

CHAPTER I

INTRODUCTION

Pueraria mirifica (Airy Shaw et Suvatabandhu, Kashemsanta and Suvatabandhu, 1952), domestically named White Kwao Krua, is an indigenous Thai herb. It has long been popularly consumed among Thai people, especially among menopausal women as a hormone replacement therapy (Suntara, 1931). The plant is widely found in Thailand, particularly in the deciduous forests in the northern part of Thailand. The plant is actually easily to be accessed by the villagers who gather its tuber parts for a preparation of traditional drugs.

The tuberous roots of *P. mirifica* had been analyzed for the chemicals containing by chromatography technique. It contains at least 13 known chemicals classified as phytoestrogens, such as miroestrol, deoxymiroestrol, daidzein, genistein, coumestrol, puerarin, kwakhurin and mirificin (Kashemsanta *et al.*, 1957; Pisetpakasit, 1976; Ingham *et al.*, 1986; 1988; 1989; Chansakaow *et al.*, 2000^a, 2000^b). Miroestrol exhibited estrogenic effect as reported in immature female mice (Jones and Pope, 1960) and ovariectomized rats (Benson *et al.*, 1961).

According to a large-scale survey on the distribution and diversity of *P. mirifica* since 1998, at least 28 provinces are confirmed to be existing habitat of the plants (Cherdshewasart, 2003^a). In addition, the recent studies found that *P. mirifica* collected from the different locations showed the different patterns in Thin Layer Chromatography band (Panriansaen, 2000). The data from High Performance Liquid Chromatography fingerprint also showed that *P. mirifica* collected from 28 provinces exhibited a highly variation in isoflavone content (Subtang, 2002). Using MCF-7 proliferation assay, *P. mirifica* collected from 28 provinces exhibited variation in proliferative effect (Trisap *et al.*, 2003).

P. mirifica has been postulated to have effects on reproductive organs. *P. mirifica* powder prepared as a crude form has shown the potential of an alternative treatment of estrogen replacement therapy for menopausal women (Muangman and Cherdshewasart, 2001). Ethanol extracted plant showed effectiveness as uterotrophic agents in both animal and clinical studies (Sukhavachana, 1949). Most reports about the effects of *P. mirifica* on reproductive organs have done only in *P. mirifica* few strains. The recent study has tested the estrogenic activity of *P. mirifica* collected from 3 provinces; Prachuab Khirikhan, Saraburi and Chiang Mai and found the variation in vaginal epithelium proliferation in ovariectomized rats (Chansri, 2002).

The present study extended those observations by comparing the estrogenic activity of cultivated *P. mirifica* (PM-III and PM-IV) collected from Ratchaburi province. To minimize the environmental factors variation of phytoestrogens content in *P. mirifica* cultivar PM-III and PM-IV, the tuberous roots used in this study were collected in rainy, winter and summer seasons, totally 12 months (From March 2005 to February 2006). The vaginal cornification assays in the ovariectomized rat were used to qualify the estrogenic activity. Vaginal cornification assay in ovariectomized rats is chosen as the criterion of the estrogenic activity in the present study. Vaginal cornification assay were widely used to evaluate the estrogenic activity of the synthetic estrogens (Ashby *et al.*, 2000; Diel *et al.*, 2001; Stroheker *et al.*, 2003; Wuttke *et al.*, 2003), xenoestrogens (Stroheker *et al.*, 2003) and phytoestrogens (Shukla *et al.*, 1987; Diel *et al.*, 2001; Balk *et al.*, 2002; Okazaki *et al.*, 2002; Chiechi *et al.*, 2003). In addition uterine gland number assay and cross section area of uterine tissue assay in ovariectomized rats is chosen as the criterion of the estrogenic activity in the present study, too, because it is sensitive, simple and inexpensive methods.

An expected benefit derived from this study is to localise the season that showed the highest and lowest activities of *P. mirifica*. Thus, collecting and preparing *P. mirifica* for the further use, applicable for human use, can be practically designed the suitable administrated dosage for the expected results. The plant materials are ranked according to their estrogenic activity the plant breeders to establish the best candidate of plant clone for plantation at a commercial scale.

The aim of this study is as follows;

This study aimed to investigate the estrogenic activity of cultivated *Pueraria mirifica* (PM-III and PM-IV) collected in 12 months, using vaginal cornification assay, uterotrophic assay, Uterine gland number assay and the cross section area of uterine tissue assay in ovariectomized rats.