# 6.0 CONCLUSIONS

## 6.1 Recommendation for Further Studies

Many researches have been performed on extracts of the fruiting body and there have been only few studies on extracts from the liquid cultivated mycelia of *Ganoderma* species. In fact, according to Smith *et al*. (2002), the reason that some of the *Ganoderma* preparations are not yet available as medicine may be from difficulties relating to mass production. There are actually a number of biologically active compounds to be found in the mycelia. Thus, isolation and purification of protein from the mycelia of *Ganoderma australe* in the present study added to the scanty literature on this research field.

The purified protein possessed a single band on Tricine SDS PAGE with molecular mass of 16.6 kDa. The protein shows significant similarity with antifungal protein from *Aspergillus giganteus* and few proteins from the Conserved Domain of Antifungal protein. The mechanism of action of antifungal proteins are varied including fungal cell wall polymer degradation, membrane channel and pore formation, damage to cellular ribosome and inhibition of the cell cycle. The mode of action of many proteins remains unknown and is the subject of active research (Selitrennikoff, 2001).

The effective concentration of the purified protein was investigated by means of minimum inhibitory concentration (MIC) assay. It was observed that a lower concentration was required to kill *E. coli* O157:H7 than *S. typhi*, indicating that the first bacterium was more susceptible to the purified protein. The inhibitory action of the purified protein also observed to be concentration dependent against susceptible bacteria and HIV-1 reverse

transcriptase. Though the activities were not comparable to the commonly used antimicrobial drugs, modifications or transformations can be performed to enhance the activities and lower the toxicity, as the purified protein is found to be very effective against Gram-negative bacteria (many Gram-negative bacteria are resistance to known antibiotics).

To identify the mechanism of action of the purified protein, further work is required to investigate the nature of the inhibitory effects, pathway and regulation analysis, possible target sites and concentration dependent effects of the purified protein. Furthermore, full analytical characterization of the purified protein such as N-terminal sequencing, structure determination and the kinetic behavior of the inhibition can provide important clues about how the protein performs its function. Study of protein – protein interaction and more analyses against other biochemical important enzyme such as angiotensin converting *enzyme* (ACE) can improves understanding of diseases and provide the basis for new therapeutic approaches.

## 6.2 Conclusions

- The present study provided an insight to the isolation and purification of bioactive protein with antimicrobial potential from indigenous *Ganoderma australe*. It demonstrated different extractions methods for protein isolation from mycelia and purification of protein through simple two chromatography techniques.
- All four indigenous *Ganoderma australe* tested were found to exhibit broad spectrum antibacterial activity but show no antifungal activity.
- Mycelium of *Ganoderma australe* cultivated in submerged fermentation is a valuable source of bioactive compounds, especially proteins.

- Remarkable antibacterial activity against *Escherichia coli* O157:H7 and *Salmonella typhi*, and also against HIV-1 reverse transcriptase are presented in the study.
- This is the first account for isolation of bioactive protein from mycelia of *Ganoderma australe*.