Chapter V

Discussion

P. mirifica collected from 28 provinces, *B. superba* collected from 24 provinces and *M. collettii* collected from 4 provinces, were analyzed for proliferative effect in MCF-7 cell cultures. There were 16 samples of *P. mirifica* express proliferative effect at 1 µg/ml, including Phitsanuloke, Nakhon Sawan, Phetchabun, Sukhothai, Nan, Chiang Rai, Chiang Mai, Prachuap Khiri Khan, Ratchaburi, Mae Hong Son, Lamphun, Chumphon, Phrae, Chaiyaphum, Lop Buri and Prachinburi There was only one sample of *B. superba*, Mae Hong Son express proliferative effect at 0.1 µg/ml. There was no *M. collettii* extract that express estrogenic activity.

Plant extracts were tested for anti-proliferative effects in MCF-7 cell cultures. There were no *P. mirifica* that exhibited IC_{50} in the range of 1,000 µg/ml. Kanchanaburi, Ratchaburi, Tak, Phitsanulok, Srisaket, Khon Kaen, Nakhon Ratchasima, Chachoengsoa, Petchabun, and Uttharadith samples of *B. superba* express anti-proliferative effect with IC_{50} less than 1,000 µg/ml. All *M. collettii* extracts expressed anti-proliferative effect with IC_{50} much more less than 1,000 µg/ml.

Comparison of Mean±S.E. of anti-proliferative effects of *P. mirifica*, *B. superba* and *M. collettii*, it revealed the possibility only in the comparison of *B. superba* and *M. collettii*. Because IC_{50} of *P. mirfica* was over 1,000 µg/ml. *M. collettii* population shows 2.39 times stronger IC_{50} than *B. superba* population.

In the study of the influence of genetics and seasonal on proliferative and anti-proliferative effects of *P. mirifica*. There was a statistical difference only in samples collected during rainy season from Chaiprakarn clone that showed higher anti-proliferative effect than Doi Tao clone. The results implied that the seasonal, not genetics, factor could affect the anti-proliferative effect of the plant extract

P. mirifica extract showed proliferative effect at the low concentrations and anti-proliferative effect at higher concentration (biphasic effect) on ER^+ breast cancer cell line, MCF-7 (Chewasopit 2001). The response pattern was similar to that of phytoestrogen such as genistein and daidzein (Wang and Kruzer, 1997; Zava and Duwe, 1997; Constantinous *et. al.*, 1998; Shao *et. al.*, 2000). Therefore the results from the experiment could confirm that *P. mirifica* extract contained phytroestrogens. In this study we found only proliferative but not anti-proliferative effect that exhibited by *P. mirifica* extracts. The difference of the results might derive from the difference in the shorter incubation time which was 3 days in this experiment as compared with 4 days in the previous study. Besides MTT was submitted to this study whereas protein analysis was submitted in the previous study with the same purpose of cell growth analysis.

The proliferative effect at low concentration might depend on the presence of ER because the effects of phytoestrogens on ER^- such as MDA-MB-231 showed no-proliferation. (Shao *et. al.*, 2000) Due to the fact that phytoestrogens could bind to estrogen receptor of MCF-7, two pathways were suggested in the marked inhibition of cellular proliferation at high doses phytoestrogens. One group suggested that the inhibitory effect was ER independent because the antiproliferative effect was shown in both ER^+ and ER^- cells. (Shao *et. al.*, 2000). It was related to the inhibition of DNA topoisomerase II, tyrosine kinase activity of growth factor (Osborne, 1999: This *et. al.*, 2001). But the other showed that the growth inhibitory effects of high extract concentrations were much higher in the presence of estradiol (Shao *et. al.*, 2000). This indicating that the extract might act in ER^+ cell the ER pathway.

B. superba tubers were submitted to proliferative and anti-proliferative response test on MCF-7 cells. There was difference in such response among different samples. Such differences might resulted from the difference in chemical composition and environment among samples (Concerdia *et. al.*, 1999 and Wang, 1994). We could classify *B. superba* in our study into 2 groups, the first group with proliferative effect. It might be a result of chemicals or phytoestrogens presence only in that sample. There was a report that phytoestrogen was found in *B. superba*;

Daidzin, Genistin and Daidzein. *B. superba* extract exhibited a significant difference increased in vaginal weight ($p< 0.01$) but no significant in uterine weight while the uterotrophic assay in *P. mirifica* extract brought about significant increase ($p<0.01$) in each weight of uterus and vagina (Kim *et al.* 2003). The second group of *B. superba* was without proliferative effect. Its chemical constituent contained few or no phytoestrogens.

The anti-proliferative effect of *B. superba* extract on MCF-7 cells might derived from the anti-cancer agent which categorized into the group of phytosterols such as β-sitosterol, campesterol and stigmasterol (Awad and Fink, 2000 and Tapiero, Townsend and Tew, 2003).

M. collttii showed no proliferative effect but strong anti-proliferative effect on MCF-7 cell line. The strong anti-proliferative effect might derive from quercertin and hopeaphenol (Wutteeraphon *et. al.*, 2001). The two chemicals exhibited low IC_{50} in cancer cell line (MCF-7 and KB) (Wang and Kuzer, 1997). The increments of kaempferol in high dose might directly increase the cytotoxic effect (Wang and Kuzer, 1997; Zava and Duwe, 1997).

The tested plant extracts showed difference degree of proliferative and anti-proliferative response. At 1 µg/ml, *P. mirifica* expressed the highest proliferative effect. At 1,000 µg/ml, *M. collettii* expressed the highest anti-proliferative effect. Such responsive difference might be due to the difference in chemical ingredient of the tested plant extracts themselves.