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ABSTRACT

Abstract of thesis presented to the Senate of Universiti Malaysia Terengganu in fulfillment of the requirement for the degree of Master of Science

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Antibacterial peptide is one of the natural antibiotics that have antibacterial activity against both Gram-negative and positive bacteria. Horseshoe crab which is a marine invertebrate has a unique defense mechanism against pathogenic microorganisms. It was reported that horseshoe crabs contain proteins in their amoebocyte (blood cells) that is involved in the immune response. Thus, a study was carried out to isolate and purify the potential of antibacterial peptide from the horseshoe crab's amoebocyte. The blood was collected from eight adult male and female horseshoe crabs and extraction of the amoebocyte lysate was done using acetic acid extraction method. The antibacterial peptide was purified using FPLC coupled with gel filtration column (HiPrep 26/60 Sephacryl S-100 High Resolution) and then subjected to SDS-PAGE for protein profiling. The purified peptide showed antibacterial activity against five test strains of Gram-negative and positive bacteria which are *Escherichia coli* (ATCC 11775), *Staphylococcus aureus* (ATCC 25923),

Staphylococcus epidermidis (ATCC 13518), *Bacillus subtilis* (ATCC 11774) and *Salmonella typhimurium* (ATCC 14128) with MIC values of 0.12 µg/ml ± 0.089, 0.98µg/ml ± 0.049, 0.24 µg/ml ± 0.033, 0.12 µg/ml ± 0.045 and 0.24 µg/ml ± 0.013, respectively. This purified antibacterial peptide is heat stable when heated from 30°C to 100°C for 30 minutes. Cytotoxicity test toward HepG2 cell line showed IC_{50} value of 42.45µg/ml indicating a non-toxic peptide was purified. The purified peptide was sequenced by LTQ Orbitrap Velos Pro Mass Spectrometry and the sequence results suggested that the purified peptide was tachystatin-A2 with a molecular weight of 7.5 kDa. The tachystatin-A2 has a potential as pharmaceutical product which have character such as stable to heat, antibacterial activity against both Gram-negative and positive bacteria and low toxicity towards cell line.