CHAPTER V

DISCUSSION

This study has investigated into total isoflvone content including puerain, daidzin, genistin, daidzein and genistein of both wild and cultivated *P. mirifica* in Thailand. According to the survey of distribution and diversity of the wild plants in the northern, north-eastern, central and southern part of Thailand, 29 provinces were found to be the sites of existing plants (Cherdshewasart unpublished data). With the hypothesis that chemical contents of the widely distributed plants may be differ from each other, the practical analytical method has to be adapted for the study to help clarify that variation.

In analytical point of view the novel protocol has been adapted from Oshima *et al.* 1988 with the changing of mobile phase by starting with 100 : 0 to 55 : 45 of 1.5 % (v/v) acetic acid : acetonitrile (45 min) gradient system instead of 95 : 5 to 50:50 (60 min) as previously described for shorten analytical time. The comparison for absorbance of isoflavone reference was performed at the wavelength of 254 nm instead of 280 nm (Table4, Figure 13). Four preparative methods including methanol, ethanol and water extract from powder of *P. mirifica* and spray dry from tuberous juice of the plant were also compared in this study. Methanol extract was found to be the most effective preparation from plant powder. It created highest amount of analyzed fingerprint including bands corresponding to 5 applied markers, puerarin, daidzin, genistin, daidzein and genistein. Spray dried tuberous juice exhibited the highest amount of isoflavone due to the fact that cell wall of the plant cells was totally discarded before submission of the tuberous juice to spray dry. This preparation method is recommended also for the purpose of establishment a standardized *P. mirifica* derived material for industrial use which is possible for both oral and topical consumed. It was found that the adapted HPLC protocol in occurrence with methanol extract of the material resulted in qualified analytical fingerprint and could be adapted for routine analysis of products derived from *P. mirifica* as demonstrated in detection of isoflavone from gamma-irradiated powder and chicken essence containing *P. mirifica* as one of the ingredient (Figure 107-108).

The widely distributed plants showed difference in isoflavone as analyzed in term of mg / 100 g powder and percentage of the fingerprint and individual ones including puerarin, daidzin, genistin, daidzein and genistein. The great difference between the sample with maximum and minimum amount of isoflavone content, 198.29 versus 18.85 mg / 100g powder of the samples from Kanchanaburi and Nan province, respectively should implied for the great difference in genetics and / or habitats of the two collected plants. The differences were observed among many samples. It might be possible to take more account into genetics difference as a key factor for such difference.

P. lobata, the well-known China-origin herb which exhibits elongated tuber with white meat similar to that of *P. mirifica* and is well grown in Thailand (Cherdshewasart unpublished data) showed less amount of isoflavone content (91.58 mg / 100g powder) than 5 collected samples but still greater than the mean value of the Thai *P. mirifica* population (80.79 mg / 100g powder). The comparison of individual isoflavone of *P. lobata* with the mean value of *P. mirifica* population revealed that there were 15 and 9 samples of *P. mirifica* contains significant higher amount of genistin and genistein than *P. lobata*, respectively. The chemical similarity of the isoflavone content might be related to the fact that *P. lobata* has been recently promoted as a key ingredient in cosmetics for breast enhancement as did by *P. mirifica* products. It might be a great interest to set up a comparative efficacy study between the 2 plant products to aid clarify this fact. It is possible to select for the clone with higher amount of isoflavone than *P. lobata* which should result in development of commercial products with more efficacy. Besides, there was no report on the presence of miroestrol and its derivatives in *P. lobata*. The difference in such chemicals as well as genistin and genistein might be related to the weaker estrogenic activity of *P. lobata* as compared with *P. mirifica.*

This study was mainly focused on provincial level for the diversity study of isoflavone. Sub-provincial (district) study was also performed in the provinces exhibiting different botanical characteristics of the plants. The samples from 5 districts in Chiang Mai province showed significant different isoflavone content from each other. The results implies that there are diversity of the active chemicals especially isoflavone in the plants. The analysis of samples collected from 3 districts in Lampang province showed slightly difference of isoflavone content but

significantly difference in each isoflavone content, namely puerarin and daidzein is observed. The analysis results of the samples collected from 3 districts in Kanchanaburi province showed the significant difference of isoflavone content among samples. The sample from Srisawat district showed the maximum amount as compared with all collected samples in Thailand. The results should result in changing the attitude of Thai people that *P. mirifica* from the northern part of the country especially Chiang Mai province is the best quality. The samples collected from 3 different sites in 2 districts of Saraburi provinces also showed the significant different in isoflavone content among samples. The results of this study confirmed the hypothesis that there is a variation in active chemicals of *P. mirifica*. Such a variation is not only provincial but also sub-provincial level. This observation should imply that there may be a widely genetic difference among sub-population of the plant even their distribution are not great apart.

To evaluate for the seasonal influence, 2 clones were cultivated in the same field at Ratchaburi province. Both clones showed difference in isoflavone content especially in clone Doi Tao with significant different in all 3 seasons. Tubers collected in the rainy season was less age than winter and summer but it showed highest amount of isoflavone in clone Doi Tao. It should imply that the harvest of tuber in clone Doi Tao for commercial uses should be done in the rainy season. Clone Chaiprakarn showed highest amount of isoflavone in winter not the rainy season. It should imply that tubers harvesting in this sub-clone should be done in winter. Besides, different genetics of the plant may highly influence the period of maximum tuber storage of isoflavone.

Clone Doi Tao cultivated in 3 provinces and harvested in the same summer showed significant different in isoflavone content with the maximum yield from the field trial in Ratchaburi province. Clone Chaiprakarn cultivated in 2 provinces and harvested in the same summer showed no statistical difference in isoflavone content. The results determined that apart from genetics, environment and physical factors of the soil plays a great role in synthesis and accumulation of active compounds especially isoflavone in the plants which could be seen clearly in clone Doi Tao but not Chaiprakarn.

To confirm for the genetics influence, 3 F_1 Doi Tao as well as Sai Yoke was cultivated in the same field at Ratchaburi province at the same day in the rainy season and harvest at the same day in summer. The one year old plants showed varied amount of isoflavone in both sub-clone. It implied that there was a great genetic variation among F_1 as they were seed-derived plants. *P. mirifica* exhibits large-size inflorescence with large number of florets. Sexual reproduction in the population should result in a great genetic diversification and the results were clearly seen in term of isoflavone of the tubers. Genetics diversification was also observed with the difference in mean value of isoflavone between the 2 clones in which F_1 of clone Sai Yoke exhibited higher amount of isoflavone.

Even the influence of genetics of the plant itself as well as environment such as physical and chemical properties of the soil and seasonal change was clearly understand in this study, one more factor was also observed. The 2 year old sub-clone Doi Tao which derived asexually from the same mother plant, cultivated in the same field with no difference in all investigated factors, including genetics, physical and chemical properties of the soil and season, still exhibited different level of isoflavone and some isoflavone content such as daidzein (Table 23-25, Figure 38-40). One main reason is the factor from tubers. One plants exhibits numerous roots which did not differentiate into tuber at the same time and the same size. The different in differentiation status of the tuber itself should take responsibility in different accumulated amount of macromolecules such as starch, protein, lipid as well as active chemicals including isoflavone. This observation should remind us that there is no any chance to produce homogeneous active chemicals from the wild and cultivated plants. Regarding miroestrol and deoxymiroestrol, they were not submitted to reference standard in this experiment according to the lack of commercial standard chemicals. If it is available in the future, all established isoflavone HPLC fingerprint could be analyzed again to aid fulfill the results of this study.

HPLC fingerprint analysis for *B. superba* in this study was not well clarified as shown in *P. mirifica* and *P. lobata.* The HPLC condition should be more validate to obtain the higher resolution of the fingerprint. Even though the method was not yet satisfied, diversity of the chemicals could be seen among samples. Some isoflavone were found present in sample from Ratchaburi province but not the others. The amount of common peaks was found in different percentage as well. We decide to select only 4 samples covered different part of Thailand to demonstrate the chemical

diversity in the plants. Further HPLC analysis should be adapted to the other collected samples for full information establishment together with bioassay for all samples.

Sample collection of *M. collettii* was successive only from 3 provinces. The adapted HPLC analysis conditions seemed to fit into the chemical analysis of the plant. Attempts was made to introduce 2 commercial markers for the analysis but the standard chemical could not present as nice peaks in our conditions. The diversity of chemicals could be relatively evaluated. Samples from Chiang Rai province showed highest total area peak as compared with samples from Lampang and Kanchanaburi province. The 2 common peaks were found with the highest concentration in the samples collected from Chiang Rai province. The analytical data confirmed the diversity in the plant chemicals.