CHAPTER V

CONCLUSIONS

In term of metabolomics study, the results demonstrated that the adapted extraction methods comprised of methanol, chloroform and butanol solvent is effectively in elimination of chlorophyll from the leaf crude extract and thus possible to determine for their major isoflavonoid contents by RP-HPLC. Only puerarin, daidzin genistin and daidzein were found in leaf crude extract. The data was different from the tuber crude extract in which genistein were also found. In addition, the tuber total isoflavonoid contents were found more than that of leaf in the 1-year harvested plant samples. In the biotechnological approach, a large scale production of puerarin, daidzin, genistin and daidzein from *P. mirifica* leaves is definitely possible. *P. mirifica* is a twinning plant that produces a lot of leaves during plant growth and differentiation. Even the plants were defoliated during late winter to early summer but cutting and watering could induce all year leaf production. Even though the tubers could accumulate 5 major isoflavonoids since the first year of age (Cherdshewasart & Sriwatcharakul 2007), the tubers were newly differentiated with small size. Normally at least 3 year-old tubers were required for harvest (Sibao et al 2007) while the leaves were produced since the first month of plant cultivation. . For the comparison of isoflavonoid yield between tuber and leaf harvested per year in general, the isoflavonoid yield harvested in leaf is higher than tuber 6.20 times. However, this conclusion is solely based on the extraction method with the main purpose of chlorophyll elimination. With the classical extraction method, eg., alcohol or water base, the yield of the tuber might be higher than that of leaf sample. Even though some of these chemicals are available from chemical synthesis but the cost is still high. Moreover, isoflavonoids from plant sources have advantage over those derived from chemical synthesis because they can be favor used in food and cosmetic industry. This will benefit to farmers to set the right harvest period for plant leaves. Moreover, metabolomics study of the plant leaves will enable to find more human benefit chemicals present in the leaves

Proteomic study of plant leaves and tubers were enlightening into key proteins or enzymes related to isoflavonoid synthesis and transformation, including glycosylation/aglycosylation of isoflavonoids in the two plant tissues. *P. mirifica* is a leguminous plant, which has not been investigated at the proteomics level. In despite of the plant, this study revealed the interesting proteome variance pattern among seasons. The protein function is categorized into 8 classes. The major protein

expression belongs to metabolism function, especially in summer. Mostly are involved with carbohydrate metabolism. For plant secondary metabolite involved proteins, this study will enlighten how the plant produced secondary metabolites proteins, especially in the seasonal isoflavonoid contents that correlated with isoflavonoid production-associated proteins expression among the seasonal change. The knowledge of major isoflavonoid production was revealed by the existence of proteins/enzymes in the biosynthesis pathway. Proteins involved in isoflavonoid biosynthesis were found including chalcone isomerase (CHI); isoflavone reductase (IFR); UDP-glycosyl transferase (UGT); and cytochrome p450, only in tuber. It might be possible that isoflavonoid were mainly synthesized within tuber rather than leaf. The result is in correlation with the metabolomic study in which isoflavonoids were found mainly in tuber not leaf. It is thus possible that the conversion aglycone to glycone is mainly occurs within tuber. The results were similar to the study in soybean leaf, in which there was no any protein involved in isoflavonoid biosynthesis detected (Xu et al 2006). The presence of UGT in tuber and leaf might confirm the hypothesis that isoflavonoid synthesis is occurred mainly in tuber not leaf. UGT itself functions as a key role in stabilizing the newly synthesized aglycone in the form of glycone isoflavonoid. The result is in correlation with the metabolomic study in which glycone isoflavonoids were found much more than aglycone isoflavonoids in tuber. It might be possible that there is no translocation of isoflavonoid from leaf (synthetic site) to tuber (accumulation site).