CHAPTER 1

INTRODUCTION

1.1 Statement of the problems

Endosseous dental implants are widely inserted in maxilla and mandible as substitutes for teeth in prosthodontics therapy to restore or replace function in partially or completely edentulous patients. As the aging population increases, a greater number of individuals are being defined as partially edentulous, and their treatment options and standards of care now include the use of dental implants. Dental implant therapy has become a very predictable method rendering excellent implant survival rates. Nevertheless, a description of the biological events occurring at the implant-bone interface may help to shed light on peri-implant osteogenesis. When inserting a medical device into a bone cavity a sequence of different biological events take place at the bone - implant interface until the implant surface appears finally integrated with a newly formed bone. The final goal of surgical procedures is controlled and guided to allow rapid healing which leads to the integration of an implant into bone. Osseointegration has been considered the most aspiration bone-implant interface. In case with insufficient amounts of bone the regeneration of bone tissue becomes a necessity. Basic science and clinical studies have documented the ability of different growth and differentiation factors to induce and enhance bone regeneration. Moreover, the mechanical and biological factors involved in the healing process of bone are certainly affected by senile and post-menopausal osteoprosis. Many clinical studies and researches have revealed that biomaterial osseointegration

is slower in osteoporotic subjects, with an increased rate of prosthetic device failures both in dental and orthognathic reconstructive surgery (Fini *et al*., 2004).

Osteoporosis is a bone remodeling disorder in adults, which results from decreased bone formation and/or increased bone resorption. Osteoblast and osteoclast are two major functional cells involved in bone remodeling. The osteoclasts are closely related to the macrophage cells that functionally remove debris or pathologic material throughout the body. The osteoblasts are fibroblast-like cells that produce structural molecules in tissues. Bone-forming osteoblasts and bone-resorbing osteoclasts are coupled functionally to keep the homeostasis of bone metabolism. Bone remodeling, as a process of bone turnovers, includes the removal of old bone and sequent formation of new bone. This process is initiated in response to physiological or pathological change including injuries, fatigue stresses, aging, inflammations and metabolic needs. The biological activity of osteoblast and osteoclasts are regulated by many factors including growth factors, cytokines, hormones, and also the mechanical stress (Koike *et al*, 2005). The pathologic bone loss underlying conditions can largely be prevented by early estrogen replacement therapy, but the mechanism by which estrogen exert their bone sparing effect has been unclear. Recent advances suggest that the female hormone 17 β-estradiol (E_2) regulates the circuitry of cytokine action that controls bone remodeling, potentially providing a more precise understanding of how E_2 exerts its action in bone. Advances in bone tissue and cell culture techniques lead to the identification of estrogen receptors in the bone forming (osteoblast) and bone resorbing (osteoclasts) cell, which in part were explained the role of estrogen on bone as a target tissue (Kennedy *et al*., 2005). Although the mechanisms of 17 β-estradiol action represent the major estrogen

in human, which is well documented at the molecular level. Similarly to other steroid hormones, estrogens bind to specific intracellular receptors and regulate the transcription of defined sets of responsive genes. Thus, estrogen promoted the expression of traits associated with the formation of bone while reducing cellular responsiveness to hormones that may trigger the resorption of bone (Majeska, Ryaby, & Einhorn, 1994).

Currently available treatments for post-menopausal osteoporosis include hormone replacement therapy, bisphosphonates, calcitonin, calcium products and selective estrogen receptor modulators (SERMs), such as raloxifene. Moreover, clinical practice has found that women undergoing treatment for osteoporosis require long-term dosing regimens that offer no symptomatic relief and may cause side effects (Radford *et al*., 2002). Consequently, many plant-derived bioactive substances with considerable therapeutic benefits have attracted interest in the scientific community over the last two decades. Among these phytochemicals are the broad classes of polyphenols including non-steroidal estrogen and phytoestrogens. The scientific interest lies the potential of phytoestrogens for medical use like in hormone replacement therapy (HRT) either as registered drug or mostly as dietary supplement. The use of certain plant derivatives might be related to their ability about binding estrogen receptor and estrogenic effects. Phytoestrogens display estrogenic effects by binding to the estrogenic receptors. It might also provide selective action on reproductive and non-reproductive estrogen target tissues depending on the expression of estrogenic receptor (ER) α and/or ER β (Morito *et al*., 2001). The common characteristic of phytoestrogenic compounds is that they are diphenolic compounds with structural similarities to natural and synthetic estrogen and anti-

estrogens. It has an aromatic A ring with one hydroxyl group and second hydroxyl group on the same plane of A ring. Shared structures include a pair of hydroxyl groups and a phenolic ring, which is required for binding to ERα and/or ERβ, and the position of these hydroxyl groups appears to be an important factor in determining their abilities to bind the ERs and activate transcription (Bail *et al*., 2001).

Phytoestrogen are divided into three classes: isoflavones, lignans and coumestans. In addition some flavonoids, such as apigenin, quercetin, kaemferol and naringenin are also classified as phytoestrogens. The most extensively studied phytoestrogens are isoflavones which are found in high concentration in soybeans (0.2-1.6 mg /g dry weight). Depending on diet, human plasma concentrations of isoflavones can vary by more than 100-fold, and can reach 6 μM (Mei, Yeung, & Kung, 2001). The soy and soy derived products have been a traditional food for eastern Asian populations for thousands of years. Daidzein, genistein and their glycoside conjugates represent the major isoflavones, along with small amounts of glycitein and its glycoside derivatives. These compounds are also present in significant concentrations in various beans and sprouts (Hendrich, 2002). One of plants the interesting is vanilla beans, which are the fruits of *Vanilla planifolia* Andrews (Orchidaceae). In the green bean, important phenolic aromatic compounds are present as glucosides. The curing process is meant to release the aglycons to set free the aroma compounds. The exact reaction during curing are not know, but apparently enzymes play an important role in flavor formation; β-glucosidase being the most important enzyme (Dignum *et al*., 2004) . The major flavor constituent of vanilla extract is vanillin. It has been shown to inhibit mutagenesis induced by chemical and physical mutagens in various models (Keshava *et al*., 1998). It also

showed chemopreventive effects in chemical carcinogenesis models in rat (Tsuda *et al*., 1994). Several reports showed that vanillin also displayed antioxidant activity (Sawa *et al.*, 1999). So far, there are no publications on experiments on the estrogenic activity towards the different glycosides naturally occurring in vanilla beans. The main objectives of the study were to characterize compounds derived from *Vanilla siamensis* Rolfe ex Downie for potential estrogenic properties. In the case, that for some compounds from *Vanilla siamensis* were positive screening results can be obtained to investigate the molecular mechanism by osteoblast cell.

1.2 Research Objectives

The main objectives of the study were:

1) To characterize compounds derived from *Vanilla siamensis* Rolfe ex Downie for potential estrogenic activities.
2) To investigate the effects of *Vanilla siamensis* Rolfe ex Downie extract on the proliferation, differentiation, and bone formation (as measured by mineralization of nodules) in human fetal osteoblast cell line (hFOB1.19).