CHAPTER 4

CONCLUSIONS

The investigation of the three medicinal plants and Triphala formula was performed based on their use by Thai traditional doctors as adaptogen for body in summer season of Thailand. It was found that all plants and Triphala formula contained antioxidant and cytotoxic activities. Thus the main objective of this study was to investigate antioxidant and cytotoxic activities against human cancer cell lines. In addition, the isolation and elucidation of pure compounds from the most active plant were carried out.

The antioxidant studies were tested by the DPPH assay which is a total chemical antioxidant screening assay. It revealed that the all extracts showed high antioxidant activities with the values of EC_{50} less than 7 µg/ml. The ethanolic extract of pulp of *P. emblica* had the highest antioxidant activity in this test with the EC_{50} value of 2.846 $\pm$ 0.83 µg/ml, followed by the ethanolic extract of seed of *T. chebula* with the EC_{50} value of 2.925 $\pm$ 0.67 µg/ml. Therefore, the EC_{50} values of all extracts were lower than BHT (EC_{50} = 12.647 $\pm$ 0.52 µg/ml) used as the standard antioxidant substance. This indicated that all extracts possessed more potentcy of antioxidant activity than the standard antioxidant substance (BHT). The ethanolic extracts of Triphala and its component showed also antioxidant activity by lipid peroxidation and it showed higher antioxidant activity than water extract like DPPH assay. There is no companative study of the method of extraction and antioxidant activity because only water extracts of Triphala were used in ayuravedic preparation. Thus, in all previous reports of Triphala was extracted by water. The extracts of Triphala and its components can be promoted in manufacturer level as antioxidant products but the method of extraction should be considered.

For cytotoxicity assay, only the ethanolic extracts of all plants showed cytotoxic activities against three types of cancer cell lines and normal cell lines. The IC_{50} value of the ethanolic extract of the pulp of *P. emblica* showed the highest cytotoxic activity against MCF-7 cancer cell line and the water and ethanolic extracts of the pulp of *T. bellerica* showed the second

most effective activity against MCF-7 cancer cell lines. The IC_{50} value of the ethanolic extract of the pulp of *P. emblica* was 29.863 $\pm$ 0.89 µg/ml and the IC_{50} value of the water and ethanolic extract of the pulp of *T. bellerica* were 38.650 $\pm$ 0.52 and 31.895 $\pm$ 2.18 µg/ml, respectively. The water extract of the pulp of *P. emblica* showed the highest cytotoxic activity against Hela cancer cell lines (IC_{50} = 30.136 $\pm$ 5.33 µg/ml) and the ethanolic extract of the pulp of *P. emblica* showed the second most effective activity against Hela cancer cell line (IC_{50} = 32.366 $\pm$ 0.51 µg/ml). As well as the water extract of the pulp of *T. bellerica* showed the highest cytotoxic activity against PC3 cancer cell line (IC_{50} = 33.785 $\pm$ 0.84 µg/ml) and the water extract of the pulp of *P. emblica* and the water extract of the seed of *T. chebula* showed the second most effective activity against PC3 cancer cell line (IC_{50} = 34.618 $\pm$ 2.69 and 34.744 $\pm$ 1.66 µg/ml, respectively). They exhibited higher specific activity against Hela and PC3 cancer cell lines than MCF-7 cancer cell lines but less active in normal cell line. The ethanolic extract of the pulp of *P. emblica* showed cytotoxicity against MCF-7, Hela and PC3 cancer cell lines and deserved for looking for the plant extracts or their active ingredients which can kill cancer cells but less harmful to normal cells. This result related to the objectives of cancer chemotherapy which can kill cancer cells but has little damage as possible to normal cells and should contain selectively active cytotoxic activity (Halliwell and Gatteridge, 1988). Triphala and its components were reported against breast and prostate cancer but they were extracted by acetone and they were reported only percentage of avaiable cell (concentrate of Triphala were 280 and 400 µg/ml with the avaible cells as 50-25% and 0-25% respectively) (Kaur *et al*., 2005). This report indicated that the cytotoxic activity depend on the method and solvent which used for extraction. The ethanolic extract should use for extraction of triphala and *P. emblica* because it showed higher antioxidant and cytotoxic activity than the water extract and acetone extracts.

Four compounds (ß-sitosterol, ß-sitosterol-3-*O*-ß-D-glucopyranoside, 5-hydroxymethylfurfural and gallic acid) were isolated from the ethanolic extract of P. emblica pulp which possessed the most active cytotoxic in this study. They were tested for antioxidant and cytotoxic against three types of human cancer cell lines (breast, cervical and prostate) and a normal cell line (MRC5). Gallic acid showed the highest antioxidant activity (EC_{50} = 0.207 $\pm$ 0.01 µg/ml) because gallic acid and its ester are used as antioxidant additives in both food and pharmaceutical products (Fiuza *et al*., 2004). ß-sitosterol, ß-sitosterol-3-*O*-ß-D-glucopyranoside

and 5-hydroxymethylfurfural exhibited no antioxidant activity. All pure compounds showed no cytotoxicity against all type cancer cell lines and normal cell line.

The results were concluded that Triphala preparation possessed a high antioxidant power and specific against cervical cancer cells. Its ethanolic extract should be promoted for industry more than water extract and *Phyllanthus emblica* should be used as a marker for standardization or biological fingerprint and chemical fingerprint. Although Triphala exhibited less cytotoxic activity but it showed selective against cancer cell and this study can show that the ethanolic extract of *Phyllanthus emblica* pulp has selective cytotoxic against breast, cervical and prostate cancer but not against normal cells. These data can support the using of Thai traditional medicine as antioxidant for health promotion products. The maker for analysis of antioxidant activity of Triphala product should be gallic acid because it was found in all plants of its formulation.

The further work should be performed on structure elucidation of C1, C6 and C7 and applied the ethanolic extract of Triphala for development health food products promote good health or treatment cancer patient or cancer prevention because it showed high antioxidant in both antioxidant assay . However the ethanolic extract of Triphala should be studied more in animal model for antitumor, antioxidant, cancer suppression, immunomodulatory and chronic toxicity because there is no reports on these aspects in animal model. The stability of Triphala extract should also be studied in preformulation study before the development of any pharmaceutical or health products in future.