CHAPTER-6

SUMMARY AND CONCLUSION

Osteoporosis is most frequent major health hazard in recent years, affecting over 2000 million people worldwide (Meryl, 1997) and characterized by low bone mass and microarchitectural deterioration of bone tissues, leading to enhanced bone fragility, which leads to a propensity to fracture with minimum trauma (Raisz, 2005). Menopause brings about increased bone turnover an imbalance between bone formation and bone resorption (Gruber et al., 1984). The most common type of osteoporosis is the bone loss associated with ovarian hormone deficiency at menopause (Riggs and Melton, 1986). Estrogen deficiency is considered as the main determinant for bone loss in postmenopausal women (Turner et al., 1994). Osteoporosis is caused by an imbalance in the normal bone remodeling process, in which there is excessive osteoclast resorption and adequate new bone formation by osteoblasts reduction. Hormone replacement therapy (HRT) has proven to be efficacious in preventing bone loss and reducing the incidence of skeletal fractures in postmenopausal women. However, long-term HRT increases the high risk of breast cancer, endometrial cancer, thromboembolic events and vaginal bleeding (Genunt et al., 1998). Traditional Indian medicines have been used from long days in prevention and treatment of postmenopausal osteoporosis. Since these medicines are prepared from medicinal plants they have fewer side effects and are suitable for long-term use.

The present study aims in exploring Pinus roxburghii and Lepedium sativum as potent antiosteoporotic plants and further establishes their pharmacognostic and phytochemical profile. The pharmacognostic profile was carried out in accordance with the standardization methods described in quality control methods for medicinal plants, WHO, 1998. The seeds of L. sativum and needles and bark of P. roxburghii were studied for the first time. The pharmacognostic parameters would serve as quality control parameters for the future studies on the plant.

Phytochemical profile of the plants was studied using GC, HPTLC and HPLC. The HPTLC analysis of the seeds of L. sativum revealed the presence of β-sitosterol and rutin. GC analysis of the volatile oil obtained by hydrodistillation of needles of P. roxburghii revealed the presence of terpenes majorly. Thunbergene, a macrocyclic diterpenoid is commonly found some species of pinus such as : P. formosana, P. armandii, P. albicaulis, was for the first time reported
in *P. roxburghii*. Other compounds mainly included monoterpenes. In Himachal Pradesh *P. roxburghii* is majorly used for tapping resin and for timber. The bark and needles are usually waste products. Quercetin was isolated from the bark of *P. roxburghii*. Quercetin is a plant derived flavanoid (Galeotti, 2008). It is thought to have potent antioxidant, antidiabetic and anti-tumour, antiviral and anti-inflammatory benefits (Spencer et al., 2008). Quercetin (3,3’,4’,5,7-pentahydroxyflavone), the primary dietary flavonol, exerts a potent inhibitory effect on the bone resorbing activity of mature osteoclasts by inducing their apoptosis (Wattel et al., 2003). Quercetin negatively regulates NFkB and AP-1 transcriptional activities in osteoclastic precursors (Wattel et al., 2004).

In the present study, *Lepidium sativum* (seeds) and *Pinus roxburghii* (needle and bark) were evaluated for anti-osteoporotic activity *in vivo* in female ovariectomized Wistar rats. Female rats were subjected to surgical ovariectomy which caused induced post menopausal syndrome. The rats were treated for a month and the results were assessed by three major parameters: biochemical, biomechanical and histopathological evaluation. The extracts: Pet.ether and methanolic extracts of *L. sativum*, methanolic extracts of needle and bark of *P. roxburghii* were evaluated for antiosteoporotic effect. The biochemical parameters include Tartarate Resistant Acid Phosphatase (TRACP), Alkaline Phosphatase (ALP), Calcium (Ca) and Inorganic phosphate (IP), while hydroxyproline content was evaluated in urine. All the treated groups showed protective effect, methanolic extract of *L. sativum* was found to be most effective in reducing serum ALP levels, methanolic extract of bark of *P. roxburghii* caused greater reduction in osteoclastic activity by reducing urine hydroxyproline levels and serum TRACP levels. Calcium and phosphate levels did not showed any marked change in serum. The biomechanical parameters were evaluated along the parameters of: three point bending of the tibia, compression of the 4th lumbar vertebra and femoral neck load testing. Methanolic extract of bark of *P. roxburghii* exhibited maximum protective effect by reversal of bone fragility correlated by the histopathological studies of the mid shaft of the femur.

The results indicate that the methanolic extract of the bark of *P. roxburghii* exhibited potent antiosteoporotic activity over the other extracts and was comparable with the standard Tamoxifene.
6.1 CONCLUSION

The present study establishes pharmacognostical profile of two potential medicinal plants – *Lepedium sativum* and *Pinus roxburghii*. Phytochemical studies of both the plants were studied and revealed the presence of β-sitosterol and rutin in Pet.ether and methanolic extracts of *L. sativum* while quercetin was isolated from methanolic extract of the bark of *P. roxburghii* for the first time. Further, antiosteoporotic activity of the different extracts of both the plants was studied in ovariectomized female rats. The *in vivo* studies reveal an associated response in all the parameters evaluated. An increase in biomechanical strength, higher osteoblastic activity and minimal osteoclastic activity account for bone formation which in turn prevents osteoporosis. The histopathological data adds a confirmatory note to the findings. The plants - *L. sativum* and *P. roxburghii* possess a marked antiosteoprototic activity with minimal toxicity and could therefore have a promising role in the treatment of osteoporosis due to estrogen deficiency.

The present study thus provides new insight to possibilities of potent herbal drugs for the treatment and management of osteoporosis. Further work is in need to ascertain the various mechanisms of action for the antiosteoporotic activities of both the plants. The present work has also established that the methanolic extract of the bark of *P. roxburghii* contains very high quantity of quercetin. Further research need to be done to exploit the plant part otherwise considered waste product in timber industry for the production of quercetin commercially.