CHAPTER I

INTRODUCTION

Increase of phytoestrogens intake in order to counter menopausal symptoms and to lower incidence of hormone-dependent diseases such as cardiovascular diseases and cancer is widely used in women. Phytoestrogen research has identified a great impact in plant consumption by humans. Most of the results concluded that routine consumption of phytoestrogen-rich plant products would benefit the body for all ages and sexes, mostly in terms of cancer protection (Murkies et al., 1998). Among the phytoestrogen-rich plants, *Pueraria mirifica* (PM) with a local name of "white Kwao Krua" might be the most interesting one because it has a great effect for rejuvenation and aphrodisiac (Bradbury and White, 1954). The crude ethanol plant extract as well as the powder were proven to be a candidate for estrogenic activity in both animal experiments and clinical trials (Muangman and Cherdshewasart, 2001; Sukawattana, 1940). However, its action in human breast cancer cells; MCF7 cells, has only ever been reported once which is the ability to antagonize estrogen action (Chansakaow et al., 2000a; Cherdshewasart, 2004).

PM consists of many phytoestrogenic compounds such as miroestrol, genistein, daidzein, coumestrol, puerarin, mirificin, kwakhurin and mirificoumestan. Interestingly, miroestrol, puerarin, mirificin and kwakhurin are found only in PM whereas genistein, daidzein or coumestrol are generally found in many plants such as soybeans or grape seeds (Chansakaow et al., 2000a; Makela et al., 1999). Deoxymiroestrol and its derivative miroestrol have been reported as phytoestrogens with high estrogenic potency (Chansakaow et al., 2000b).

Indeed, phytoestrogens are secondary metabolites produced in a wide variety of plants that induce biological responses in vertebrates and can mimic or modulate the actions of endogenous estrogens, usually by binding to estrogen receptors (ERs) (Chansakaow et al., 2000a; Makela et al., 1999). Phytoestrogens have the ability to bind the ER because of their biphenolic structure and they can act like partial ER agonists or antagonists. The varying degrees of agonist-antagonist activity of phytoestrogen depend on the cell types, target genes and types of ER distributed in the tissues (Benassayag et al., 2002; Paech et al., 1997). The two ERs,

ER-α and ER-β revealed and appeared to have unique tissue distributions and their own sets of specific functions. ER-β has been found to have a wide tissue distribution with the richest expression in the central nervous system, cardiovascular system, mammary glands, colon and reproductive organs (Enmark et al., 1997). In many organs including bone and mammary gland, ER-β acts negatively to regulate cellular proliferation which is the effect mediated by ER-α. Therefore, the absence or decrease of ER-β by substances may cause many diseases including cancer. In addition, there is great interest in identifying ER-β specific agonists for therapeutic uses in several diseases, including osteoporosis and breast cancer (Gustafsson, 1999). Several phytoestrogens show a pattern of differential binding to the two ER subtypes, for example genistein has been reported to bind more strongly to ER-β than to ER-α (Kuiper et al., 1998). Therefore, it was strongly suggested as the promising substance impacts on estrogen-sensitive cancer. Even though PM was recently suggested to benefit for treatment of estrogen-sensitive cancer such as breast cancer (Cherdshewasart, 2004), the mechanism of PM action on ER in the target organs is not known. Moreover, the effects of PM in normal reproductive cells have not been investigated.

From all above, it brought about the questions that: 1) What is the effect of ethanol-extracted *Pueraria mirifica* root on the proliferation of normal endometrial cells and endometrial cancer cells? 2) Is the estrogen receptor modulated by ethanol-extracted *Pueraria mirifica* root?

This study was then conducted based on the following hypothesis. 'Ethanol-extracted *Pueraria mirifica* root has the proliferative effect in normal endometrial cells and cytotoxic effect on endometrial cancer cells, and PM has modulator effect on the ER-α or ER-β expression.'

The objectives of the present study were:

1) To examine the proliferative effect of ethanol-extracted *Pueraria mirifica* root compared with daidzein, genistein and 17β-estradiol on the normal endometrial cells and RL-95, endometrial cancer cells.

2) To quantitate estrogen receptor protein expression in normal endometrial cells and RL-95, endometrial cancer cells treated with ethanol-extracted *Pueraria mirifica* root, daidzein, genistein and 17β-estradiol.