ABSTRACT

The present study attempted to purify bioactive protein from mycelia of *Ganoderma* culture using organic extraction method (dichloromethane and 50% ethanol) and selection of chromatographic techniques. By using crude ethanolic extract, four strains of indigenous *Ganoderma australe* and one from *Ganoderma tsugae* were assessed for their antagonistic activity against human pathogenic bacteria and yeast. All *Ganoderma* strains tested exhibited different levels of antibacterial activity against both Gram-positive and Gram-negative bacteria. The ethanolic extract from G. *australe* strain KUM60813 was selected for further protein purification on the basis of intense antibacterial activity compared to proteins from other strains tested. The protein was further isolated using ammonium sulfate precipitation and gel filtration on Sephacryl S-100 and Superdex 75 HR column. This resulted in a purification of a protein with a molecular weight of 16.6 kDa, visualized on Tricine SDS PAGE. The purified protein was assessed for its antibacterial activity using microtitre broth dilution method. The protein showed inhibitory activity towards *Salmonella typhi* and *Escherichia coli* O157:H7 with IC_{50} of 5.21 mg/ml and 4.04 mg/ml, respectively. It also inhibited HIV-1 reverse transcriptase with an IC_{50} of 1.08 mg/ml and deoxyribonuclease activity towards herring sperm DNA. The protein exhibited no antifungal activity against the plant pathogenic fungi tested and also had no ribonuclease activity. Finally, the identity of the protein was further analyzed using Mass Spectrometry and showed significant similarity to antifungal protein from *Aspergillus giganteus*. The study has demonstrated a simplified approach of purification of bioactive protein from mycelia of *G. australe*. Hence, should be adopted for convenient purification of proteins from other species of mushroom.

ABSTRAK

Kajian ini bertujuan untuk menulenkan protein daripada miselium kultur *Ganoderma* menggunakan kaedah pengekstrakan organik (diklorometan dan etanol 50%) dan pemilihan teknik kromatografi. Dengan menggunakan ekstrak etanol, empat strain cendawan *Ganoderma australe* tempatan dan *Ganoderma tsugae* telah dinilai untuk melihat aktiviti antagonistik terhadap bakteria dan yis yang patogenik terhadap manusia. Semua strain *Ganoderma* yang dikaji menunjukkan tahap aktiviti antibakteria yang berbeza terhadap kedua-dua jenis bakteria Gram-positif dan Gram-negatif. Ekstrak etanol daripada *G. australe* strain KUM60813 telah dipilih untuk penulenan protein peringkat seterusnya disebabkan oleh aktiviti antibakteria yang lebih kuat berbanding protein dari strain lain yang dikaji. Protein tersebut kemudiannya diasingkan melalui pemendakan ammonium sulfat dan penurasan gel menggunakan kolom Sephacryl S-100 dan Superdex 75 HR. Ini menghasilkan penulenan protein dengan berat molekul 16.6 kDa yang boleh dilihat di atas gel Tricine SDS PAGE. Protein yang telah ditulenkan telah diuji tahap aktiviti antibakterianya menggunakan kaedah pencairan kaldu mikrotiter. Protein tersebut menunjukkan aktiviti perencatan terhadap *Salmonella typhi* dan *Escherichia coli* O157:H7 dengan IC_{50} masing-masing sebanyak 5.21 mg/ml dan 4.04 mg/ml. Ia juga merencatkan aktiviti enzim reverse transcriptase HIV-1 dengan nilai IC_{50} sebanyak 1.08 mg/ml serta aktiviti deoksiribonuklease terhadap DNA sperma ikan Herring. Protein tersebut tidak menunjukkan sebarang aktiviti antikulat apabila diuji terhadap beberapa jenis kulat yang patogenik terhadap tumbuhan dan juga aktiviti ribonuclease. Akhir sekali, analisa bagi mengenalpasti identiti protein tersebut telah dijalankan menggunakan spektrometri massa dan menunjukkan persamaan yang signifikan dengan protein antikulat daripada *Aspergillus*

giganteus. Kajian ini telah menunjukkan satu pendekatan yang mudah bagi penulenan protein daripada miselium *G. australe*. Justeru, kaedah ini boleh diadaptasi untuk tujuan penulenan protein daripada spesis cendawan lain.