosis that reduces fracture rates, but each exhibits its own advantages and disadvantages (F. Li et al., 2013).

The present research suggests that modifications in the environmental factors in the intra-uterine life may increase the risk of developing various chronic diseases including cardiovascular disease, diabetes and osteoporosis etc. in later part of the life. The availability of nutrients *in utero* may directly influence the foetal development at critical points, via acting on biologically plastic processes during development, with potential long-term health outcomes (Barker, 2012; Christian & Stewart, 2010). Therefore, programming of bone growth in utero could be the important contributor to the later risk of osteoporotic fracture (E
M Dennison, Cooper, & Cole, 2010). It has been evidenced that a low protein diet in utero affected the osteogenic environment in the offspring during the pregnancy and the programming of skeletal development which persist into late adulthood (S. A. Lanham, Roberts, Cooper, & Oreffo, 2008).

Hence, natural extracts and its constituents derived from medicinal plants with high efficacy but few side effects are urgently required as alternative approach for the prevention and treatment of osteoporosis. With the increasing side effects of the conventional system of medicine in the management of postmenopausal bone loss, alternative or complementary medicines are gaining demand for the prevention and curation of osteoporosis with the reputation of being both safe and efficacious. Phytoestrogens such as isoflavones, lignins, flavonoids, and coumestans, share structural and functional similarities with naturally occurring or synthetic estrogens. They are potential alternatives in the Hormone replacement Therapy to the synthetic selective estrogen receptor modulators (SERMs). They are scientifically known to bind the estrogen receptor sites and trigger the processes of estrogenic activity, hence preventing postmenopausal osteoporosis and cardiovascular risks, by improving the defensive system (Thomson, Mundy, & Chambers, 1987).

Since, *Sesbania grandiflora* possess rich source of phytoestrogens (Ching & Mohamed, 2001; Kasture, Deshmukh, & Chopde, 2002; Mustafa, Hamid, Mohamed, & Bakar, 2010). It is proposed that supplementation of ethanolic and aqueous extracts of *Sesbania grandiflora* may beneficially prevent bone loss caused by estrogen deficiency and in other medications. have beneficial effect on the bone health even in diabetic state. Howbeit, *S. grandiflora* has never been investigated as an alternative preventive medicine for osteoporosis and hence, the present study was designed with this objective.
1.7 Aim and Objectives

Management of Osteoporosis is a major global public health problem in elderly people and women within 10-15 years after menopause. With the advent of modern treatments and diagnostic techniques it has become easier for its management, but also associated with side-effects. Hence, supplementary treatments with lesser side effects including traditional medicinal system are urgently required. With this objective, the study was performed to evaluate the potential of *Sesbania grandiflora* (SG) as an antiosteoporotic agent.

Objectives:

1. Procurement, Identification and Authentication of *Sesbania grandiflora*.

2. Isolation, standardization and characterization of the different extracts of the *Sesbania grandiflora*.

3. Phytochemical analysis of the extracts.


5. *In vitro* evaluation of the standardized extracts for antioxidant and reduction activities.

6. *In vivo* Studies

   - Evaluation of the standardized extracts for Safety studies by oral route as per OECD 423.
   - Evaluation of SG extracts for anti-osteoporotic activity in ovariectomy induced osteoporosis in adults.
   - Biochemical, histopathological, morphometrical and biomechanical evaluation of SG extracts in Glucocorticoid Induced Osteoporosis in adults.
   - Evaluation of prenatal exposure of SG extracts on the neonatal bone growth, mechanical strength and reproductive outcome in adult offspring.
   - To evaluate Anti-fertility activity of SG extracts in female rats.
   - Evaluation of bone parameters – BMD and BMC using DEXA and Micro-CT analysis.
1.8 Organization of the thesis

The organization of the thesis has been structured under following chapters:

- Chapter 1 includes introduction related to the importance of the herbal medicine in the management of osteoporosis, background of the research work, relevance of the study and objectives. It also discuss the comparative prevalence of the disease globally and in India.
- Chapter 2 discusses in-depth literature review about the disease, mechanism of action, different diagnostic techniques and early detection markers. It also explains the various pharmacological molecules approved by Internationally acclaimed governing bodies.
- Chapter 3 outlines the research methodology based on literature review. It has been followed from the different sources with reference to preclinical models and safety studies.
- Chapter 4 describe the detailed explanation of the results, of different assays and preclinical studies as performed in the research work. The results are then compared with the existing literature and discussed in detail for its validity and reliability.
- Chapter 5 summarizes the generalized and specific conclusion of the research work, along with the limitations of the study and scope for future work.