

## CHAPTER VI

### CONCLUSIONS

- It is found that five clones of *P. mirifica* exhibits highly variation in isoflavonoid contents including puerain, daidzin, genistin, daidzein and genistein. Such a variation is affected by various factors including genetics and environmental factors.
- *P. mirifica* collected from three seasons showed significantly different in the bioassays. The difference in harvesting season was convincing to be the main cause.
- Climatic factors; amount of rain and temperature were higher influence than plant genetic on the tuberous isoflavonoid contents.
- The five clones exhibited difference in MCF-7 cell proliferation, PM-I, PM-II and PM-V collected in summer and PM-III collected in winter have proliferation effect while PM-II collected in winter has anti-proliferation effect.
- MCF-7 proliferation assay was much influenced by the presence of S9 mixture which could increase the proliferation response.
- It had been confirmed that *P. mirifica* phytoestrogens influence the vaginal epithelium, part of the female reproductive organ, in the ovariectomized rats.
- The estrogenic potency was ranked by 2 parameters; day of appearance of cornified cell and duration of cornified cell. From this study it was found that at the dose of 1000 mg/kg BW all clones collected in winter and PM-III collected in summer and rainy season, at the dose of 100 mg/kg BW PM-III collected in winter exhibited the strongest estrogenic activity.
- The uterus weight was increased in a dose dependent manner. It exhibited highly estrogenic activity, especially at the dose of 1,000 mg/kg BW.
- In the analysis of endometrial tissue, it was found that uterus in the treatment with *P. mirifica* showed higher degree of glandular proliferation as well as endometrial area while the lumen was smaller. It is a first time demonstration that phytoestrogen treatment could initiate a better quality differentiation of uterine endometrium.

- This study should be a conventional guide to select the plant with a comparable estrogenic effect with  $17\beta$ -estradiol. This will open a possibility to introduce the plant product to be used for an alternative to ERT (Estrogen Replacement Therapy) in menopausal women.

### **Perspectives of the studies**

The results from the study should be an applicable guide to select plant materials or plant clones for plantation of *P. mirifica* for commercial scale and to harvest tubers with high isoflavonoid contents or estrogenic activities. The effective harvesting period can be monitor by HPLC analysis of tuberous isoflavonoid storage. The bioassay methods are not complicate and expensive but practical methods. The simplified method of ovariectomized rats in this study is a conventional method with a similar pattern as use as human oral consumption of plant products. Evaluation for the high quality material as a key of success in the comercialized products development and to ensured for the efficacy and safety of such products, the more data still needed.

The influence of climatic factors; temperature and amount of rainfall on isoflavonoid contents in tubers collected in three seasons give sketchy knowledge so should collected samples in monthly to complete the data.