CHAPTER 4
CONCLUSION

Before the advent of synthetic chemical pesticides, natural pesticides were in common use. In recent years it has been realized that the synthetics, although highly effective, often possess undesirable side effects such as toxicity to mammals and pollution of the environment (Marrs and Ballantyne, 2004; Morgan, 1992; Wilson, 2003). As awareness of the potential health and environmental hazards of many residual synthetic pesticides increases, and pests become resistant to more and more synthetic compounds, hence interest in plant-derived pesticides were in focused. *Stemona* alkaloids have been described for insecticidal activities with selective agricultural pests (Brem *et al.*, 2002; Kaltenegger *et al.*, 2003).

The convenient and effective use of *Stemona* extract as pesticidal products in agriculture requires the development of suitable formulations and factors such as extractability with different solvents, stability of the extract in solution or in formulations, and associated financial costs need to be taken into account. These type of studies often require the isolation and identification of the main pesticidal components, since this would then allow their use as 'markers' in further scientific studies, and eventually in quality control/assurance of the finished product.

The result from our study demonstated that the crude extract of roots and rhizomes of *S. curtisii* collected from Chumphon Province contained pyridostemin (stemocurtisine), a pyrido[1,2-a]azepine as the major compound. Pyridostemin was used as the marker and external standard for this research. The stability indicating HPLC method for quantitative analysis of pyridostemin, the major active alkaloid in *S. curtisii* extracts, was developed and validated and subsequently successfully used to study the stability of the partially purified extracts of *S. curtisii* or of their formulations. The stationary phase was C_{18} and the mobile phase was a mixture of acetonitrile-water-triethylamine (30:70:0.12, v/v/v). The flow rate of the mobile phase was 1.25 ml/min and the injection volume was 20 µl. The detection wavelength was fixed at 300 nm. The retention time of the pyridostemin was 11.2 min.

The amount of pyridostemin in the *S. curtisii* root extract macerated with two different organic solvents was determined. Methanol extracts were found to contain significant higher absolute amount of pyridostemin per 100 g of dried plant than dichloromethane extracts. However pyridostemin content per 100 g of crude extract which extracted by dichloromethane was higher than using methanol. Although dichloromethane extract gave higher pyridostemin content than methanol, the pyridostemin content in crude extract was still too low for use in pesticide formulation development since unwanted constituents in crude extract may have an affect in formulation development. Furthermore isolation of the pure pyridostemin from plant materials would not be economically viable for formulation. Therefore, partial purification of the crude extract was required. The partially purification process for the methanol extract was more costly than partially purified process for dichloromethane extract, in term of time and cost of solvents and silica gel. In addition, dichloromethane seem to be suitable extraction solvent than methanol for this purpose. The partially purified dichloromethane extract by silica gel column chromatography resulting in much higher pyridostemin content (71.40±5.51 %w/w) than the crude dichloromethane extract (18.77±1.22 %w/w).

Degradation behavior of pyridostemin was fitted with the first-order kinetics. Decomposition pathways mainly occur through acid-base hydrolysis, oxidation, and photolysis. Therefore, formulations of *S. curtisii* extract should be carefully protected from moisture and light during storage to conserve the active ingredient. The photo labile of the extract indicated that this natural pesticide could be oxidized after exposed to sunlight. Furthermore, an antioxidant should also be added in the liquid formulation to prevent oxidation process.

For convenience in agricultural application, the water dispersible granules and emulsifiable concentrate containing *S. curtisii* extract had been developed. The developed formulations were evaluated for physicochemical properties and also investigated their stabilities for three months. Both formulations were developed using non-toxic excipients that met the formulation requirements in term of satisfactory handling, ease of application, safety to the applicators and environment. The water dispersible granules, had many advantages such as low inhalation hazard to applicator, good flowability, and suitable for water-insoluble pesticide when compared to other dry formulations. In the case of liquid

formulation, emulsifiable concentrate was chosen because this formulation is suitable for water-insoluble pesticide, simple in preparation, low cost, and could enhance chemical stability. Both formulations containing *S. curtisii* extract exhibited good physicochemical properties but the chemical stability of the water dispersible granules showed faster degradation rate than the emulsifiable concentrate. These developed formulations containing *S. curtisii* extract could be diluted with water in 1-3% range (depending on target pest) before application and should be occasionally shake during application.

Although, the under expectation of chemical stability of the developed formulations was found when stored at ambient temperature, storage in the cold place (at 4°C) was compulsory which would keep the active component in the *S. curtisii* extract longer than three months. Recommended storage conditions for the developed water dispersible granules and emulsifiable concentrate containing *S. curtisii* extract are the dark, cold, and dry place.

In addition, efficacy of the formulation is exactly importance for product development. Therefore, the toxicity against crop pest of the developed formulation should be proved.

Natural pesticidal plants in Thailand are available as alternatives to synthetic pesticide. However, a major obstacle to widespread use of natural pesticides is a lack of ready-to-use, easily applied and stable formulation. This research provides an idea for the future agriculture which should concern about human and environmental safety. The two developed pesticide formulations containing *S. curtisii* extract would be the example models that encourage the natural pesticide formulation development and utilizing in agricultural countries.