

## CHAPTER VIII

### 8.1. Conclusion

The antibacterial activity of honey at low concentration can be confirmed quantitatively using methods microtiter plates, nanophotometer and plate count assay for determine and confirm the antibacterial activity of honey. The current study proved that honey was able to inhibit the target multiple antibiotic resistant pathogens even at low concentrations. Tualang and Acacia honeys from Malaysia were able to give very high antibacterial activity compared to other tested honeys and that support the use of these honeys in medical and preservation purposes.

The antibacterial activity of honey samples was affected by the different pH, heat and enzymatic treatments. The activity of honeys was increased at pH 3, while reduced at the other pH adjustment (5 to 9). The inhibitory activity of all tested honeys was heat stable at high temperatures even at 121 °C. The enzymatic treatments of honey samples resulted variability in the antibacterial activity, which suggested that proteins are present in honey samples and affect its antimicrobial activity.

Sugars, phenolic compounds, proteins and peptides were detected present in all honey samples at variable amounts, which may relate to the origin and type of honey. Dilution and heating honey samples before the antibacterial activity tests, confirm that the activity of these honey samples was not from sugars, phenolic compounds or hydrogen peroxide.

Proteins were confirmed present and extracted from all honey samples using acetone precipitation. Both essential and non-essential amino acids with variable amounts depend on the source of honey were determined, and proline was the major amino acid present. Fractionation of the proteins using RP-HPLC showed the antibacterial activity and peptides content, but no proteins or peptides were detected on SDS-PAGE gel.

The fractions obtained from Sephadex G-50 column chromatography showed antibacterial activity against *S. aureus* and high peptide content with clear bands and smears on the SDS-PAGE gel. The fractions with antibacterial activity and high peptide content from Tualang and Acacia honey contained high number of novel peptides. Fifteen novel peptides were obtained from Tualang honey, while five novel peptides were detected from Acacia honey. The sequenced peptides from Tualang honey were almost double the number of peptides from Acacia honey. This study confirmed that honey contained known and unknown peptides with antibacterial activity and mostly the source of those peptides were from bees, plants and flowers.

## 8.2. Recommendations

The current study confirmed the presence of novel and identified antibacterial peptides in Tualang and acacia honeys from Malaysia. However, further studies are needed to elucidate the functions of these peptides in their health promoting role other than antibacterial activity.