CHAPTER IV
DISCUSSION

The peels of mangosteen, *Garcinia mangostana* Linn., have been used as a traditional medicine for treatment of skin infection and wounds in Southeast Asia for many years, and xanthones (α- mangostin, β- mangostin, isomangostin, magostenol, mangostenone A,mangostenone B, trapezifolixanthone, tovophyllin B, etc.) from the peels have attracted attention as a source of chemopreventive or therapeutic agents, due to their antibacterial, anti–inflammatory, and anticancer activity.

The cytotoxicities of mangosteen peel extracts (butanol, ethanol, ethyl acetate, hexane, methanol, and ethanol with BSA precipitate) on leukemic cell lines were different in each type of leukemic cell. Ethyl acetate, hexane, ethanol, and butanol fractions had higher levels than methanol and ethanol with BSA, and when compared to the active ingredients of mangostin (mangostin and xanthone), the cytotoxicities effect of these fractions were similar to these of mangostin, especially the ethyl acetate fraction, while the cytotoxicity of methanol and ethanol with BSA were similar to that of xanthone. This result suggests that mangostin was the main active compound in the butanol, ethanol, ethyl acetate, and hexane fractions while xanthone was the active compound in the methanol and ethanol-BSA extracts. However, these fractions should be analyzed by HPLC for further analysis.

The percentages of yield of mangosteen peel fraction after extraction varied among organic sovents. The highest percentage of magosteen peel fraction was due to ethanol, as it can dissolve many compounds of mangosteen peel.

Mookranrdi *et.al* [187] showed that mangosten peel had antiproliferation, antioxidation and induction of apoptosis effects on human breast cancel cell lines, but the mangosteen peel was extracted only with absolute methanol. While this studied showed the cytotoxicity effect of mangosteen peel extracts on leukemic cell lines (excluding methanol extracts), the effective fraction may be extracted by ethanol,

following from the high cytotoxicity to leukemic cell lines associated with the ethanol fraction.

In the K562 cell line, WT1 mRNA levels were decreased in butanol, ethanol, and ethyl acetate fractions. The WT1 mRNA levels were inhibited by 54, 45, and 31%, respectively while the non–cytotoxicity concentrations of these fractions were 3.26, 31.5, and 8.6 µg/mL, respectively. From these results, the ethyl acetate fraction appeared to be more effective for the K562 cell line. The strongest inhibitory fraction on Molt4 and U937 was the ethanol fraction. Non–cytotoxic concentrations were 8.9 and 9.1 µg/mL, respectively. The WT1 mRNA levels were inhibited by 44 and 35%, respectively. However, the strongest inhibitory effect on HL-60 was via the butanol extracts, but the greatest cytotoxicty was associated with ethyl acetate. The non–toxic concentration of butanol was 20.1 µg/mL and the mRNA level was 43%, while the non–toxic concentration of ethyl acetate was 5.8 µg/mL and the mRNA level was 11%.

A decrease in *WT1* gene expression with increasing ethyl acetate fraction concentrations (5-15 µg/mL) on K562 cell line indicated that ethyl acetate fraction inhibited the levels of WT1 mRNA. The experiment also showed that treatment of cells with ethyl acetate fraction at the concentration of 10 µg/mL for 1 to 3 days inhibited the *WT1* gene expression in a time–dependent manner. Furthermore, *WT1* gene expression was decreased by increasing selected fractions on remainder cell lines (15–25 µg/mL of butanol fraction on HL-60 cell line, 3–7 µg/mL of ethanol fraction on U937 cell line, and 3–10 µg/mL of ethanol fraction on Molt4 cell line).

The WT1 protein was detected in 2 leukemic cell lines, the K562 and Molt4 cell lines but not detected in U937 and HL-60 due to the WT1 protein levels in these cell lines being too low to be detected by this method. However, different mechanisms of *WT1* gene expression at the translational level of each type of leukemic cell will be subsequently investigated. The WT1 protien decreased with increasing concentrations of the ethyl acetate fraction in K562 cells and the ethanol fraction in Molt4 cells and with increasing duration of treatement in both cell lines. These findings supported the supposed role of *WT1* gene in leukemogenesis.

The effective components of mangosteen peel need further investigation. The current results suggest that mangosteen peel can potentially be used as a

chemotherapeutic agent in human leukemic cancer. This study may lead to clinical trials in the future. Moreover, the mechanism of mangosteen peel extract inhibition should yield valuable insights into the application of medicinal plants as anticancer drugs.