



UNIVERSITI PUTRA MALAYSIA

***BIOLOGICAL ACTIVITIES OF METHANOLIC EXTRACTS OF
SELECTED LOCAL MUSHROOMS***

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**BIOLOGICAL ACTIVITIES OF METHANOLIC EXTRACTS OF
SELECTED LOCAL MUSHROOMS**

By

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**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in
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of the requirement for the degree of Master in Science

**BIOLOGICAL ACTIVITIES OF METHANOLIC EXTRACTS OF
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Chairman: Wan Zuhainis Saad

Faculty : Biotechnology and Biomolecular Sciences

Local selected mushrooms; *Ganoderma boninense*, *Auricularia auricula judae*, *Pleurotus cystidiosus* and one new unidentified (BS01) were evaluated for the antioxidant, antimicrobial, anti-tyrosinase, anti-hyaluronidase, anti-inflammatory and insulin secretion activities. The antioxidant activity was measured using the DPPH (1,1-diphenyl-2-picryl hydrazyl) radical scavenging activity assay and ferric reducing antioxidant power assay (FRAP). In antioxidant activity, both *G. boninense* and *A. A. judae* showed the highest activity for DPPH and FRAP assays with the lowest IC₅₀ value. The IC₅₀ of *G. boninense* and *A. A. judae* for DPPH

were 129.8 ± 1.8 and 198.5 ± 1.5 while for FRAP were 25.8 ± 5.0 and 52.7 ± 3.8 , respectively. Anti-inflammatory activity was determined by inhibition of nitric oxide (NO) and measuring the nitrite (NO_2^-) formation using Griess assay. However, only *G. boninense* showed inhibitory effect of NO inhibition with IC_{50} value at $151.3 \mu\text{g/mL}$ but the extract was also toxic to the RAW 264.7 cell at $500 \mu\text{g/mL}$ with cell viability percentage of $39.28 \pm 2.5\%$ only.

Tyrosinase inhibitory was determined by a spectrophotometric method using L-3,4-dihydroxyphenylalanine (L-DOPA) as a substrate. BS01 exhibited significant ($p < 0.05$) inhibition with the IC_{50} value at $279.4 \mu\text{g/mL}$ and *G. boninense* at $474.4 \mu\text{g/mL}$. The colorimetric Morgan-Elson method was carried out for hyaluronidase assay but all of the mushrooms were tested negative. Insulin secretion activity was measured using rat pancreatic β -cell line, BRIN-BD11 cells and the insulin level produced by the cell line was measured by an enzyme-linked immunosorbent assay using a commercial rat insulin ELISA. Among the mushrooms, *G. boninense* and *P. cystidiosus* extracts showed significant ($p < 0.05$) increased in insulin secretion at the concentration of 62.5, 125, 250 and $500 \mu\text{g/mL}$. Overall, from the four mushrooms tested, *G. boninense* seem to exhibit more bioactive compounds and the further work could be done on the isolation, characterization and purification of the active compounds from the crude extract.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

**AKTIVITI BIOLOGI EKSTRAK METANOL CENDAWAN TEMPATAN
TERPILIH**

oleh

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Cendawan tempatan, *Ganoderma boninense*, *Auricularia auricula judae*, *Pleurotus cystidiosus* dan sejenis spesies yang tidak dikenali (BS01) dinilai untuk aktiviti antioksidan, anti-mikrob, anti-tyrosinase, antihyaluronidase, anti-radang dan aktiviti perembesan insulin. Aktiviti antioksidan diukur menggunakan kaedah 1,1-diphenyl-2-picryl hydrazyl (DPPH) pemerangkapan radikal dan pengurangan antioksidan ferrik (FRAP). Dalam aktiviti antioksidan, kedua-dua *G. boninense* dan *A. A. judae* menunjukkan aktiviti tertinggi bagi ujian DPPH dan FRAP dengan nilai IC_{50} terendah. Nilai IC_{50} *G. boninense* dan *A. A. judae* untuk DPPH adalah 129.8 ± 1.8 dan 198.5 ± 1.5 manakala untuk FRAP 25.8 ± 5.0 dan 52.7 ± 3.8 , masing-

masing. Aktiviti anti-radang ditentukan oleh perencatan nitrik oksida (NO) dan mengukur pembentukan nitrit (NO_2^-) dengan menggunakan kaedah *Griess*. Walau bagaimanapun, hanya *G. boninense* menunjukkan kesan perencatan terhadap NO dengan nilai IC_{50} 151.3 $\mu\text{g/mL}$ tetapi ekstrak tersebut juga toksik kepada sel RAW 264.7 pada kepekatan 500 $\mu\text{g/mL}$ dengan peratusan sel yang hidup hanya $39.28 \pm 2.5\%$.

Perencatan tyrosinase telah ditentukan oleh kaedah spectrophotometrik menggunakan L-3,4-dihydroxyphenylalanine (L-DOPA) sebagai substrat di mana BS01 menunjukkan perencatan yang ketara dengan nilai IC_{50} pada 279.4 $\mu\text{g/mL}$ dan *G. boninense* 474.4 $\mu\text{g/mL}$. Pengukuran warna Morgan-Elson telah dijalankan untuk cerakin hyaluronidase tetapi semua cendawan yang diuji menunjukkan aktiviti negatif. Aktiviti rembesan insulin adalah diukur menggunakan β -sel pankreas tikus dikenali sebagai BRIN-BD11 dan tahap insulin yang dihasilkan oleh sel diukur oleh cerakin immunosorben enzim yang menggunakan ELISA insulin. Antara semua cendawan, ekstrak *G. boninense* dan *P. cystidiosus* menunjukkan peningkatan yang ketara dalam perembesan insulin pada kepekatan 62.5, 125, 250 dan 500 $\mu\text{g/mL}$. Secara keseluruhan, dari empat cendawan yang diuji, *G. boninense* menunjukkan sifat bioaktif yang lebih dan kajian selanjutnya boleh dilakukan bagi pengasingan dan penulenan komponen aktif dari ekstrak mentah.

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I certify that an Examination Committee has met on 2nd July 2012 to conduct the final examination of Mahfuzatunajla Hashim on her Master of Science thesis entitled “**Biological activities of methanolic extracts of selected local mushrooms**” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The committee recommends that the candidate be awarded Master of Science.

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DECLARATION

I declare that the thesis is my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or other institutions.



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Date: 2 July 2012



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