

Thesis Title Effect of Temperature on Biological Activities of Bee Venom from 3 Different Species of Honey Bee in Thailand

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ABSTRACT

Bee venom (BV) is one of well-known honeybee products, used as traditional alternative medicine. Due to its protein components such as melittin, BV has been reported to possess pharmaceutical properties including anticancer and antimicrobial activities. The activities of BV would be varied due to different species and temperature. In this study, BV from 3 species of honeybee (*Apis mellifera*, *A. dorsata* and *A. florea*) were investigated for its antibacterial activities against five skin pathogens and anticancer activity against B16F10 melanoma cell. All BV samples were prepared using 10 kDa molecular weight cut-off column to separate complex components according to molecular weight. Prior to determine the effects of temperature on MIC, BV samples were incubated at 40°C, 60°C, 80°C for 5 minutes and unincubated (control). The results indicated that *A. mellifera* BV showed the lowest MIC (6.25-12.5 µg/ml) at 40°C against *Streptococcus pyogenes* and *Staphylococcus aureus* while *A. dorsata* BV showed the lowest MIC (12.5-25 µg/ml) at control and 40°C against *S. aureus*. Meanwhile, BV from *A. florea* was indicated the MIC over 100 µg/ml among temperature levels against all bacteria species.

Further to determine the anticancer activities of BV, BV peptide focused on melittin was generated the interaction simulation with anti-apoptotic protein of cancer cell by computational *in silico* study. We found that melittin have potential to block the activity of anti-apoptotic protein by referring to hydrogen bond and hydrophobic interaction with anti-apoptotic protein together with docking energy score. Additionally, BV samples were tested for inhibitory concentration (IC) against melanoma cells and AO/PI fluorescence staining. The result indicated that BV from each species were presented different inhibitory concentration causing 20 percent of death cell (IC₂₀) and different stages of cell apoptosis could not be observed but secondary necrosis which cells completely dead could be observed for all BV samples. Moreover, samples at IC₂₀ were BV incubated at 40°C, 60°C, 80°C for 5 minutes and unincubated (control) and tested for cell cytotoxicity. BV from all species had an activity to decrease cell viability of melanoma compared to control after incubated at 40 to 80°C. BV incubated at 60°C from *A. mellifera*, at 40 and 60°C from *A. dorsata* and 40°C to 80°C from *A. florea* showed the lowest cell viability. In conclusion, each species demonstrated different activities on gram-positive bacteria skin pathogens and melanoma cells at different temperatures. Therefore, BV have shown potential biological activities which could be used as alternative component aiming for anticancer and antimicrobial properties.

Keywords: Bee Venom Activity, Cell Viability, Minimum Inhibitory Concentration