ISOLATION & CHARACTERIZATION OF ANTIMICROBIAL PROTEIN FROM MUSHROOM

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THIS DISERTATION PRESENTED TO FULFILL
PART OF THE REQUIREMENTS IN OBTAINING
THE BACHELOR DEGREE IN SCIENCE WITH HONOURS

PROGRAM BIOTEKNOLOGI
SEKOLAH SAINS DAN TEKNOLOGI
UNIVERSITI MALAYSIA SABAH

MAY 2008

ABSTRACT

Antimicrobial proteins or peptides are universal feature of the defense systems of virtually all forms of life, with representatives found in organisms ranging from bacteria to plants and mammals. Antimicrobial peptides are typically relatively short, positively charged, and amphiphilic. In this experiment, soluble proteins were extracted from three types of mushrooms which were *L. edodes*, *A. polytricha* and *P. sp.*, but only two samples (*L. edodes* and *A. polytricha*) possessed antimicrobial properties. Two extraction methods that were used in this experiment such as distilled water extraction and salt solution extraction. The efficiency of these two methods was compared by the determination of protein recovery using Bradford assay. From the experiment, the salt solution extraction was found better due to the higher protein recovery of 20.37% and 18.52% for sample L.e.2 and P.sp.2 respectively. In order to analyze the antifungal and antibacterial activities of the proteins, disk diffusion assays was applied for each sample and the MIC values were determined for each sample with antimicrobial activities. The extracted protein of all samples were found less effective against Gram-negative bacterium *Pseudomonas aeruginose* and fungus *Botrytis cinerea*, but *L. edodes* and *A. polytricha* demonstrated inhibitory activity against Gram-positive bacteria *Streptococcus aureus* and *Bacillus cereus*. The proteins *in vitro* exhibited effective antibacterial activities against the both strains of bacteria at minimum concentration of 0.5mg/ml, except sample *L. edodes* that inhibited the growth of *S. aureus* even with concentration of 0.25mg/ml. Apart from that, the antioxidant activities of the proteins were investigated by applying DPPH free radical-scavenging assay. There was only low potency of the antioxidant activities showed by all the protein extracts with the highest 29.44%. The protein extracted from the both samples was purified using DEAE-cellulose chromatography. However, there was low protein recovery shown by the Bradford assay. The purity and molecular mass of the extracted and purified proteins were then determined by SDS-PAGE analysis. The purified proteins of sample *L. edodes* and *A. polytricha* were found appear as few protein bands with range of size 18-55kDa and 15-50kDa respectively.