

CHAPTER 4

CONCLUSIONS

The investigation of the twelve medicinal plants was based on their use by Thai traditional doctors for the treatment of cancer patients. It was found that at least five plants were reported for cytotoxic activity. Thus the objective of this research was the use of *in vitro* bioassays to detect cytotoxicity against human cancer cell lines and related activities with cancer such as antioxidant activity and apoptosis. It also describes the isolation and elucidation of active compounds from the active plants.

The result on cytotoxicity assay revealed that the water extract of all plants species exhibited no cytotoxic activity against two cancer cell lines and normal cells. The ethanolic extract of *Dioscorea membranacea* rhizomes showed the highest ($IC_{50} = 4.63 \mu\text{g/ml}$) and *Curcuma zedoaria* rhizome showed the second effectiveness ($IC_{50} = 6.05 \mu\text{g/ml}$) against the lung cancer cell line but less cytotoxicity against the normal cell line ($IC_{50} = 66.05, 55.50 \mu\text{g/ml}$ respectively). The ethanolic extract of *Rhinacanthus nasutus* root showed the highest activity against prostate cancer cells ($IC_{50} = 2.01 \mu\text{g/ml}$) and nonspecific cytotoxicity because it can kill all cell types including normal cell line ($IC_{50} = 10.95 \mu\text{g/ml}$). These results support using of this formula for treatment of cancer patients.

These twelve species were also tested for antioxidant activity because the treatment of cancer may use antioxidant agents for reducing toxicity from oxygen species or free radicals. The antioxidant products may also improve the resistance of tissue to oxidative damage, promote healing, increase immune stimulant properties (Thurnham, 1993 and Gordon, 1996). From these reasons, the twelve medicinal plants were tested by the DPPH assay which is a total antioxidant screening assay. The results found that the water extracts of *Erythrophleum teysmannii* and *Sapindus rarak* showed the highest antioxidant activity in this test ($EC_{50} = 0.625, 0.625 \mu\text{g/ml}$ respectively) and they also exhibited stronger activity than BHT ($EC_{50} = 11.583 \mu\text{g/ml}$). In addition, the four ethanolic extracts (*Curcuma zedoaria*, *Dioscorea*

membranacea, *Erythrophleum teysmannii* and *Smilax corbularia*) showed strong antioxidant activity ($EC_{50} < 20 \mu\text{g/ml}$). Therefore, these plant may used as an antioxidant product and support using this preparation to treat cancer patients.

Four compounds and the oil were isolated from the ethanolic extracts of *Curcuma zedoaria* and *Dioscorea membranacea*, they were the oil (CZV), curcumin (CZS1) and β -sitosterol 3-*O*- β -*D*-glucopyranoside (CZS2) isolated from the rhizome of *Curcuma zedoaria*, dioscorealide A (DMS2), dioscoreanone (DMS3) and also β -sitosterol 3-*O*- β -*D*-glucopyranoside (DMS1) isolated from the rhizome of *Dioscorea membranacea*.

Dioscorealide B, stigmasterol, β -sitosterol, diosgenin 3-*O*- α -*L*-rhamnopyranosyl(1 \rightarrow 2)- β -*D*-glucopyranoside and diosgenin 3-*O*- β -*D*-glucopyranosyl(1 \rightarrow 3)- β -*D*-glucopyranoside taken from Itharat, A. and all compounds isolated in this present work were tested for cytotoxicity against two human cancer cell lines (lung and prostate) and one normal cell line (human fibroblast cells or 10FS). Curcumin, dioscoreanone and diosgenin 3-*O*- α -*L*-rhamnopyranosyl-glucopyranoside showed cytotoxic activity. Dioscoreanone showed the highest cytotoxicity against lung cancer cell line ($IC_{50} = 2.45 \mu\text{M}$) the ratio of IC_{50} of normal cell line/ IC_{50} of cancer cell line was 8.76. Diosgenin 3-*O*- α -*L*-rhamnopyranosyl (1 \rightarrow 2)- β -*D*-glucopyranoside exhibited the highest cytotoxicity against prostate cancer cell line ($IC_{50} = 5.88 \mu\text{M}$) and the ratio of IC_{50} of normal cell line/ IC_{50} of cancer cell line was 6.47. It also showed the second effectiveness against lung cancer cell line ($IC_{50} = 5.61 \mu\text{M}$) and the ratio of IC_{50} of normal cell line/ IC_{50} of cancer cell line was 6.77. Curcumin showed cytotoxicity against prostate cancer cell line ($IC_{50} 18.29 \mu\text{M}$) but less toxic in against human fibroblast cell line and the ratio of IC_{50} of normal cell line/ IC_{50} of cancer cell line was 4.71. The results indicated that diosgenin 3-*O*- α -*L*-rhamnopyranosyl-glucopyranoside had selective cytotoxic activity against prostate cancer cell line but less toxicity against the normal cell line. Dioscoreanone had selective cytotoxic activity against lung cancer cell line but it had no toxicity against the normal cell line.

The cytotoxic compounds; curcumin, dioscoreanone and diosgenin 3-*O*- α -*L*-rhamnopyranosyl(1 \rightarrow 2)- β -*D*-glucopyranoside were tested for apoptosis by the TUNEL assay. The results showed that curcumin and dioscoreanone induced

apoptosis in lung and prostate cancer cell lines whereas diosgenin 3-*O*- α -*L*-rhamnopyranosyl(1 \rightarrow 2)- β -*D*-glucopyranoside induced cell death via apoptosis only in prostate cancer cell line and it may induced the death of lung cancer cell line through necrosis or another pathway. Based on the obtain results. It is likely that these compounds should be promising candidate for further study. All these results can conclude that there are at less the ethanolic of five plants which showed cytotoxic activity against lung and prostate cancer and five plants showed antioxidant activity. These data can support using of Thai traditional medicine to treat cancer. The maker of analyzed cytotoxic activity of this cancer preparation were curcumin, dioscoreanone and diosgenin 3-*O*- α -*L*-rhamnopyranosyl(1 \rightarrow 2)- β -*D*-glucopyranoside. The further work should study on combination preparation by using all these active ingredients as the maker for analysis and should continued to study in animal model especially lung and prostate cancer for supporting using of folk doctor.