Chapter V

Discussion

MCF-7

The classical estrogenic response (Wang *et. al.*, 1996) to estradiol was showed in **Figure 8**. At low concentration (10^{-12} - 10^{-10} M), the proliferative effect was showed with the maximum response but not significant proliferation at 10^{-11} M. At high concentration, the antiproliferative effect (cytotoxic effect) was showed at the concentration of 10^{-4} M. The ED_{50} was not calculated as this experiment was aimed to find out the optimum concentration for plant extracts and estradiol combination.

P. mirifica extract showed proliferative effect at the low concentrations and antiproliferative effect at higher concentration (biphasic effect) on ER^+ breast cancer cell line, MCF-7. The response pattern was similar to that of phytoestrogen such as genistein and daidzein (Wang *et. al.*, 1996; Wang and Kruzer, 1997; Zawa 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

The effects of *P. mirifica* extract were similar to that of phytoestrogens as the effect of the extracts on MCF-7 were found to be depended on the concentration of the extract in the culture medium (Pagliacci *et. al.*, 1994: Constantinous *et. al.*, 1998). 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 showed in both ER^+ and ER^- cells. (Wang *et. al.*, 1996; Shao *et. al.*, 2000). It was related to the inhibition of DNA topoisomerase II, tyrosine kinase activity of growth factor (Markovits *et. al.*, 1989: 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.

P. lobata, which was reported to contain high amount of puerarin as well as daidzein (Kaufman *et. al.*, 1997: Gurerry *et. al.*, 2000) but showed no proliferative effect. It might result from the absent of miroestrol and its derivative which was reported to be a potent estrogen agonist. (Bound and Pope, 1960: Jones and Pope, 1960: Chansakaow *et. al.*, 2000).

B. superba showed surprising no proliferative effect because its chemical constituent contained no phytoestrogens. The antiproliferative effect of the extract on MCF-7 cells might derived from the fact that the chemical constitute contained the anti-cancer agent which categorized into the group of phytosterols such as β-sitosterol, campesterol and stigmasterol (Raksilapa, 1995; Awad, Downie, and Fink, 2000; Awad, Downie, Fink and Kim, 2000; Awad, Williams and Fink, 2001).

M. collttii showed no proliferative effect with strong antiproliferative effect on MCF-7 cell line. The strong antiproliferative 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;Oyama *et. al.*, 1999) The increments of kaempferol in high dose extract might directly increase the cytotoxic effect. (Wang and Kuzer, 1997; Zava and Duwe, 1997)

The tested plant extracts showed difference degree of proliferative and antiproliferative response. At 1 μg/ml, *P. mirifica* expressed the highest proliferative effect. At 1000 μg/ml, *M. collettii* expressed the highest antiproliferative effect. Such responsive difference might be due to the difference in chemical ingredient of the tested plant extracts themselves.

P. mirifica extract showed high estrogenic and high cytotoxic. At cytotoxic dose (high dose), the combination of the plant extract with estradiol showed the increment of cytotoxicity. In the presence of estradiol, phytoestrogens behaved as a competitive inhibitor with low affinity for the binding of ER and thus decreasing the proliferation effect of the eatradiol (This *et. al.*, 2001)

P.lobata extract showed weak estrogenic effect as well as weak cytotoxic effect. Thus the combination of the extract with estradiol showed non-significant response on both cellular proliferation and cytotoxicity

B. superba extract showed no proliferative effect at low concentration but cytotoxic effect at high concentration. The chemicals content in *B. superba* were found to be in the category of flavonoid and flavonoid glycoside (Raksilapa, 1995 Yavada and Reddy, 1998; Roengsamran *et. al.*, 2000) which were not classified as the group of phytoestrogens. The extract showed only antiproliferative effect because the chemical constitute contain phytosterols which were proved to be anticancer agents (Awad, Downie, and Fink, 2000; Awad, Downie, Fink and Kim, 2000; Awad, Williams and Fink, 2001). The addition of estradiol did not therefore influence the response of MCF-7 to *B. superba* extract. It might possible to develop *B. superba* into an anti-breast cancer product. Due to the fact that the ED_{50} value is greater than 100 μg/ml, the product might not suitable to be a crude drug but should be in a form of purified phytochemical.

M. collettii extract showed no estrogenic effect but high cytotoxic effect. The combination of the extract at high concentration showed the increment of cytotoxic. The addition of low dose estradiol might also add up the estrogenic effect of high dose phytoestrogen, kaemferol and resulted in higher cytotoxic effect of the treated phytoestrogen from the plant extract.

HeLa

The classical biphasic effect of estradiol did not express in HeLa cell line due to the fact that HeLa was an ER^- cell line

P. mirifica extract showed no proliferative effect on the growth of HeLa cell line as HeLa was an ER^- cell line. At high concentration, *P. mirifica* extract showed markedly inhibition of the cellular proliferation This effect is similar to that happen in MCF-7 and was probably related to the inhibition of tyrosine kinase activity of growth factor receptor. (This *et. al.*, 2001)

P. lobata extract showed no proliferative effect and antiproliferative effect. It might derived from the fact that *P. lobata* contained low content of phytoestrogens (Kaufman *et. al.*, 1997; Gurerry *et. al.*, 2000) as compared with of *P. mirifica*. Thus *P. lobata* did not show the high antiproliferative effect.

B. superba at high concentration (100 and 1000 μg/ml) showed markedly inhibition of the cellular proliferation. It might draw out from the experiment that *B. superba* extract exhibited anti-cancer agent as it contained phytosterol (β-sitosterol, stigmasterol and campasterol) which were the group of anti-cancer plant chemicals (Raksilapa, 1995; Awad, Downie, and Fink, 2000; Awad, Downie, Fink and Kim, 2000; Awad, Williams and Fink, 2001).

M. collettii showed markedly antiproliferative effects because it contained qucertin and hopeaphenol (Wutteeraphon *et. al.*, 2001) which were very toxic compound (Ohyama *et. al.*, 1999). Thus, the antiproliferative effect of *M. collettii* on MCF-7 (ER^+ cell) and HeLa (ER^- cell) were quite similar.

The comparisons of the four types of extracts on HeLa cell line at the same concentration showed no different proliferative effect. *P. lobata* extract showed the lowest antiproliferative effect as its chemical constituents contained low amount of the high potential phytoestrogen such as genistein. *M. collettii* extract showed the highest antiproliferative effect as it contained the high cytotoxic compounds, qucertin and hopeaphenol. (Ohyama *et al.*, 1999: Roengsamran *et. al.*, 2000)

MCF-7 versus HeLa

The results from this study provided the evidence that *P. mirifica* inhibition of the ER^+ cell, MCF-7 and ER^- cell, HeLa may not occur through ER as the extract showed antiproliferative effect on both cell lines. This study and others had demonstrated that phytoestrogens inhibited proliferation similarly in ER^+ and ER^- human breast cancer (Wang *et. al.*, 1996: Wang and Kurzer, 1997: Constantinous *et. al.*, 1998: Shao *et. al.*, 2000). But the growth inhibitory effects of the high extract concentrations were much higher in the presence of estradiol. It should indicated that the ER pathway was the pathway by which the extract act in ER^+ cell. So the anitiproliferative of the phytoestrogens are still not clear and the study in the molecular level will help to explain the mechanism of this effect.