CHAPTER 4

CONCLUSION

Protein components from the venom of *Trimeresurus macrops* have been separated by sodium-dodecyl sulfate polyacrylamide-gel electrophoresis (SDS-PAGE), two-dimensional electrophoresis (2-D electrophoresis), and gel filtration chromatography (GFC). The protein pattern of SDS-PAGE gel shows a wide range of molecular mass from 14 to 66 kDa and a large quantity of two major proteins with approximate masses 14.4 kDa and 45 kDa. The 2-D electrophoresis result shows many protein spots, which are mostly acidic proteins at molecular mass ranges between 14 kDa and 66 kDa. The analysis of enzymatically in-gel digested protein was performed using the MALDI/Tof mass spectrometer and followed by protein database searching via MASCOT program. Due to the result from searching shows no reasonable protein matching, the 2-D protein spots could not be identified. The *Trimeresurus macrops* venom was applied to Sephacryl S-100 HR column for gel filtration chromatography. The twelve peaks of proteins were obtained. The molecular masses of protein fractions of VI, IX, and X determined by MALDI/Tof mass spectrometer were 13732.02, 13255.68, and 13341.09 *m/z*, $[M+H]^+$. The protein fractions of II and III showed proteolytic activity with specific activity 0.18 and 0.15 unit/ml, respectively. The Phospholipase A activity was found in the fraction VI protein. For protein identification, the N-terminal sequence of protein fraction X (13340.09 Da) was analyzed using Edman degradation. The N-terminal sequence is HVLQLGLYIL. The peptides, obtained by trypsin cleavage of fraction VI protein (13731.02 Da), were identified by ESI-Q-Tof tandem mass spectrometry. The product ion spectra of four precursor ions, which are doubly charged ions at *m/z* of 659.3, 844.3, 984.0, and 1224.0, were obtained. The molecular masses, $[M+H]^+$ of these four peptides, are 1317.6, 1687.6, 1965.0, and 2447.0 Da. The partial sequence of protein fraction VI was obtained since two product ion spectra of precursor at *m/z* of 659.3 and 844.3 could be manually interpreted. The first peptide sequence is WAVQCSQQPNR, which is composed of 11 amino acids. The calculated protonated peptide mass is 1316.51 Da. The second peptide sequence is EAVGEDPWYNHQ(I/L)K, which is composed of 14 amino acids. The calculated protonated mass for this peptide is 1686.05 Da.