CHAPTER 5

CONCLUSION

5.1 Conclusion

In this project, three *Stemona* spp. i.e. *Stemona* curtisii Hook F., *Stemona* sp. (unknown 1) and *Stemona* sp. (unknown 2) were selected and collected from different places in Thailand. The plant selection depended on their insecticidal properties from literature reports. These plants were studied in order to discover new insecticidal compounds and to develop plant extract formulations for use as less harmful pesticides. The bioactive compounds from *Stemona* spp. were relatively complex alkaloid compounds. According to the strategies of extraction, isolation, purification, and structure elucidation, the results could be concluded as follows:

Three known *Stemona* alkaloids stemocurtisinol, oxyprotostemonine and stemocurtisine were isolated from the roots of *S. curtisii*. Their biological activities were studied on *Artemia salina* Leach (brine shrimp) and the results showed that these alkaloids exhibited toxic activities with LC_{50} values of 57, 18 and 27 ppm, respectively, while the ethanolic extract showed toxic activities with a LC_{50} value of 37 ppm.

Pure compounds of two new tuberostemonine alkaloids, tuberostemonine L and tuberostemonine M and a new stemofoline alkaloid, (3′*S*)-hydroxystemofoline, along with three known alkaloids, neotuberostemonine, (2′*S*)-hydroxystemofoline and stemocurtisine were isolated from the roots of *Stemona* sp. (unknown 1).

Unfortunately, biological activities of neotuberostemonine, tuberostemonine L and tuberostemonine M on *Artemia salina* Leach (brine shrimp) could not study due to instability of these compounds, while biological activities of (3′*S*)-hydroxystemofoline could not perform due to sample limitation. However, the ethanolic extract showed toxic activities on *Artemia salina* Leach (brine shrimp) with a LC_{50} value of 98 ppm.

Pure compounds of six new stemofoline alkaloids, methylstemofoline, (2′*R*)-hydroxystemofoline, (3′*R*)-stemofolenol, (3′*S*)-stemofolenol, stemofolinoside, and 1′,2′-didehydrostemofoline-*N*-oxide and three known stemofoline alkaloids, (2′*S*)-hydroxystemofoline, (11*Z*)-1′,2′-didehydrostemofoline and (11*E*)-1′,2′-didehydrostemofoline were obtained from the roots of *Stemona* sp. (unknown 2). The biological activities of these new compounds have not been studied on brine shrimp due to the sample limitation except the mixture of (3′*R*)-stemofolenol and (3′*S*)-stemofolenol which showed a LC_{50} value of 155 ppm, while the toxic activities of known compounds comprise of (11*Z*)-1′,2′-didehydrostemofoline and (2′*S*)-hydroxystemofoline were 58 and 82 ppm, respectively. In addition, the ethanolic extract showed a LC_{50} value of 50 ppm.

The results from the crude ethanolic extracted from *S. curtisii* had the highest activity against brine shrimp with a LC_{50} value of 37 ppm, while the highest activity of pure compound was shown by oxyprotostemonine which had toxic activities against brine shrimp with a LC_{50} value of 18 ppm. Therefore, it could be concluded that the crude ethanolic extract of *S. curtisii* was the most appropriate to prepared a bioinsecticidal formulation because of its toxic activity and from economical considerations.

A bioinsecticidal formulation was investigated using the ethanolic crude extract of *S. curtisii* as the main component. Different solvents and other supplement substances were considered to be used for producing the bioinsecticidal formulation. The percentage of the crude extract, ethanol, methanol, water, pine oil and tween 80 for the bioinsecticidal formulation were 30, 30, 10, 10, 10 and 10 %w/w, respectively.

The efficiency of the bioinsecticidal formulation was studied in the laboratory to realize the insecticidal activities by bioassay methods i.e. the leaf disk choice test and the topical application method. The results of the leaf disk choice test exhibited a strong antifeedant activity against *S. littoralis* at 0.015%, while repellent activity against *S. littoralis* was seen at higher concentrations above 0.030%. Moreover, the results of the topical application method exhibited 0% mortality of *S. littoralis*, while the chemical pesticide showed 100% mortality. It was concluded that mode of action of the bioinsecticidal formulation as a repellent substance which did not kill the pests at the same concentration.

Bioinsecticide production on a pilot scale was investigated and the bioinsecticidal formulation was analysed by HPTLC from which the components showed a specific chromatographic pattern (fingerprint) under UV light at 254 nm and using a mixture of dichrolomethane, methanol and ammonia solution, in a ratio of 95: 4: 1, as the mobile phase.

The efficiency of the bioinsecticide produced on a pilot scale was tested on *Brassica oleracea* L. CV. (Chinese kale) in an experimental field of the Department of Agronomy, Chiang Mai University, in the period from 6 June 2006 to 13 July 2006. The quantity items comprised an analysis of the height, the weight, the number of leaves, and the amount of insects i.e. leaf eating beetles, diamondback moths, green

aphids, cabbage loopers, common cutworms and predators. The total vegetable quality of products was also considered.

The bioinsecticidal formulation of the plant extract, the synthetic pesticide (methomyl) and the control (water) were applied to Chinese kale from the first week after transplantation until harvesting. Most of the quantity items showed the same results except for the number of leaves for which the bioinsecticidal formulation gave the better results both in terms of the value and statistical significance than the chemical pesticide and the control. For quality results, the bioinsecticidal formulation exhibited slightly better results against diamondback moths, cabbage loopers and common cutworms but the results were also not statistically significantly different from the chemical pesticide and the control. Moreover, the bioinsecticidal formulation gave a statistically significant better result for the amount of predators than both the chemical pesticide and the control. However, the bioinsecticidal formulation was ineffective to green aphid compared with the chemical pesticide. Consequently, the bioinsecticidal formulation was effectively similar to the chemical pesticide for controlling pests in terms of both the quality and the quantity of the product.

5.2 Future work

The bioinsecticidal formulation should be studied for its mode of action, toxicity against mammals, its physical properties and also its shelf life.

In order to maintain and improve the effectiveness of the bioinsecticidal formulation and to prevent resistance by pests or plant pathogenic molds, the new active compounds should be synthesized and their structure should be modified to give higher activity.

Chemically modify *Stemona* alkaloids should be undertaken to produce both rare alkaloids and novel derivatives for structure-biological activity studies. Biological testing should be done to find out their anti-feedant and insecticidal activities against several plant pathogenic molds. The phytochemical and biological testing of other fractions and parts of *Stemona* spp. should also be investigated.