STUDIES ON ANTIOXIDANT AND CYTOTOXIC ACTIVITIES OF DICHLOROMETHANE EXTRACT OF *MARASMIUS* SPECIES

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ABSTRACT

Although much research has been focused on the therapeutic and medicinal effects of wild mushrooms, there is still paucity of information on the pharmacological properties of *Marasmius* species. The present study was undertaken to evaluate the cytotoxic and antioxidant activities of *M. guyanensis* (KUM 20044, KUM 20222, and KUM 20117), *M. kanchingnensis* (KUM 20160), *Marasmius* sp. (KUM 20067), *M. ruforotula* (KUM 20111, KUM 20112) and *M. selangorensis* (KUM 20181). Crude dichloromethane extracts were prepared from the mycelial biomass grown in liquid Glucose-Yeast-Malt-Peptone (GYMP) using a soxhlet extractor system. The cytotoxic effect of the *Marasmius* extracts was screened using Neutral Red assay (NR). At 20 µg/ml, all the extracts demonstrated less than 50% inhibition against the cancer cell lines tested, namely human mouth epidermal carcinoma cell line (KB), human epidermal carcinoma of cervix cell line (CaSki), human colon cancer cell line (HT 29), human intestinal colon cancer cell line (HCT 119), human colorectal cancer cell line (SKOV 3), human breast cancer cell line (MCF 7) and also on normal human fibroblast cell (MRC 5). These extracts were therefore regarded as not actively cytotoxic against the cancer cell lines. At 20 µg/ml, crude dichloromethane extract of *M. guyanensis* (KUM 20044) showed the highest percentage inhibition activity of 37.7% ± 1.82 against SKOV 3 cells followed by *M. ruforotula* (KUM 20111) (34.6% ± 2.22) and *M. guyanensis* (KUM 20222) (33.8% ± 3.35). Crude extracts of *M. guyanensis* (KUM 20222) exhibited the highest percentage inhibition against MCF 7 and HT 29 cancer cells with inhibition of 23.2% ± 1.30 and 40.7% ± 3.76 respectively at 20 µg/ml. For HCT 119 cells, crude extracts of *M. ruforotula* (KUM 20111, KUM 20112) and *M. selangorensis* (KUM 20181) gave the highest percentage of inhibition of 37.0% ± 3.72, 35.5% ± 3.58 and 33.4% ± 3.67, respectively. Similarly, *M. ruforotula* (KUM 20111) exhibited the

highest percentage of inhibition of 47.2% ± 2.04 towards KB cells followed by *M. ruforotula* (KUM 20112) with a value of 46.9% ± 0.84 and 46.9% ± 2.20 for *M. selangorensis* (KUM 20181). Among the extracts, *Marasmius* sp. (KUM 20067) showed the highest percentage of inhibition of 32.0% ± 2.59 against CaSki cells. The antioxidant potency was investigated by employing three established *in vitro* systems such as 2,2-diphenyl,1-picrylhydrazyl (DPPH) radical scavenging, reducing power and metal chelating assays. The extracts exhibited slow kinetic reaction with the DPPH radicals with extracts taking about 60 minutes to reach a steady state scavenging abilities. Based on the EC_{50} values, the effectiveness as DPPH radical scavengers were arranged in descending order: *M. kanchingnensis* (KUM 20160) 67.49 ± 0.004> *M. guyanensis* (KUM 20222) 77.56 ± 0.004 > *M. ruforotula* (KUM 20111) 79.90 ± 0.004 > *M. ruforotula* (KUM 20112) 91.70 ± 0.004 > *M. selangorensis* (KUM 20181) 99.50 ± 0.022> *M. guyanensis* (KUM 20117) 102.65 ± 0.037 > *M. guyanensis* (KUM 20044) 141.80 ± 0.007> *Marasmius* sp. (KUM 20067) 150.78 ± 0.015. Based on the reducing power assay, *M. selangorensis* (KUM 20181) exerted good reducing power abilities of 1.772 nm at 20 mg/ml. In the metal chelating assay, the absorbance reading of all the crude extracts are relatively low which indicates they are exhibiting weak chelating effects on ferrous ion at all concentration tested.

ABSTRAK

Walaupun banyak kajian memberi fokus kepada kesan terapeutik dan perubatan cendawan liar, namun kandungan farmakologi bagi spesies *Marasmius* belum mendapat liputan yang meluas. Kajian ini dijalankan bertujuan untuk mengkaji aktiviti sitotoksik dan antioksidan bagi *M. guyanensis* (KUM 20044, KUM 20222 dan KUM 20117), *M. kanchingnensis* (KUM 20160), *Marasmius* sp. (KUM 20067), *M. ruforotula* (KUM 20111, KUM 20112) dan *M. selangorensis* (KUM 20181). Ekstrak mentah diklorometana daripada miselia yang ditumbuhkan dalam cecair 'Glucose-Yeast-Malt-Peptone' (GYMP) dihasilkan mengunakan sistem soxhlet ekstrak. Esei sitotoksik 'Neutral Red' (NR) digunakan untuk menguji aktiviti sitotoksik ekstrak *Marasmius.* Pada 20 µg/ml, semua ekstrak menunjukkan kurang daripada 50% perencatan terhadap sel kanser yang diuji, iaitu pada sel terbitan mulut epidermal manusia (KB), sel terbitan kanser servik (CaSki), sel terbitan kanser kolorektal (HT 29), sel terbitan kanser kolon manusia (HCT 119), sel terbitan kolorektal kanser (SKOV 3), sel terbitan kanser payudara (MCF 7) termasuk juga pada sel terbitan normal peparu manusia (MRC 5). Ini menunjukkan bahawa kesemua estrak tidak mempunyai kesan sitotosik yang aktif terhadap sel kanser yang diuji. Pada 20 µg/ml, ekstrak mentah diklorometana *M. guyanensis* (KUM 20044) menunjukkan kesan peratus perencatan yang tinggi iaitu 37.7% ± 1.82 pada SKOV 3 sel diikuti dengan *M. ruforotula* (KUM 20111) (34.6% ± 2.22) dan *M. guyanensis* (KUM 20222) (33.8% ± 3.35). Ekstrak *M. guyanensis* (KUM 20222) mempamerkan peratus perencatan tinggi terhadap sel kanser MCF 7 dan HT 29 dengan kadar sebanyak 23.2% ± 1.30 dan 40.7% ± 3.76 pada 20 µg/ml. Bagi sel HCT 119, ekstrak *M. ruforotula* (KUM 20111, KUM 20112) dan *M. selangorensis* (KUM 20181) memberikan peratus perencatan yang tinggi pada 37.0% ± 3.72, 35.5% ± 3.58 dan 33.4% ± 3.67. *Maramius ruforotula* (KUM 20111) menunjukkan peratus

perencatan tinggi sebanyak 47.2% ± 2.04 terhadap sel KB diikuti dengan *M. ruforotula* (KUM 20112) dengan nilai 46.9% ± 0.84 dan 46.9% ± 2.20 untuk *M. selangorensis* (KUM 20181). Perbandingan antara semua ekstrak menunjukkan *Marasmius* sp. (KUM 20067) memberi peratus perencatan tinggi sebanyak 32.0% ± 2.59 terhadap sel CaSki. Potensi antioksidan dikaji dengan menggunakan pelbagai sistem *in vitro* seperti esei radikal 2,2-diphenyl,1-picrylhydrazyl (DPPH), esei tenaga reduksi dan kuasa antipengoksidaan penurunan ion logam. Ekstrak - ekstrak tersebut menghasilkan tindakbalas kinetik yang lambat dengan radikal DPPH dan memerlukan masa selama 60 minit untuk mencapai keadaan stabil. Nilai- nilai EC_{50} antioksidan: *M. kanchingnensis* (KUM 20160) 67.49 ± 0.004 > *M. guyanensis* (KUM 20222) 77.56 ± 0.004 > *M. ruforotula* (KUM 20111) 79.90 ± 0.004 > *M. ruforotula* (KUM 20112) 91.70 ± 0.004 > *M. selangorensis* (KUM 20181) 99.50 ± 0.022> *M. guyanensis* (KUM 20117) 102.65 ± 0.037 > *M. guyanensis* (KUM 20044) 141.80 ± 0.007> *Marasmius* sp. (KUM 20067) 150.78 ± 0.015. Berdasarkan kepada esei tenaga reduksi, *M. selangorensis* (KUM 20181) menunjukkan kuasa penurunan yang baik iaitu sebanyak 1.772 nm pada 20 mg/ml. Kesemua ekstrak menunjukkan kuasa antipengoksidaan penurunan ion logam yang lemah.

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LIST OF ABBREVIATIONS

ATCC	American Tissue Culture Collection
BHA	Butylated hydroxyanisole
CAT	catalase
cm	centimetre
CO_2	carbon dioxide
BHA	butylated hydroxynanisole
DCM	dichloromethane
DMEM	Dulbecco's Modified Eagle's Medium
DMSO	Dimethyl sulfoxide
DNA	deoxyribonucleic acid
DPPH	1,1diphenyl-2-picrylhydrazyl or α,α-diphenyl-β-picrylhydrazyl
CaSki	human epidermal carcinoma of cervix cell line
EC_{50}	Effective concentration to give 50% antioxidant activity
ED_{50}	Effective dosage to inhibit 50% of proliferation cell cultures
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme Linked Immunosorbent Assay
Fe^{2+}	ferrous ion
Fe^{3+}	ferric ion
FRAP	Ferric-reducing ability of plasma
g	Gram
GAE	Gallic acid equivalents
GYMP	Glucose-Yeast-Malt-Peptone
GSH.Px	glutathione peroxidase
HCT 119	human intestinal colon cancer cell line
H_2O_2	hydrogen peroxidase
HLPC	high performance liquid chromatography
HT 29	human colon cancer cell line
KB	human mouth epidermal carcinoma cell line
KUM	Kulat Universiti Malaysia
LDH	lactate dehydrogenase leakage
LOO•	lipid peroxyl radical
M	Molar
MCF 7	human breast cancer cell line
MEA	Malt Extract Agar
MEM	Medium Essential Medium
min	minutes
MRC 5	human fibroblast cell
MTT	methyl tetrazolium
nM	Nanometer
NO	nitric oxide radical
NR	Neutral Red
O_2	superoxide anion radical
OD	Optical Density
OH	hydroxyl radical
PBS	Phosphate Buffered Saline
PSK	protein-bound polysaccharide
PSP	polysaccharide-peptide
mg	Milligram

mg/ml	milligram per litre
ml	Milliliter
µg/ml	Microgram per milliltre
µl	Microlitre
µm	Micrometre
RPMI	Rosewell Park Memorial Institute
ROS	reactive oxygen species
RNS	reactive nitrogen species
rpm	rotation per minute
spp.	species
SKOV 3	human colorectal cancer cell line
TCA	trichloroacetic acid
UV	ultraviolet
%	percentage
^{o}C	degree celcius
wt	weight