CHAPTER VI
CONCLUSION

Pueraria candollei or White Kwao Krua is a commonly known as Thai herbal medicine used for its rejuvenation. Two plants varieties were found in Thailand (*P. candollei* var. *mirifica* and *P. candollei* var. *candollei*). Their tuberous roots contain bioactive compounds, known as phytoestrogen, such as major isoflavonoid and minor chromene (miroestrol, deoxymiroestrol and isomiroestrol).

Deoxymiroestrol possess the highest estrogenic activity among the known phytoestrogens due to structural similarity to 17β-estradiol. 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 (Matsumura et al., 2004). The pharmacological activity of isoflavonoid were reported to decrease the incident of the breast, prostate and intestine cancer (Watanabe et al., 2002), improved hot flushes and frustration in postmenopausal women (Taylor, 2003), preventive cardiovascular disease (Davis et al., 1998) and also reduce blood sugar level in STZ-diabetic rats (Khitkal et al., 2009).

Many commercial products of *P. candollei* are available in market. Nowadays domestic and global demand for the raw materials from *P. candollei* tubers has increased, resulting in intense harvesting of the plant and encroach on forests of Thailand. Consequence of Cherdshewasard et al. (2007b) found the variation of isoflavonoid containing in each *Pueraria* spp. depend on their cultivation and harvesting season. The application of plant tissue culture techniques was used for enhancing highly and continuously production of plant secondary metabolite with quality control and also protection encroach on forests of Thailand. Many technological advances have been made in several cases and cell cultures have been shown to produce higher amounts of the products than the intact plants from which they are derived.

Until now we have successful established the callus of *P. candollei* var. *mirifica* which were derived from stem explants in MS medium supplemented with 0.1 mg/l TDZ, 1 mg/l NAA and 0.5 mg/l BA. Characteristic of cell suspension was green, friable and rapid growth. Although the growth pattern of cell suspension culture was similar to previously report. But after several subculture cause of callus instability due to decreasing of the chromenes and total isoflavonoids accumulation in cell suspension at the 4th week from our study about 2 and 5-fold, respectively, compare with Udomsuk et al. (2011).

The hairy roots of *P. candollei* var. *mirifica* have been established using *Agrobacterium rhizogenes* ATCC 15834 transformation. The hairy root was fast growth and lateral branching. Hairy root were produced the highest chromene content 9.17±0.73 µg/g dry wt. and total isoflavonoid content 9.56±0.29 mg/g dry wt at the last third week.

Previously many studies reported the use of elicitors to enhance only isoflavonoid in *Pueraria* spp. In this present study we found effect of elicitors on chromene and total isoflavonoid production in both cell suspension and hairy root culture. Although the level of total isoflavonoid accumulation in cell suspension culture was lower than previously reports about 5-fold (Korsangruang et al., 2010 and Udomsuk et al., 2011). However elicitors enhanced both compounds can be observed. Moreover, only deoxymiroestrol, the highest estrogenic activity, can be found in our experiment. In cell suspension culture 200 µM methyl jasmonate significantly increased highest chromene production after 3 days of elicitation (11.61±0.55, dry wt, 2-fold higher than control). Yeast extract at 0.5 mg/ml for 6 days showed the highest enhancing of total isoflavonoid content (9.61±0.50 mg/g dry wt, 3-fold higher than control). For hairy root culture, the results found 200 µM methyl jasmonate and 0.5 mg/ml yeast extract significantly increased chromene production after 3 days and 6 days of elicitation, respectively. While 200 µM methyl jasmonate and 0.5 mg/ml yeast extract highly significantly increased total isoflavonoid production after 3 days of elicitation (16.06±1.28 and 14.65±1.44 mg/g dry wt, 2-fold higher than control, respectively). From our result chitosan was less effective elicitor on chromene and total isoflavonoid production in *P. candollei* var. *mirifica* cell suspension and hairy root culture. The variations of chromene and isoflavonoid production and treatment interval of

elicitation may be due to variation of both *P. candollei* varieties, characteristic of plant cell and also culture condition.

Furthermore, our present study demonstrates the use of phytohormone as enhancer for isoflavonoid production. As a result, addition various concentrations of CPPU and TDZ significantly increased the production of total isoflavonoid in both PM and PC hairy root cultures after 5 days and 10 days of elicitation. All treatments with ABA showed significantly increased the production of total isoflavonoid in PC hairy root culture. However, only of ABA 0.1 mg/l for 5 days significantly increased total isoflavonoid content in PM hairy root culture. Moreover, TDZ-treated PM hairy root and ABA-treated PC hairy root showed effect of dose response when increasing hormone-concentrations, the total isoflavonoid content was decreased.

The comparative analysis of each phytoestrogen between hairy root and native root found chromene and isoflavonoid biosynthesis pathway may be related. In chromene biosynthesis pathway, the prenylation reaction needs isoflavan which was the substrate derived from isoflavonoid biosynthesis pathway. These resulting in decreasing of substrate that was turn to isoflavonoid then seeing the biosynthesis alteration of two compounds content in this experiment. When compare the level of isoflavonoid content were not difference. This indicated that isoflavonoid biosynthesis can be occurred and accumulated in root part. Whereas higher chromenes accumulation in native roots than hairy roots, it is possible that its biosynthesis may take place in plastid of leaves part then being localized and accumulated to the root part. In addition, the structures of chromenes are more complex than those of isoflavonoid and chromene biosynthesis pathway still doubt. However, to confirm these findings we need further study in-depth.

Finally, we have successful established the callus, cell suspension and hairy root of *P. candollei* var. *mirifica* with ability to produce chromene and isoflavonoid. Although cell suspension culture is getting a highly and continuously production of bioactive compounds but it was somaclonal variation limitation after several subculture. Then hairy root culture was an alternative source because of its stability higher than cell suspension culture. Moreover we can use elicitors (methyl jasmonate and yeast extract) for increasing production of both compounds in cell suspension and hairy root culture of *P. candollei* var. *mirifica*. We also use of phytohormone (CPPU,

TDZ, ABA) as enhancer for isoflavonoid production in two varieties of *P. candollei* hairy root cultures.