

CHAPTER I

INTRODUCTION

White Kwao Krua *Pueraria mirifica*, a well-known Thai herb becoming popular in Asia into commercialized development of the plant products in various forms including dietary supplements, traditional medicine, cosmetics and beverages. It was long recorded to be consumed as folklore remedies among Thai people with the purpose of improving human physical appearance such as skin enrichment, enlarging breast, re-growing hair, promote black hair, improving body complexion including hormone replacement therapy in menopause women (Suntara, 1931). The plant was found to contain high amount of phytoestrogens with potent estrogenic effect (Cain, 1960; Chansakaow *et al.*, 2000^b). The crude powder was confirmed to be an effective alternative treatment for menopausal symptoms (Muangman and Cherdshewasart, 2001) with negative results of toxicity tests in animals and humans (Cherdshewasart, 2003). In addition; the cellular action of the plant extract needed metabolic activation (Lee *et al.*, 2002) with anti-proliferation effects to HeLa cells and MCF-7 (Cherdshewasart, Cheewasopit and Picha, 2004^{a,b}).

Analysis of the plant active ingredients by HPLC is an accepted analytical method for herbal materials used in preparation of traditional medicines, dietary supplements, plant extracts as well as cosmetics. Thus this method has been adapted for studying the isoflavonoid contents in *P. mirifica* and resulted in finding of highly diversified isoflavonoid contents in *P. mirifica* population (Cherdshewasart, Subtang and Dahlan, 2007).

According to a large-scale survey of the distribution and diversity of the plant since 1998, at different locations are confirmed to be exist habitat of the plants (Cherdshewasart, Subtang and Dahlan, 2007) with a varied degree of estrogenic activity in vaginal cornification epithelium proliferation in ovariectomized rats (Cherdshewasart, Kitsamai and Malaivijitnond, 2007), antioxidant activities (Sutjit, 2003) and anti-proliferation effects to MCF-7 (Trisap, 2003).

The cultured cells could act as model of physiological function *in vivo*. The estrogen dependency of human breast cancer has been used to study the role of estrogenic organic compounds from plants. MCF-7 is an estrogen receptor alpha-positive human breast cancer cell line. Several studies have shown that low

concentrations of phytoestrogens could induce proliferation of MCF-7 cells. Conversely, high concentrations of phytoestrogens could inhibit proliferation of human breast carcinoma cells (Trisap, 2003). The vaginal cornification assay and uterotrophic assay in the ovariectomized rats are chosen as the criterion of the estrogenic activities in the present study, because they are sensitive, simple and inexpensive methods.

This study will focus on the seasonal analyzed isoflavonoid contents in relation with antioxidant assay, estrogenic activities by MCF-7 cell proliferation assay, vaginal cornification assay, uterine weight assay, uterine gland number assay, cross section area of uterine tissue assay in ovariectomized rat.

Purposes of the studies are as follows:

- To evaluate isoflavonoid contents from five clones of *P. mirifica* tubers collected in three seasons in 2005 by HPLC.
- To evaluate the cytotoxicity of seasonal collected tubers from five clones of *P. mirifica* extracts by MCF-7 cell proliferation assay.
- To evaluate the estrogenic activity of seasonal collected tubers from five clones of *P. mirifica* extracts by using vaginal cornification assay, uterotrophic assay, uterine gland number assay and the cross section area of uterine tissue assay in ovariectomized rats.
- To correlate isoflavonoid contents with temperature and rainfall amount of the field trial site.
- To correlate isoflavonoid contents with estrogenic activities of the plant samples.