#### **CHAPTER 5**

#### CONCLUSIONS

## **5.1 Conclusions**

In this research project, two different *Stemona* spp. i.e. *Stemona curtisii* Hook. F. and *Stemona aphylla* Craib. were collected from different regions in Thailand. To investigate biological activities such as insecticidal properties, acetylcholinesterase inhibitory activities, antimicrobial activities and antioxidant activities led to the extraction, isolation and purification of the bioactive compounds.

One new stemofuran i.e. stemofuran L together with three known stemofurans, stemofuran K, stemofuran J, stemofuran F and one known alkaloid, stemocurtisinol were isolated from the non-alkaoid crude extract of *S. curtisii* from Trang Province whereas, dehydro- $\gamma$ -tocopherol and stigmasterol were isolated from the petroleum spirit crude extract.

One new stemofuran i.e. stemofuran S along with three known alkaloids, oxystemokerrin, oxystemokerrin-*N*-oxide and oxyprotostemonine were obtained from the dichrolomethane crude extract of *S. curtisii* from Petchaboon Province.

Three known compounds, stemofuran J, stigmasterol and dehydro-δtocopherol were also isolated from the petroleum spirit crude extract of *S. aphylla* from Lampang Province and stemofuran L was obtained from the non-alkaoid crude extract, corresponding to that isolated from *S. curtisii* from Trang Province. The structure elucidation of the isolated compounds were carried out by spectroscopic techniques.

The toxic activities of each pure compound from *Stemona* spp. on *Artemia salina* Leach (brine shrimp) were investigated. From the results, stemofuran J had the highest activity against brine shrimp with a LC<sub>50</sub> value less than 1.0 ppm, followed by stemofuran S, stemofuran L, stemofuran K, stemofuran F, oxystemokerrin, oxyprotostemonine, stemocurtisinol dehydro- $\delta$ -tocopherol, dehydro- $\gamma$ -tocopherol, oxystemokerrin-*N*-oxide and stigmasterol with LC<sub>50</sub> values of 2.8, 4.9, 7.2, 10.6, 13.4, 21.7, 56.1, 82.6, 89.5, 211.2 and 285.6 ppm, respectively.

The effective insecticidal activities of different *Stemona* crude extracts were studied on *Spodoptera littoralis* (3 <sup>rd</sup> instar lavae) in the laboratory by the leaf disk choice method. The results indicated that the alkaloid crude extracts of *S. curtisii* and *S. aphylla* exhibited strong antifeedant activity at the concentration of 0.5%. However, the alkaloid crude extracts of *S. curtisii* also showed repellent activity at the higher concentration. The higher antifeedant activity of the crude alkaloid extracts of *S. curtisii* was responsible for the activity due to the alkaloid substances contained in this fraction. Morever, most of the secondary metabolites such as alkaloids were reported as bioinsecticide that could be an effective alternative for insect pest control. However, their specific mode of action is not yet clearly understood. In comparison the commercial insecticide, methomyl, showed strong antifeedant inhibition at the concentration of 0.1% while such tests on pure samples could not perform because of the limited amount of samples.

From a study of the acetylcholinesterase inhibitory activities of the isolated compounds from *Stemona* species, it was found that stemofuran S and

117

oxystemokerrin-*N*-oxide were the most active compounds with a minimum inhibitory requirement of 100 ng. Whereas stemofuran K, stemocurtisinol and oxystemokerrin displayed weak activity with a minimum inhibitory requirement of 500-1000 ng.

For the antimicrobial activities, stemofuran J showed the highest efficacy against *Cryptococcus neoformans* and methicillin-resistant *Staphylococcus aureus* (MRSA) with minimum inhibitory concentration (MIC) values of 7.8 and 15.6  $\mu$ g/mL, respectively. Whereas, stigmasterol, dehydro- $\delta$ -tocopherol, dehydro- $\gamma$ -tocopherol, stemofuran F, stemofuran K, stemofuran L, stemofuran S, oxystemokerrin, oxystemokerrin-*N*-oxide and oxyprotostemonine displayed weak inhibitory activity with MIC values ranging from 15.6 to 125  $\mu$ g/mL. For the antifungal activities, all compounds could inhibit *Cr. neoformans* at a concentration of 31.3  $\mu$ g/mL. While dehydro- $\delta$ -tocopherol possessed the least activity against MRSA with a minimum bactericidal concentration (MBC) value of 500  $\mu$ g/mL.

Efficiency of antioxidant activities from each *Stemona* species crude extract and some pure compounds were investigated. The results indicated that stemofuran S displayed the highest antioxidant capacity with an IC<sub>50</sub> value of 30.1 µg/mL. These results could be correlated to their chemical structures. It could be assumed that the antioxidant capacity was dependent upon the number and position of hydroxyl groups in the molecule. Furthermore, the non-alkaloid crude extracts of *S. aphylla* and *S. curtisii* showed ability to scavenge DPPH radical with IC<sub>50</sub> values of 113.8 and 148.7 µg/mL, respectively followed by the ethanolic crude extract and the alkaloid crude extract of *S. aphylla* (IC<sub>50</sub> = 539.3 and 671.5 µg/mL). While an alkaloid crude extract

118

and ethanolic crude extract of *S. curtisii* showed lower antioxidant capacity with  $IC_{50}$  values of 760.0 and 1,017.3 µg/mL, respectively.

In summary, the results obtained from this study indicated that *Stemona* crude extracts and some pure compounds could be used as alternative sources for medicinal purposes and might be effectively used in pest management.

### 5.2 Future work

Isolation and purification of compounds from other parts of *Stemona* spp. such as leaves, fruits etc. could be examined. Additionally, the study of new species of *Stemona* plants would be of great interest for the investigation of new compounds.

The effective insecticidal activity of different *Stemona* crude extracts should be done using other methods i.e. the topical application method for detection of contact toxicities.

The active role of the crude extract against insects should be considered, including the mode of insecticidal action.

The acetylcholinesterase inhibitory activities should be possible confirmed by using another method i.e. Ellman's method.

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