CHAPTER V

DISCUSSION AND CONCLUSION

S.venosa tuber is often used in Thai traditional medicine as anticancer remedy in the preparations of boiled water or alcoholic soaking solution. This led to the aim of this study in order to compare antitumor activity of both preparations of this herbal plant. Cytotoxic, antiproliferative and apoptotic activities on human PBMCs of water and ethanol extracts of *S.venosa* tuber were chosen to study to suit the purpose.

From this study, the ethanol extract demonstrated more potent cytotoxic effect than the water extract. Its IC_{50} value was ten folds higher from trypan blue dye exclusion methods and 5 folds from alamarBlue reduction assay. The higher cytotoxicity of the ethanol extracts was also found against brine shrimp and breast adenocarcinoma cell line (MCF-7) *(16)*. It is known that the tuber of *S.venosa* contains isoquinoline alkaloids which mostly are poor water solubility. Palmatine and crebanine, purified from the ethanol extract of *S. venosa*, were found to possess the most cytotoxic activity in MCF-7 cells *(16)*. It is possible to suggest that these two cytotoxic alkaloids are more soluble in ethanol than in water *(62-64)*. The results from this study confirmed cytotoxic activity of *S.venosa* both in ethanol and water extracts, as previously reported *(15,16)*. It can conclude here that cytotoxicity is a part of anticancer activities of *S.venosa* tuber in traditional medicine as anticancer remedy.

Trypan blue dye exclusion method is more sensitive than alamarBlue reduction method for detecting cytotoxicity in this study. The possible explanation may come from the difference principles of both methods. When a cell become cytotoxic, its membrane permeability is defect or loss. However, the mitochondrial function of this cell may either remain or loss depend on the severity of cell damage. AlamarBlue can be changed to reduced form in a cytotoxic cell which retain mitochondrial function. On the contrary, this dye is still in oxidized form in a severe damage cell. From this reason, all cytotoxic cells can not exclude trypan blue while some of these cells can still change alamarBlue to reduced form. This gives rise to the higher sensitivity of trypan blue dye exclusion method when compared to alamarBlue reduction assay.

The cytotoxicity of both extracts of *S.venosa* were also investigated on human lymphoblastic leukemic T-cell (Jurkat cells). Because of the higher sensitivity as mentioned before, only trypan blue dye exclusion was used for detecting cytotoxic of these cells. Both extracts demonstrated cytotoxic activity against Jurkat cells with less sensitivity than on human PBMCs. The ethanol extract was found to be more potent than the water extract. The higher IC_{50} values of both extracts on Jurkat cells led to omit these cells for evaluating the other activities, antiproliferative and apoptotic induction, of the extracts.

Plumchai *(15)* demonstrated antiproliferative activity of the water extract of S.venosa against PHA stimulated human PBMCs with the IC_{50} value of 7.5 folds lower than its cytotoxic activity. It was described that apoptotic induction may be a part of antiproliferative effect of S.venosa. Mitogen stimulated PBMC proliferation was also used in this study. The results demonstrated that the ethanol extract was more potent than the water extract in antiproliferative action against PHA *(65-72)*, SPA *(73-75)* and PWM *(76-77)* stimulated PBMCs. It is reasonable to suggest that this effect of both extract comes from palmatine and crebanine alkaloids which are poorly soluble in water.

Apoptosis or programmed cell death is a set of ordered events that enables the selective removal of cells from tissue and is essential for homeostasis and proper function of multicellular organisms. Many component involving apoptotic events are targets of drug development in cancer research and other disease *(77-79)*. Some current chemotherapeutic drugs have been reported to act in part via apoptotic induction *(80-81)*. Apoptotic induction activity was also demonstrated in many herbal plants claimed to have anticancer action *(82-86)*. This activity of the water extract of *S.venosa* was also found by Plumchai using TUNEL assay *(15)*. The present study aimed to confirm this apoptotic activity and compare to the activity of the ethanol extract. FACS analysis with using Annexin V-FITC and PI staining was the method used in this study. The method can evaluate both the amount and the patterns of cell death Annexin V positive cells are apoptotic cells which necrotic cells are PI positive. Annexin V positive or PI positive cells are still controversy type of cell death *(56-58)*. They are define as either mixed type of cell death or only necrosis *(87)*. This results demonstrated that both extracts could induced apoptosis in PBMCs. The ethanol extract was more

potent than the water extract. It is very interesting to notice the different patterns of cell death between both extracts. The ethanol extract could induced cell death predominantly via apoptosis. On the contrary, apoptosis was not the predominant pattern induced by the water extract. Necrosis was detected in almost the same extent as apoptosis at the lower concentration of the extract used in this study (100 and 200 µg/ml).Even though at the high concentration the apoptotic was observed more distinctly than the lower ones. The proportion of Annexin V positive/PI positive was also distinct in the water extract. As mentioned before that apoptosis is less harmful than necrosis. It is possible to suggest that the ethanol extract may cause less unwanted effect involving cell death than the water extract. The detail about the different patterns of cell death induced by both extracts needed to investigate further.

The mechanisms underlying apoptotic induction of *Svenosa* were first investigated by Tonsomboon *(88)*. Increased in caspase-3 activity, cytochrome c level and intracellular calcium were observed in Jurkat cells treated with the water extract. In the present study, it is possible to suggest that apoptosis mechanisms of *S.venosa* should be better confirmed and further investigated in the ethanol extract or its active cytotoxic compound, palmatine and crebanine alkaloids.

In conclusion, this study compared activities involving antitumor action between the ethanol and the water extract of *S.venosa* tuber. The ethanol extract was more potent in cytotoxic, antiproliferative and apoptotic induction activities when compare to the water extract. Apoptotic cell death was the predominant pattern found in the effect of the ethanol extract. It is possible to conclude that both extracts possess not only the different potency but also the different in unwanted effects involving cell death. So, cautions should be considered and addressed when it was prescribed in traditional medicine.