ANTIOXIDATIVE POTENTIAL OF SELECTED EDIBLE WILD MUSHROOMS FROM SABAH

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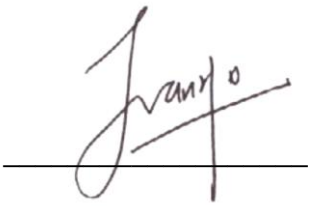

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ABSTRACT

ANTIOXIDATIVE POTENTIAL OF SELECTED EDIBLE WILD MUSHROOMS FROM SABAH

The objectives of the present study were to evaluate the *in vitro* antioxidant properties of four species of edible wild mushrooms namely, *Pleurotus porrigens*, *Schizophyllum commune*, *Hygrocybe conica*, and *Lentinus ciliatus*; optimization of the extraction conditions for antioxidant activity of the mushrooms; identification of the potent antioxidative components from the selected mushrooms; and the evaluation of the oxidative stability of cooking oil supplemented with selected mushroom extracts. The antioxidant properties of edible wild mushrooms were evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging ability, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation inhibition, ferric reducing antioxidant power (FRAP), and β-carotene-linoleate bleaching (β-CB). Total phenolic content (TPC) was determined using Folin-Ciocalteu's method. Response surface methodology (RSM) was employed to optimize the extraction time and temperature of *P. porrigens* and *S. commune* for the maximal yields of antioxidant activity. Fractionation by means of column chromatography was carried out and the identification of major antioxidative components was performed by liquid chromatography-mass spectrometry analysis. The oxidative stability of sunflower oil was determined by peroxide value, iodine value, p-anisidine value, and thiobarbituric acid-reactive substances. Results showed that *P. porrigens* had significantly higher ($p < 0.05$) DPPH radical scavenging ability and FRAP than that of butylated hydroxyanisole (BHA), while *S. commune* showed comparable DPPH radical scavenging ability and ABTS radical cation inhibition with BHA. Total phenolic content was found in a descending order of *P. porrigens* > *L. ciliatus* = *P. ostreatus* > *H. conica* = *S. commune*. Positive correlations were found between TPC and DPPH; ABTS; FRAP; and β-CB, respectively, indicating that the presence of phenolic compounds contribute to the antioxidant activities of the edible wild mushrooms. The optimized extraction time and temperature of *P. porrigens* and *S. commune* were 315.5 min and 37.4°C; and 213.2 min and 41.5°C, respectively. The values obtained experimentally agreed well with the predicted values, indicating the suitability of respective RSM models for maximal yields of antioxidant activity. Both sub-fraction (SF)-III of *P. porrigens* and *S. commune* showed consistently higher DPPH radical scavenging ability, FRAP, and TPC with two flavones glucosides, namely luteolin 7-*O*-β-glucoside and apigenin 7-*O*-β-glucoside were identified in both SF-III. The sunflower oils supplemented with *P. porrigens* and *S. commune* extracts were found to be able to prolong the shelf-life between 1 and 2 years by retarding the formations of primary and secondary oxidation products, and reducing losses of polyunsaturated fatty acids. In conclusion, the selected edible wild mushrooms showed promising antioxidant activity, and luteolin 7-*O*-β-glucoside and apigenin 7-*O*-β-glucoside were identified in SF-III of *P. porrigens* and *S. commune*. Thus, edible wild mushrooms from Sabah can be promoted as antioxidant-rich foods as well as potential sources of natural antioxidants for food industry application.

ABSTRAK

Objektif-objektif kajian ini adalah untuk menilai aktiviti antioksidan secara in vitro empat spesis cendawan liar yang boleh dimakan (Pleurotus porrigens, Schizophyllum commune, Hygrocybe conica dan Lentinus ciliatus); pengoptimuman keadaan pengekstrakan untuk aktiviti antioksidan; pengenalpastian komponen antioksidan yang berpotensi; dan menilai kestabilan pengoksidaan minyak masak yang ditambah dengan ekstrak cendawan terpilih. Aktiviti-aktiviti antioksidan dinilai melalui keupayaan pemerangkapan radikal 2,2-difenil-1-pikrilhidrazil (DPPH), perencatan radikal kation asid 2,2'-azino-bis(3-ethilbenzothiazoline-6-sulfonik) (ABTS), kuasa antioksidan penurunan ferik (FRAP) dan pelunturan β-karotena-linoleate (β-CB). Jumlah kandungan fenolik (TPC) ditentukan melalui kaedah Folin-Ciocalteu. "Response surface methodology" (RSM) digunakan untuk mengoptimumkan masa dan suhu pengekstrakan P. porrigens dan S. commune untuk menghasilkan aktiviti antioksidan yang maksimum. Pengasingan dilakukan dengan kromatografi kolum dan pengenalpastian antioksidan dilakukan melalui kromatografi cecair-spektrometri jisim. Kestabilan pengoksidaan minyak bunga matahari ditentukan melalui nilai peroksida, nilai iodin, nilai p-anisidin dan bahan reaktif-asid thiobarbituric. Keputusan menunjukkan P. porrigens mempunyai keupayaan pemerangkapan radikal DPPH dan FRAP yang signifikan tinggi ($p < 0.05$) berbanding dengan butylated hydroxyanisole (BHA), sementara S. commune menunjukkan keupayaan pemerangkapan radikal DPPH dan aktiviti perencatan radikal kation ABTS yang setanding dengan BHA. Jumlah kandungan fenolik didapati dalam urutan menurun seperti berikut: P. porrigens > L. ciliatus = P. ostreatus > H. conica = S. commune. Korelasi yang positif ditemui antara TPC dan DPPH; ABTS; FRAP dan β-CB masing-masing, menunjukkan kehadiran kompaun fenolik menyumbang kepada aktiviti-aktiviti antioksidan cendawan liar yang boleh dimakan. Masa dan suhu pengekstrakan yang optimum bagi P. porrigens dan S. commune adalah 315.5 min dan 37.4°C; dan 213.2 min dan 41.5°C masing-masing. Nilai yang diperolehi secara eksperimen merapati nilai yang diramalkan, menunjukkan kesesuaian model RSM tersebut untuk menghasilkan aktiviti antioksidan dan TPC yang maksimum. kedua-dua sub-fraksi (SF)-III P. porrigens dan S. commune menunjukkan nilai tertinggi bagi keupayaan pemerangkapan radikal DPPH, FRAP dan TPC dengan dua flavone glukosida, iaitu luteolin 7-O-β-glukosida dan apigenin 7-O-β-glukosida dikenalpasti hadir dalam kedua-dua SF-III. Kestabilan pengoksidaan minyak bunga matahari yang ditambahkan dengan ekstrak-ekstrak P. porrigens dan S. commune didapati berupaya memanjangkan tempoh penyimpanan selama 1 dan 2 tahun melalui perencatan pembentukan produk-produk primer dan sekunder, dan berupaya mengurangkan kehilangan asid-asid lemak politaktepu. Kesimpulannya, cendawan-cendawan liar yang boleh dimakan menunjukkan aktiviti antioksidan yang baik, dan kandungan luteolin 7-O-β-glukosida dan apigenin 7-O-β-glukosida didapati hadir dalam SF-III P. porrigens dan S. commune. Oleh itu, cendawan-cendawan liar yang boleh dimakan dari Sabah boleh dipromosikan sebagai makanan yang kaya dengan antioksidan semulajadi dan berpotensi sebagai sumber antioksidan untuk aplikasi industri makanan.

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LIST OF ABBREVIATIONS/SYMBOLS

$A^{\bullet}$	Antioxidant radical
ABTS	2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
AH	Antioxidant
$AlCl_3.6H_2O$	Aluminium chloride 6-hydrate
ANOVA	Analysis of variance
ANT	Antioxidant activity
α	Alpha
β	Beta
BCB	Beta carotene bleaching
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluen
C	Carbon
CE	Catechin equivalent
CAT	Catalase
CCD	Central composite design
CoQ_{10}	Coenzyme Q_{10}
CTC	Condensed tannin content
CV	Coefficient of variation
°C	Degree Celsius
DNA	Deoxyribonucleic acid
DPPH	2,2-diphenyl-1-picylhydrazyl
DW	Dried weight
EA	Ethyl acetate
EC_{50}	Half maximal effective concentration
EGCG	Epigallocatechin-3-*O*-gallate
ET	Electron transfer
FA	Formic acid
FBB	Fast blue B salt
FC	Folin Ciocalteu
FCR	Folin Ciocalteu reagent
FE	Fe^{2+} equivalent
$Fe(II)/Fe^{2+}$	Ferrous ion
$Fe(III)/Fe^{3+}$	Ferric ion
$FeCl_3.6H_2O$	Ferric trichloride hexahydrate
FRAP	Ferric reducing/antioxidant power
GAE	Gallic acid equivalent
GPx	Glutathione peroxidase
GRAS	Generally Recognized as Safe
g	Gram
HAT	Hydrogen atom transfer
HCl	Hydrochloric acid
H_2O	Water
H_2O_2	Hydrogen peroxide
HPLC-DAD	High-performance liquid chromatography-diode array detector
HPTLC	High-performance thin layer chromatography

K	Potassium
$K_2S_2O_8$	Potassium persulphate
LC-MS	Liquid chromatography-mass spectrometry
LDL	Low-density lipoprotein
LLP	Liquid-liquid partitioning
Ln	Natural logarithm
Mn-SOD	Manganese superoxide dismutase
µg	Microgram
µg/ml	Microgram per milliliter
µl	Microliter
µM	Micromolar
mg	Milligram
mg/g	Milligram per gram
ml	Milliliter
ml/g	Milliliter per gram
mm	Millimeter
mM	Millimolar
min	Minute
M	Molar
Mo	Molybdenum
NaOH	Sodium hydroxide
$NaNO_2$	Sodium nitrate
n-BUT	n-Butanol
nm	Nanometer
NMR	Nuclear magnetic resonance
NO	Nitric oxide
O_2	Oxygen
$O_2^{\bullet-}$	Superoxide anion
O_3	Ozone
$OH^{\bullet}$	Hydroxyl radical
ORAC	Oxygen radical absorption capacity
%	Percentage
p	Probability
PG	Propyl gallate
$R^{\bullet}$	Alkyl free radical
R^2	Coefficient of determination
RH	Antioxidant
$ROO^{\bullet}$	Peroxyl radical
$RO^{\bullet}$	Alkoxyl radical
ROOH	Alkyl peroxides
ROS	Reactive oxygen species
rpm	Rotation per minute
RSA	Response surface analysis
RSM	Response surface methodology
SET	Single electron transfer
SF	Sub-fraction
SOD	Superoxide dismutase
TBHQ	*tert*-Butyl hydroquinone
TE	Trolox equivalent

TEAC	Trolox equivalent antioxidant capacity
TFC	Total flavonoid content
TPC	Total phenolic content
TPTZ	2,4,6-Tripyridyl-s-triazine
TOTOX	Total oxidation
TRAP	Total peroxyl radical trapping potential
UV	Ultraviolet
v/v	Volume per volume
w/v	Weight per volume
X_1	Extraction time
X_2	Extraction temperature

CHAPTER 1

INTRODUCTION

1.1 Background

The oxidation process is essential for energy production in most of the vital metabolic processes for living organisms including human beings. However, these processes produce free radicals and other reactive oxygen species (ROS) that have been linked to degenerative processes associated with aging, as well as with the onset of many diseases such as cancer, rheumatoid arthritis, and atherosclerosis (Halliwell and Gutteridge, 1984). Free radicals and ROS are also derived from external sources such as exposure to air pollutants, industrial chemicals, radioactivity rays, ozone, and cigarette smoking (Dean *et al.*, 1997). The increased rate of oxidation within a biological system disturbs the balance between prooxidant and antioxidant state, which often is referred as oxidative stress (Dubost *et al.*, 2007; Li *et al.*, 2009).

The major concern in the food industry and a real challenge for the food scientist is the deterioration of food quality because of the exerted oxidative stress. Free radicals often oxidize lipid components within foods leading to rancidity which results in off-odors and flavors. The formation of secondary oxidation products that are potentially toxic decreases the nutritional quality and safety of foods (Chanwitheesuk *et al.*, 2005). Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and *tert*-butyl hydroquinone (TBHQ) have been used extensively in the past to preserve food quality by preventing oxidative deterioration. However, their usage is restricted due to the documented carcinogenicity effects in laboratory animals (Zheng and Wang, 2001). Butylated hydroxyanisole has been shown to induce fore-stomach squamous cell carcinomas in rodents at high doses above 3000 ppm (Williams *et al.*, 1999), and BHT is suspected to be the main factor for the development of liver damage and carcinogenesis in laboratory animals (Saad *et al.*, 2007).

The application of powerful TBHQ in foods is prohibited in countries like Japan, Canada and Europe (Mohdaly *et al.*, 2010); whereas, BHA has been de-listed from the generally recognized as safe (GRAS) compound in the United States of America (Goli *et al.*, 2005). Most animal experimental studies had shown carcinogenesis effects of synthetic antioxidants at very high doses (> 3000 ppm), however, no human data has been reported on such high doses thus far. Nevertheless, there are controversies arising from the public pertaining to the probable side-effects of synthetic antioxidants on human health. These controversies had sparked the interests among food scientists to research into naturally occurring antioxidants that are considered to be safer for use in food or nutraceuticals applications. Bioactive compounds derived from natural resources have been reported to possess physiological activities such as antioxidative, antibacterial, antiviral, antiulcer, antimutagenic, antiallergic, and anticarcinogenic properties; in addition to protecting the cell from free radical damage (Moure *et al.*, 2001).

Almost all organisms including human beings possess an innate protective mechanism against free radical damage by antioxidative enzymes (such as superoxide dismutase and catalase) or compounds (such as ascorbic acid, tocopherols, and glutathione) (Niki *et al.*, 1994). Nevertheless, the aging process can alter the mechanism of antioxidant protection that affect many bodily physiological functions that eventually resulting in disease state and further acceleration of aging. The antioxidants present in human diet and those derived from natural resources are of great interest as possible protective agents to help the human body fight against or to reduce the negative effects resulting from oxidative damage. There is a wide array of natural antioxidants that have been isolated from different types of plant materials such as oilseeds, cereal crops, vegetables, fruits, leaves, roots, spices, and herbs (Ramarathnam *et al.*, 1995). Chinese herbs are well-known for their therapeutic effects for several millennia, and some of them are found to exhibit significant antioxidant activity (Su, 1992; Kim *et al.*, 1994).