CHAPTER I
INTRODUCTION

1. Rationale and background

Pueraria candollei or White Kwao Krua is a commonly known as Thai herbal medicine belonging to family Leguminosae, subfamily Papilionoideae. In Thailand there are two varieties of *P. candollei* , *P. candollei* Wall. ex Benth var. *mirifica* (Airy Shaw & Suvat.) Niyomgham (PM) and *P. candollei* Wall. ex Benth var. *candollei* (PC) (Smitinand, 2001). These two varieties have similar botanical characteristic but both vary in chemical component containing (Yusakul et al., 2011). Their tuberous roots contain chemical compounds, known as phytoestrogen, such as major isoflavonoid and their glycoside (daidzin, daidzein, genistin, genistein and puerarin), coumestan, pterocarpan and minor chromene (miroestrol, deoxymiroestrol and isomiroestrol). Deoxymiroestrol possess the highest estrogenic activity among the known phytoestrogens due to structural similarity to 17β-estradiol (E2), the main estrogen in the human body.

Local communities in Thailand have used White Kwao Krua for well over one hundred years, specifically for its rejuvenating qualities. It is stated in the pamphlet (Suntara, 1931) that *P. candollei* can serve as skin moisturizer, improve growth of hair, improve body flexibility and sexual performance, firm and enlarge the breasts.

According to the various phytoestrogens containing in tuberous roots of *P. candollei*, the estrogenic activities were investigated in cell line, animals and in clinical trial. The studies of estrogenic activities of this plant affected on both female and male animals sexual reproductive organs (Sukhavachana, 1941; Jones et al., 1961; Malaivijitnond et al., 2006; Trisomboon et al., 2007a, 2007b; Cherdshewasart et al., 2007a, 2007b), prevention of bone lose (Urasopon et al., 2007a, 2007b) and reducing postmenopausal symptoms (Lamlertkittikul and Chandeying, 2004 and Chandeying and Sangthawan, 2007; Muangman and Cherdshewasart, 2001; Hosoyama et al., 2007). Furthermore the antioxidant and antihyperglycemic activities (Cherdshewasart and Sutjit, 2008 and Khitkal et al., 2009) were also investigated.

Isoflavonoid are phytoestrogens commonly found in family Leguminosae or soy bean and pea subfamily. In *P. candollei* also found isoflavonoid, the major compounds containing in tubers roots of this plant. The pharmacological activities of isoflavonoid were reported to decrease the incident of the breast, prostate and intestine cancer (Watanabe et al., 2002), improve hot flushes and frustration in postmenopausal women (Taylor, 2003), preventive cardiovascular disease in term of decrease LDL and increase HDL level (Davis et al., 1998) and also reduce blood sugar level in STZ-diabetic rats (Khitkal et al., 2009).

Miroestrol and its derivative like deoxymiroestrol and isomiroestrol are a chromene compounds which possess highest estrogenic activity among the known phytoestrogens due to structural similarity to 17β-estradiol (E2). As reported previously (Matsumura et al., 2004), the estrogenic activity of deoxymiroestrol and miroestrol when compared to 17β-estradiol in proliferation of MCF-7 cell was estimated to be about 0.3 and 0.05 fold after 7 days, respectively. While in proliferation of MCF-7 cell after 14 days, estrogenic activity of deoxymiroestrol and miroestrol was about 1 and 0.25 fold of 17β-estradiol, respectively. In human trial (Cain, 1960), ten women with postmenopausal symptom were treated orally with 1 mg or 5 mg of pure miroestrol for 14 days. Both dose levels caused significant vaginal cornification as well as effects such as an enlargement but even small quantities of miroestrol cause unacceptable side effects (malaise, headaches, nausea/vomiting) in the majority of patients. Although Chansakaow et al. (2000b) found very low content of miroestrol and its derivative in *P. candollei* var. *mirifica*, each compound estimated to be about 0.002% dry weight compare with other isoflavone like genistein to be about 0.040 % dry weight but both miroestrol and deoxymiroestrol having potent estrogenic activity. Thus when claim to *P. candollei* var. *mirifica* the most estrogenic activity was affected from predominance of miroestrol derived from deoxymiroestrol or, more probably, the combined effects of both compounds.

Commercial products from *P. candollei* are continually introduced into the world market and have become popular in Thailand, Korea, and Japan. Most commercial products are available as topical rejuvenating, antiaging, or skin-lightening creams/gels, soaps, capsules or tablets. Until now domestic and global demand for the

raw materials from *P. candollei* roots or tubers has increased, resulting in intense harvesting of the plant and encroach on forests of Thailand. Concerning about this plant species is liable to extinct and exported their seeds to rivalry countries. Therefore White Kwao Krua is announced to be one of conserved plant lists in Plant Offspring Act B.E. 2518. (1975). The Act is convention on international trade in endangered species of wild fauna and flora.

Normally it is waste time on White Kwao Krua cultivating until harvesting season at least three years. And consequence of Cherdshewasard et al. (2007b) found the variation of isoflavonoid containing in each *Pueraria* spp. depend on their cultivation and harvesting season. Nowadays the application of plant tissue culture techniques in herbal medicines is extensively, in case of enhancing pharmacologically active compounds in medicinal plants. Due to getting a highly and continuously production of plant secondary metabolite with quality control, many techniques were used improving active compounds accumulation in plant such as elicitation, immobilization, permeabilization and genetic modification or controlling biosynthesis pathway. Moreover using plant tissue culture techniques for White Kwao Krua cultivation was decreased harvesting time and also protection endangered species of this plant.

Cell suspension and hairy root culture are alternative method to produce isoflavonoid and chromene because characteristic of cell suspension and hairy root is fast growth and continuously produce plant secondary metabolite. Previously Udomsuk et al. (2009b) reported total isoflavonoid content in *P. candollei* var. *candollei* (PC) hairy root culture higher than that found in native root.

Elicitation is one of extensively techniques to enhance secondary metabolites production in plants tissue culture. According to no appropriate determination method of chromene compound until Yusakul et al. (2010) have published HPLC method, therefore only studies on abiotic and biotic elicitors enhancing isoflavonoid production in both varieties of *P. candollei* culture have been reported (Thanonkeo, 2006; Khitkal et al., 2009; Udomsuk et al., 2009a, 2009b, 2010, 2011; Boonsnongcheep et al., 2010; Korsangruang et al., 2010).

Furthermore using of plant hormones as elicitors to increase flavonoid and terpenoid production in various plants have been studied (Cristiane et al., 2009;

Inthima et al., 2009; Nagira et al., 2006; Gagne' et al., 2010, Sun et al., 2011). Recently, production of isoflavonoid in callus culture of *P. candollei* var. *mirifica* (PM) was reported to be influenced by plant hormone, thiadiazuron (Udomsuk et al., 2010). Therefore using plant hormone to enhance isoflavonoid production in this plant is interesting.

In the present study, (1) we established cell suspension and hairy root culture of *P. candollei* var. *mirifica* (PM) and optimal conditions for growth, and investigated the effects of plants elicitors (methyl jasmonate, yeast extract and chitosan) on enhancing isoflavonoid and chromene accumulation in cell suspension and hairy root culture of *P. candollei* var. *mirifica* (PM). (2) We also investigated the effects of plant growth regulator N-(2-chloro - 4- pyridyl)-N- phenylurea (CPPU), thidiazuron (TDZ) and abscisic acid (ABA) on plant growth and total isofavonoid production in hairy root culture in both varieties of *P. candollei* culture. (3) Finally we comparative studied on isoflavonoid and chromene production between hairy root and native root in both varieties of *P. candollei*.

2. Objectives of the study

2.1 To establish the callus and cell suspension of *P. candollei* and compare chromene and isoflavonoid content in cell suspension culture of *P. candollei* var. *mirifica*.

2.2 To establish the hairy root of *P. candollei* and compare chromene and isoflavonoid content in hairy root culture of *P. candollei* var. *mirifica*.

2.3 To investigate the optimal conditions, effect of elicitors and plant hormone on chromene and isoflavonoid production in cell suspension and hairy root culture of *P. candollei* var. *mirifica*.

3. Expected outcomes

The establishment of optimal conditions possibility led to high producing of chromenes and isoflavonoid content in cell suspension and hairy root culture of *P. candollei* var. *mirifica*.

4. Place for study

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