How to Cite:
Al-Salihi, A. S. R., & Lau, B. F. (2022). Antioxidant activities and chemical composition of hot water-soluble extracts and fractions of selected Schizophyllum commune Fr. strains. *International Journal of Health Sciences*, *6*(S8), 3976–3994.
https://doi.org/10.53730/ijhs.v6nS8.13035

Antioxidant activities and chemical composition of hot water-soluble extracts and fractions of selected Schizophyllum commune Fr. Strains

Alyaa Salah Rashid Al-Salihi
Institute of Biological Sciences, Faculty of Science, Universiti Malaya, 50603 Kuala Lumpur, Malaysia
Corresponding author email: alyaa.alsalihi86@gmail.com

Beng Fye Lau
Institute of Biological Sciences, Faculty of Science, Universiti Malaya, 50603 Kuala Lumpur, Malaysia

***Abstract*---**Oxidative stress is a conjoint pathological mechanism that can be triggered by free radicals leading to cellular senescence or apoptosis. Antioxidants have the potential to inhibit free radicals' generation and balance the irregulated oxidative stress to restore cellular homeostasis. *Schizophyllum commune*, also known as the split-gill mushroom, is well-known for its nutritional values and bioactive compounds. This study aimed to investigate the antioxidant activity and chemical composition of the hot water extracts of selected strains of *S. commune* including natural, gamma-irradiated and hybrid strains. The fruiting bodies of different strains of *S. commune* were subjected to hot water extraction and the extracts were evaluated by radical scavenging, and reducing capacity assays. Results showed that, overall, the antioxidant activity of the various strains was comparable. The natural strain W and the strain obtained when strain W was subjected to gamma-irradiation at a dose of 4000 Gy (W4000) consistently exhibited high antioxidant activities in all assays and they were either comparable or higher than common edible mushrooms, *Pleurotus pulmonarius* and *Agaricus bisporus*. Moreover, the gamma-irradiated strains R2000 and R4000 exhibited higher reducing activities (0.40 and 0.41 mmol Fe^{2+}/g extract) than the parental strain R (0.27 mmol Fe^{2+}/g extract). Furthermore, the hybrid strains WR and RW showed higher scavenging activities towards 2,2 diphenyl-1-picylhydrazyl radicals (1.40 and 1.45 mmol TE/g extract) than the parental strain R (1.17 mmol TE/g extracts), but lower than the parental strain W (1.78 mmol TE/g extract). There is a strong

correlation between the antioxidant activity of the extracts and the levels of flavonoid and phenolic compounds ($p < 0.01$). In order to determine the nature of antioxidative components in the hot water extracts of *S. commune*, the extracts of strains W and W4000, were fractionated to yield fractions with distinct chemical components. Results indicated that the phenolics- and flavonoids-rich ethyl acetate and ethanol fractions exhibited higher scavenging activities towards DPPH radicals, and ferric reducing activities compared to the fractions containing mostly water-soluble components. Similar trends were observed for both W and W4000 strains. Our findings suggest that the observed antioxidant activities of the hot water extracts of *S. commune* is mainly attributed to the water-soluble, low- molecular-weight compounds rather than the water-soluble components such as polysaccharides and proteins which make up the bulk of the water extracts.

***Keywords*---**Schizophyllum commune, hot water extracts, fractionation, antioxidants.

Introduction

Oxidative stress is a physiological state triggered by an exposure to excessive levels of free radicals or reactive oxygen species (ROS) such as superoxide anion ($O_2\bullet^-$), hydrogen peroxide (H_2O_2) and hydroxyl radical (•OH) (Mittal et al., 2014). These reactive species are molecules or atoms having one or more unpaired electron in the external electron shell, capable of stabilizing themselves by abstracting an electron from a nearby target molecule (Di Meo et al., 2016; Phaniendra et al., 2015). The normal cell processes such as oxidative phosphorylation and exposure to toxic environmental contaminants can result in the formation of elevated concentrations of free radicals which can hamper the functionalities of important cell biomolecules, mainly proteins, DNA, and lipids. Rising evidence indicated that the severe impairment caused by the accumulation of ROS contributes to several chronic disorders (Mittal et al., 2014; Rahal et al., 2014; Wei et al., 2009). Antioxidants play a significant role in neutralising the oxidative stress by minimising the levels of free radicals and consequently enabling the body cell to carry out useful biological functions without excessive damage. Balance must be reached in the body between free radicals and antioxidants to avoid the risk of oxidative stress (Al-Joudi, 2013; Halliwell, 2006).
Mushrooms have gained significant attention for their nutritional value (Rathee et al., 2012). Mushrooms provide a remarkable group of nutritional substances, such as vitamins B1, B2, B12, C, D and E (Feeney et al., 2014). Besides, due to its high protein contents, mushrooms can be used as a substrate to avoid protein malnutrition (Miles & Chang, 2004). Similarly, their low cholesterol level as well as carbohydrate contents make it a suitable substitute food for diabetic and cardiac patients (Lee et al., 2019). In addition to mineral compositions of mushroom includes K, P, Ca, Mg, Na, Fe and some other trace elements (Wang et al., 2014).

Numerous types of mushrooms have been reported to possess antioxidant properties, which enable them to neutralize free radicals. Mushroom's antioxidant components are found in the fruiting bodies, mycelium and culture broth. For example, Gasecka et al. (2018) have estimated the content of macroelements (Ca, K, Mg, Na) as well as ascorbic acid and phenolic compounds in several mushrooms, and correlated the presence of these components to the ability of the extracts to act as free radical scavengers. Likewise, certain types of medicinal mushrooms and their extracts are alternative sources of antioxidant components, and their defense abilities against oxidative effects have been demonstrated by De Silva et al. (2012). While, Bozdogan et al. (2018) have worked on two edible mushrooms *Lactarius deliciosus* and *Pleurotus ostreatus* and found that the ion reducing capability of the isolated extracts is correlated to the presence of phenolic compounds and lycopene content.

Among the species of basidiomycetes with notable medicinal significance is *Schizophyllum commune*, a fungus which is typically found on diverse trees and rotting wood across the world (Chowdhary et al., 2013). It is a non-sporulating, wood-decaying and endophytic fungus which is also known as split gills due to the splitting appearance of its underside gills when it is dehydrated (Sim, 2014). Several researches have showed that various extracts of *S. commune* exhibited high scavenging activities toward free radicals and acted as ion reducing agents (Chandrawanshi et al., 2017; Devi et al., 2014; Emsen et al., 2017; Mirfat et al., 2010) but in-depth studies into the chemical constituents are lacking. Therefore, the aim of this research was to compare the antioxidant activities of the hot water extracts and fractions of the fruiting bodies of selected *S. commune* strains. As well as to determine the chemical compositions of the *S. commune* crude hot water extracts and fractions of the selected strains, and correlate the antioxidant activities of *S. commune* extracts and fractions with their chemical compositions.

Methodology

The mycelium cultures of *S. commune* were obtained from the Mycology Laboratory, Institute of Biological Sciences, Faculty of Science, Universiti Malaya. The strains used in this study have minor differences with respect to the size and colour of the fruiting bodies. The fruiting bodies of *P. pulmonarius* (grey oyster mushroom) and *A. bisporus* (white button mushroom) were purchased from a local supermarket in Petaling Jaya, Selangor, Malaysia. The dried mushroom samples were powdered using a Waring blender. The powdered fruiting bodies were soaked in 80% (v/v) aqueous ethanol at a ratio of 1:10 (w/v) for two days at room temperature under shaking condition. The extracts were then filtered and the residues were air-dried before subjected to hot water extraction at a ratio of 1:20 (w/v) at 121°C for 30 min under high pressure in an autoclave.

Mushroom samples used in this study

Sample	Description
Natural strains of *Schizophyllum commune*	
W	Natural strain isolated from Malaysia
R	Natural strain isolated from Thailand
Gamma-irradiated strains of *S. commune*	

W2000	Strain W subjected to gamma irradiation at a dose of 2000 Gy
W4000	Strain W subjected to gamma irradiation at a dose of 4000 Gy
R2000	Strain R subjected to gamma irradiation at a dose of 2000 Gy
R4000	Strain R subjected to gamma irradiation at a dose of 4000 Gy
Hybrid strains of *S. commune*	
WR	Hybrid of strain W and R with dominant characteristics of W
RW	Hybrid of strain W and R with dominant characteristics of R
Common edible mushrooms	
Pleurotus pulmonarius	Obtained from commercial sources
Agaricus bisporus	Obtained from commercial sources

*The fruiting bodies of all *S. commune* strains were cultivated using sawdust as the main substrates.

Fractionation

The crude hot water extracts of the *S. commune* strains W and W4000 were selected for the study. The powdered fruiting bodies (20 g) of the selected strains were again subjected to aqueous ethanol and hot water extraction as described above. The resulting hot water extracts were partitioned with ethyl acetate (1:1, v/v) to give the ethyl acetate-soluble and water fractions. The ethyl acetate fractions of strains W and W4000 were termed as W-EA and W4000-EA, respectively. The water fractions of strains W and W4000 were termed as W-H_2O and W4000-H_2O, respectively. Powdered ammonium sulphate (60.3 g) was gradually added into the aqueous fraction until 90% (w/v) saturation was achieved and the mixture was left at 4°C. These fractions were termed as WS1. The fractions obtained from strains W and W4000 were termed as W-WS1 and W4000-WS1, respectively. The samples were kept at -20°C prior to analysis. While Ethanol was added to the water fractions until the final concentration of ethanol was 80% (v/v). The mixture was left overnight at 4°C without stirring. Following that, the mixture was centrifuged at 10,000 ×g for 20 min and the pellet was washed with acetone and ethanol, Theses fractions were termed as WS2. The fractions obtained from strains W and W4000 were termed as W-WS2 and W4000-WS2, respectively.

Determination of chemical compositions of the crude hot water extracts and fractions

Total sugar content

Total sugar content of the extracts was assayed by the phenol-sulphuric acid method (Masuko et al., 2005). A 50 µl of sample was added in a well followed by 150 µl of concentrated sulphuric acid and 30 µl of 5% (w/v) phenol in water.

Total protein content

Total protein content of the extracts was estimated using the Micro BCA™ Protein Assay Kit (Thermo Scientific, Rockford, IL, USA). A 200 µl of the working reagent, prepared by mixing 50 part of reagent A and 1 part of reagent B, was added to 25 µl of sample. The mixture was incubated at 37°C for 30 min.

Total phenolic content

Total phenolic content of the extracts was measured using the Folin-Ciocalteu method by Singleton and Rossi (1965) with modifications. A 25 µl of 1 N Folin-Ciocalteu reagent was added into 50 µl of sample and the mixture was incubated for 5 min. Then, 100 µl of sodium carbonate solution (0.57 M) and 75 µl of milliQ water were added respectively. The final volume was 250 µl.

Total flavonoid content

Total flavonoid content (TFC) of the extracts was determined using the aluminium chloride colorimetric method by Liu et al. (2008) with slight modifications. A 100 µl of extracts was mixed with 10 µl of 5% (w/v) sodium nitrite. After incubation for 5 min, 10 µl of 10% (w/v) aluminium chloride was added. Following 6 min incubation, 100 µl of 1 M sodium hydroxide and 30 µl of milliQ water were added respectively. The final volume was 250 µl. The absorbance was read at 510 nm. A calibration curve of rutin (0-0.1 mg/ml) was prepared and results were expressed as mg rutin equivalents (RE)/g extract.

Assessment of antioxidant activity of crude hot water extracts and fractions

The Diphenyl-2-pricryl-hydrazyl (DPPH) free radical scavenging activity of the extracts was measured according to the method of Brand-Williams et al. (1995). The 3-ethylbenzothiazoline-6-sulphonic acid (ABTS) is a stable radical cation used to measure the trolox equivalent antioxidant capacity. The assay was done according to Re et al. (1999). Ferric reducing antioxidant power (FRAP) assay was performed according to the method of Benzie and Strain (1996). The FRAP reagent was prepared by adding 300 mM acetate buffer, 10 mM of TPTZ in 40 mM HCl and 20 mM of ferric chloride solution at a ratio of 10:1:1 (v/v/v). The Cupric ion-reducing antioxidant capacity (CUPRAC) assay was done according to Apak et al. (2005) with slight modifications. The CUPRAC test solution was prepared by adding 50 µl of copper(II) chloride (10 mM), neocuproine (7.5 mM) and acetate buffer (1000 mM, pH 7).

Statistical analysis

All analyses were performed in triplicates. Results were expressed as mean ± standard deviation (n=3). All data were statistically analyzed using Statistical Package for the Social Sciences software, version 25.0 (SPSS Inc., Chicago, Illinois, USA). One-way ANOVA was used to compare means among the groups. Pearson's correlation was used to analyse the relationship between chemical components and antioxidant activities of the extracts. A p value < 0.05 will be regarded as significant.

Results and Discussion

Table 1: The yields of crude hot water extracts of selected mushroom samples

Samples	Yield (%, w/w)
Schizophyllum commune strains	
W	9.02
W2000	13.07
W4000	12.76
R	9.32
R2000	11.88
R4000	13.38
WR	10.92
RW	12.76
Common edible mushrooms	
P. pulmonarius	11.24
A. bisporus	8.57

*W; natural strain from Malaysia; W2000 and W4000, two gamma-irradiated strains; WR and RW, two hybrid strains; R, natural strain isolated from Thailand; R2000 and R4000, two gamma-irradiated strains.

Table 2: The yields of fractions of *S. commune* strains W and W4000

Fraction	Yield (%, w/w)	
	S. commune strain W	*S. commune* strain W4000
Ethyl acetate (EA)	0.07	0.05
Water (H_2O)	9.41	6.01
Water-soluble component (WS1)	18	22.71
Water-soluble component (WS2)	53.36	71.28
Ethanol (EtOH)	3.0	2.3

*The fractions were derived from the crude hot water extracts of *S. commune* strains W and W4000.

Table 3: The chemical compositions of crude hot water extracts of selected mushroom samples

Mushrooms	Chemical Composition			
	Total sugars (mg carbohydrate/g extract)	Total proteins (mg protein/g extract)	Total phenolic content (mg GAE/g extract)	Total flavonoid content (mg RE/g extract)
S. commune				
W	327.99 ± 4.36^{e}	539.69 ± 6.05^{c}	25.78 ± 0.57^{a}	13.45 ± 0.31^{b}
W2000	400.73 ± 1.55^{d}	442.42 ± 12.20^{e}	20.14 ± 0.79^{bc}	7.78 ± 0.95^{de}
W4000	414.85 ± 17.11^{c}	430.60 ± 39.31^{d}	22.07 ± 0.87^{b}	7.01 ± 0.73^{de}

R	229.95 ± 1.61^{f}	526.66 ± 40.98^{cd}	15.95 ± 0.89^{def}	4.04 ± 0.62^{f}
R2000	596.22 ± 1.72^{a}	480.00 ± 19.68^{de}	19.21 ± 0.22^{c}	8.76 ± 0.93^{cde}
R4000	395.14 ± 13.27^{de}	438.18 ± 26.78^{e}	18.27 ± 0.49^{cde}	9.51 ± 0.46^{bcd}
WR	449.65 ± 9.07^{bc}	440.90 ± 8.33^{e}	18.59 ± 0.36^{d}	9.92 ± 0.65^{bc}
RW	468.97 ± 12.61^{b}	395.75 ± 6.82^{e}	15.63 ± 0.21^{ef}	6.41 ± 0.37^{ef}
P. pulmonarius	219.06 ± 14.98^{fg}	666.96 ± 12.60^{b}	13.87 ± 1.95^{f}	1.40 ± 0.64^{g}
A. bisporus	204.85 ± 8.25^{g}	684.24 ± 63.58^{a}	29.39 ± 0.16^{a}	24.18 ± 0.89^{a}

a, b, c.... means with different superscripts on the same column differ significant ($p < 0.05$).
*Data are expressed as mean ± standard deviation (n = 3).
*GAE, gallic acid equivalent; RE, rutin equivalent.
*W; natural strain from Malaysia; W2000 and W4000, two gamma-irradiated strains; WR and RW, two hybrid strains; R, natural strain isolated from Thailand; R2000 and R4000, two gamma-irradiated strain

Table 4: The chemical compositions of the fractions derived from the selected strains of *S. commune*

Fractions	Chemical composition			
	Total sugars (mg carbohydrate/g extract)	Total proteins (mg protein/g extract)	Total phenolic (mg GAE/g extract)	Total flavonoid (mg RE/g extract)
S. commune strain W				
W crude	224.16 ± 11.06^{c}	457.87 ± 5.01^{b}	22.28 ± 0.28^{b}	8.69 ± 0.42^{d}
W-EA	ND	ND	34.59 ± 2.46^{a}	84.55 ± 4.09^{a}
W-H_2O	400.53 ± 6.81^{b}	458.18 ± 7.10^{b}	19.45 ± 0.24^{cd}	12.52 ± 1.18^{c}
W-WS1	549.46 ± 12.65^{a}	729.39 ± 25.26^{a}	29.89 ± 1.22^{a}	42.69 ± 1.74^{b}
W-WS2	597.30 ± 10.30^{a}	267.87 ± 7.35^{c}	12.11 ± 1.44^{d}	12.60 ± 0.66^{c}
W-EtOH	16.81 ± 0.45^{d}	133.93 ± 4.67^{cd}	6.43 ± 0.26^{e}	12.25 ± 0.87^{c}
S. commune strain W4000				
W4000 crude	263.57 ± 13.34^{c}	395.45 ± 8.08^{b}	18.46 ± 0.62^{b}	8.77 ± 0.80^{cd}
W4000-EA	ND	ND	37.87 ± 9.70^{a}	71.44 ± 2.94^{a}
W4000-H_2O	273.57 ± 24.92^{c}	415.45 ±	17.80 ±	5.77 ± 0.40^{d}

		13.39[a]	0.09[bc]	
W4000-WS1	409.38 ± 6.97[b]	490.30 ± 9.64[ab]	19.94 ± 1.14[b]	18.21 ± 2.68[bc]
W4000-WS2	628.38 ± 38.93[a]	296.06 ± 10.45[c]	11.70 ± 0.44[d]	12.70 ± 0.61[c]
W4000-EtOH	51.12 ± 2.50[d]	312.12 ± 8.15[bc]	18.17 ± 1.75[bc]	29.81 ± 2.38[b]

[a, b, c....] means with different superscripts on the same column differ significantly ($p < 0.05$).

*EA, ethyl acetate; H_2O, water-soluble fraction; WS1, water-soluble protein; WS2, water-soluble polysaccharides; EtOH, ethanol fractions.

Table 4.5: Antioxidant activities/capacities of crude hot water extracts of selected mushroom samples

Mushrooms	Antioxidant Activities			
	DPPH (mmol TE/g extract)	$ABTS^+$ (mmol TE/g extract)	FRAP (mmol Fe^{2+}/g extract)	CUPRAC (mmol TE/g extract)
S. commune				
W	1.788 ± 0.04[a]	0.158 ± 0.01[ab]	0.505 ± 0.01[a]	0.147 ± 0.02[a]
W2000	1.392 ± 0.02[cd]	0.126 ± 0.01[bc]	0.391 ± 0.08[bc]	0.122 ± 0.00[bc]
W4000	1.682 ± 0.02[ab]	0.141 ± 0.01[b]	0.419 ± 0.07[b]	0.125 ± 0.08[b]
R	1.177 ± 0.07[d]	0.119 ± 0.09[c]	0.274± 0.01[c]	0.083 ± 0.03[cd]
R2000	1.646 ± 0.06[ab]	0.131 ± 0.03[bc]	0.404 ± 0.02[b]	0.111 ± 0.08[bc]
R4000	1.543 ± 0.08[c]	0.126 ± 0.09[bc]	0.415 ± 0.02[b]	0.107 ± 0.06[c]
WR	1.454 ± 0.06[c]	0.142 ± 0.02[b]	0.410 ± 0.01[b]	0.111 ± 0.00[b]
RW	1.392 ± 0.03[cd]	0.117 ± 0.02[cd]	0.351 ± 0.03[bc]	0.107 ± 0.00[c]
P. pulmonarius	1.353 ± 0.08[cd]	0.081 ± 0.01[d]	0.278 ± 0.01[d]	0.064 ± 0.00[d]
A. bisporus	2.013 ± 0.01[a]	0.198 ± 0.01[a]	0.721 ± 0.07[a]	0.168 ± 0.01[a]

[a, b, c....] means with different superscripts on the same column differ significant ($p < 0.05$).

*Data are expressed as mean ± standard deviation (n = 3).

*TE, Trolox equivalent.

* W; natural strain from Malaysia; W2000 and W4000, two gamma-irradiated strains; WR and RW, two hybrid strains; R, natural strain isolated from Thailand; R2000 and R4000, two gamma-irradiated strains.

Table 4.5: Antioxidant activities/capacities of the fractions derived from the selected strains of *S. commune*

Fractions	Antioxidant Activities			
	DPPH (mmol TE/g extract)	$ABTS^{+}$ (mmol TE/g extract)	FRAP (mmol Fe^{2+}/g extract)	CUPRAC (mmol TE/g extract)
S. commune strain W				
W crude	0.123 ± 0.00^{b}	0.229 ± 0.01^{a}	0.201 ± 0.01^{c}	0.125 ± 0.00^{a}
W-EA	0.477 ± 0.02^{a}	0.159 ± 0.00^{b}	1.177 ± 0.08^{a}	ND
W-H_2O	0.094 ± 0.00^{c}	0.189 ± 0.06^{a}	0.227 ± 0.01^{c}	0.121 ± 0.00^{ab}
W-WS1	0.032 ± 0.01^{d}	0.080 ± 0.03^{c}	0.186 ± 0.01^{cd}	0.126 ± 0.00^{a}
W-WS2	0.043 ± 0.03^{d}	0.073 ± 0.00^{cd}	0.126 ± 0.00^{d}	0.096 ± 0.01^{c}
W-EtOH	0.423 ± 0.01^{a}	0.042 ± 0.02^{d}	0.389 ± 0.05^{bc}	0.041 ± 0.04^{d}
S. commune strain W4000				
W4000 crude	0.099 ± 0.00^{bc}	0.202 ± 0.00^{a}	0.140 ± 0.00^{cd}	0.103 ± 0.00^{ab}
W4000-EA	0.473 ± 0.01^{a}	0.165 ± 0.02^{b}	1.093 ± 0.09^{a}	ND
W4000-H_2O	0.080 ± 0.01^{c}	0.188 ± 0.08^{a}	0.156 ± 0.03^{cd}	0.108 ± 0.00^{ab}
W4000-WS1	0.039 ± 0.00^{d}	0.057 ± 0.01^{cd}	0.105 ± 0.00^{d}	0.112 ± 0.03^{bc}
W4000-WS2	0.025 ± 0.00^{d}	0.064 ± 0.00^{cd}	0.080 ± 0.00^{d}	0.079 ± 0.00^{c}
W4000-EtOH	0.457 ± 0.1^{a}	0.037 ± 0.00^{d}	0.251 ± 0.00^{b}	0.122 ± 0.01^{a}

$^{a, b, c....}$ means with different superscripts on the same column differ significant ($p < 0.05$).

*Data are expressed as mean ± standard deviation (n = 3).

*TE, Trolox equivalent. *ND = not determined

*CUPRAC-reducing capacity of ethyl acetate fractions were not determined due to the low yield of the fractions.

*EA, ethyl acetate; H_2O, water-soluble fraction; WS1, water-soluble protein; WS2, water-soluble polysaccharides; EtOH, ethanol fractions.

Table 5: Pearson's correlation coefficients between various chemical compositions parameters and antioxidant activities of the crude hot water extracts

Parameters	DPPH (mmol TE/g extract)	ABTS (mmol TE/g extract)	FRAP (mmol Fe^{2+}/g extract)	CUPRAC (mmol TE/g extract)
Total sugar content	0.374	-0.130	0.233	0.139
Total protein content	0.285	0.518**	0.305	0.333
Total phenolic content	0.782**	0.796**	0.827**	0.826**
Total flavonoid content	0.723**	0.672**	0.894**	0.728**

** Correlation is significant at the 0.01 level (2-tailed)

Table 6: Pearson's correlation coefficients between various chemical compositions parameters and antioxidant activities of the fractions

Parameters	DPPH (mmol TE/g extract)	ABTS (mmol TE/g extract)	FRAP (mmol Fe^{2+}/g extract)	CUPRAC (mmol TE/g extract)
Total sugar content	-0.778**	-0.005	-0.532**	-0.128
Total protein content	-0.549**	0.176	-0.361	0.753**
Total phenolic content	0.332	0.012	0.686**	0.823**
Total flavonoid content	0.626**	-0.181	0.900**	0.479*

** Correlation is significant at the 0.01 level (2-tailed)
*Correlation is significant at the 0.05 level (2-tailed)

The yields of *S. commune* hot water extracts and their selected fractions

The yields (w/w) of crude hot water extracts of *S. commune* ranged between 9.02 and 13.38%, Furthermore, the yields of *S. commune* crude extracts were slightly higher than that of *Agaricus bisporus* (8.57%) but comparable to the yield of *Pleurotus pulmonarius* (11.24%). It is noticeable that *S. commune* strains subjected to gamma irradiation showed higher yield than the natural or hybrid strains. Similarly, Rashid et al. (2015) have found that the yields of *Pleurotus sajor-caju* irradiated mycelium increased gradually as the dose of γ-irradiation increased from 23.10% to 39.82%. Likewise, the extraction yields of *Antrodia camphorate* mycelia increased with higher doses of gamma irradiation up to 15 kGy from 47.6% to 53.1% (Huang & Mau, 2007).

The extraction yields of the fractions derived from crude hot water extracts of *S. commune* strains W and W4000 varied from one strain to another. The highest yields were obtained from the water-soluble fractions W-WS2 (53.36%) and W4000-WS2 (71.28%). The yields from semi-polar solvents such as ethyl acetate were found at very low levels that ranged between 0.05 to 0.07% compared to polar solvents (ethanol and water). This suggests that the crude extracts consist of mainly water-soluble components with a small proportion of secondary metabolites that are likely to be retained in the ethyl acetate fractions. The findings here are comparable to a previous study conducted by Kim (2012) who reported that the yield of the ethyl acetate fraction was (1.05%) which was lower than the yield of water-soluble fraction (35.68%).

Chemical compositions and antioxidant activities of crude hot water extracts of *S. commune*

The chemical composition of mushroom species may be affected by several variables such as genetic structure, strains, maturation stage, environmental conditions as well as irradiation and hybridisation (Appels et al., 2018; Bellettini et al., 2019; Cardoso et al., 2019; Mleczek et al., 2020; Oropeza-Guerrero et al., 2018; Sakamoto, 2018;). The results obtained in the current work have shown that crude hot water extracts of different strains contain carbohydrates, protein, phenolic and flavonoid compounds. However, the total content of these components varied from one strain to another.

Different doses of gamma irradiation have influenced the total sugar contents of *S. commune* strains W and R. The total sugar content increased with increased doses of gamma irradiation up to 2 and 4 kGy. Gamma irradiation might cause genetic mutation leading to an overexpression of certain genes encoding polysaccharides synthesis (Mahmood et al., 2019). Similarly, the carbohydrate content of *Pleurotus osteratus* ethanol extracts increased gradually as the dose of gamma irradiation increased (San et al., 2019). The two hybrid strains with different dominant characteristics (WR and RW) also yielded higher sugar content than the two natural strains of *S. commune* (W and R). These results are consistent with those of other studies and suggest that the increase of total sugar contents of the hybrid strains is most likely related to the changes in the genetic structure leading to an overexpression of genes encoding information of polysaccharides synthesis (Liu et al., 2017; Raman et al., 2021).

Generally, gamma irradiation either decreased or increased the total phenolic content. For the natural strain W, different doses of gamma irradiation have slightly decreased the total phenolic content. Surprisingly, for the natural strain R, gamma irradiation doses 2 and 4 kGy significantly influenced the phenolic content in comparison with non-irradiated strain. The reason for such changes is still unclear but based on previous studies, the effect of gamma irradiation on the chemical compositions of mushroom may be influenced by factors such as genetic mutation that may cause certain changes in the synthesis of bioactive components, species type, geographical and environmental conditions, and dose of gamma irradiation (Tsai et al., 2014).

Furthermore, the total flavonoid contents of the *S. commune* crude hot water extracts were lower than their total phenolic contents. The findings of the current study are consistent with those of Emsen et al. (2017) who reported that the amount of total flavonoid content of *S. commune* water extract was significantly lower than total phenols. Another important finding was that gamma irradiated strains (R2000 and R4000) have higher flavonoid contents than the parental strain (R). Both hybridised strains (WR and RW) have higher flavonoid contents in comparison to the natural strain R. Our results are in agreement with a previous study conducted by Oropeza-Guerrero et al. (2018) who reported that the hybrid strains of *Pleurotus djamor* exhibited higher flavonoid content (6.89 mg QE/100 g extract) than that of the parental strains (2.22 mg QE/100 g extract).

The $ABTS^+$ and DPPH assays were utilized to measure the radical scavenging activities of crude hot water extracts of selected mushrooms. Our results showed that the natural strain W possessed the highest phenolic and flavonoid contents among the different *S. commune* strains and showed higher scavenging activities than *P. pulmonarius* water extract. Comparable results were reported by a study conducted on five different mushrooms including *S. commune* reported that hot water extracts of *S. commune* exhibited higher DPPH radical scavenging activity than *P. pulmonarius* extracts (Tepsongkroh et al., 2019). It has been reported that the IC_{50} of *S. commune* crude hot water extract (19.36 µg/µl) was higher than the methanol (24.82 µg/µl) and ethanol (18.56 µg/µl) extracts (Chandrawanshi et al., 2017). Furthermore, several studies showed that crude hot water extracts of *S. commune* had the ability to scavenge DPPH radicals (Emsen et al., 2017; Kumar et al., 2018). Nonetheless, the $ABTS^+$ scavenging activities of *S. commune* crude hot water extracts were lower than that of Tepsongkroh et al. (2019). This result may be explained by the fact that bioactive compounds of the water extracts may act differently towards different free radicals. Also, these assays use different chromogenic redox reagents with different standard potentials (Apak et al., 2007). Our data showed that gamma irradiated strains R2000 and R4000 exhibited better scavenging activity and reducing power in all assays than the natural strain R. Similarly, San et al. (2019) found that the antioxidant activity of *Pleurotus osteratus* extract increased with higher doses of gamma irradiation. The observed increase in the antioxidant activities of the extracts could be attributed to genetic mutation because gamma rays are high-energy electromagnetic waves that have the ability to penetrate cell walls of mushroom's mycelia causing a breakdown in DNA structure and changing the purine and pyrimidine bases (Wang et al., 2007; Zhao et al., 2015). Thus, gamma irradiation may induce mutations in terms of chromosome number, morphological structure, and growth behavior (Mohajer et al., 2014). According to Djajanegara (2008), mutation might cause changes in the gene expression encoding biosynthetic enzymes resulting in an increase in the production and accumulation of bioactive components with antioxidative activity. Moreover, Jyothi and Thara (2021) reported the DNA variation of the mutants from single base pair change to repeated sequences. The polymorphism analysis using random amplified polymorphic DNA (RAPD) markers revealed that *P. djamor*, *P. florida* and *P. ostreatus* had 16.7%, 25% and 22% polymorphism with their respective improved strains.

Hybridisation is a method used to exchange the genetic information between two compatible nuclei and generate a recombinant genome with a probable expression for a desirable trait (Kaur et a., 2008). Furthermore, the exchange of genes between two nuclei during hybridisation may produce hybrids with diverse genetic materials and this may also result in differential expression of genes between the hybrid strains compared to the parental strains. According to Raman et al. (2021), the genetic profile of ten hybrids was detected using inter-simple sequence repeats (ISSR) and RAPD markers, the results depicted those hybrids contain polymorphic bands indicating the rearrangement and deletion in genetic structure. Moreover, Liu et al. (2017) reported that certain hybrids have higher concentrations of polysaccharides and triterpenes than that of parental strains. Similarly, in the current study, higher antioxidant activity of the hybrid strains (WR and RW) than the natural strain R may be due to the differences in the chemical composition between the strains.

Based on the results of the correlation analysis in Table 5, it is pertinent to suggest that total phenolic contents and total flavonoid contents present in the *S. commune* crude hot water extracts were the main contributors to the observed antioxidant effect. This is in agreement with previous reports by Cheung et al. (2003) and Shah et al. (2018). Both reported that the water extracts showed a significant correlation between the scavenging activity and the total phenolic and flavonoid contents.

Chemical compositions and antioxidant activities of the fractions derived from the crude hot water extracts of *S. commune* strains W and W4000

Overall, according to the results obtained, the water-soluble fractions of *S. commune* are mainly enriched with carbohydrates and proteins. The WS2 of the gamma irradiated strain W4000 have higher total sugar content compared to the WS2 of the natural strain W. It is encouraging to compare this result with those of Wang et al. (2005) who reported that gamma-irradiated strains have higher polysaccharides than non-irradiated strain. These findings further support the idea that mutagenesis such as gamma rays might cause changes in the genetic structure, hence increases the synthesis of bioactive compounds. Moreover, the water-soluble protein fraction of *S. commune* strain W possesses the highest phenolic and flavonoid contents among all water-soluble fractions. Similarly, the total phenolic contents of the water-soluble protein fractions obtained from *P. pulmonarius* was higher than the total phenolic contents of water-soluble polysaccharides and the crude extracts (Abidin et al., 2016).

This study confirms that ethyl acetate fractions W-EA and W4000-EA contain the highest phenolic content among all fractions. The findings of the current study are consistent with those of Rajput et al. (2020) who worked on different fractions derived from *Ophiocordyceps sinensis* water extracts, and found that the ethyl acetate fractions have higher content of phenolic compounds than that of the crude extracts. Furthermore, the ethyl acetate fractions showed the highest level of flavonoid contents among all water-soluble fractions and crude extracts.

Based on the current results, the ethyl acetate fractions exhibited strong scavenging activities towards free radicals amongst water-soluble fractions and crude extracts, similar to the obtained findings by Kim (2011). In addition, the total reduction activities served as a significant indicator of the potential antioxidant activity. The ethyl acetate fractions showed the highest reducing activities amongst all tested fractions. Similar observations on the potency of the ethyl acetate fractions, representing the compounds with intermediate polarity, have been reported earlier by several researchers (Mishra et al., 2018; Rajput et al., 2020).

The fact that ethyl acetate fractions derived from hot water extracts exhibited the highest contents of organic compounds and antioxidant activities, is related to several hypotheses. For instance, Jeong et al. (2004) suggested that soluble polyphenols can be liberated by heat treatment. Moreover, Liu et al. (2014) reported that during hot water extraction, the viscosity and surface tension of water decrease, while diffusivity and solubility of the polyphenols increase. According to Minatel et al. (2017), polyphenols can be found either combined with

mono- and polysaccharides in the cell wall or linked to one or more phenolic group. Thus, thermal processing method may liberate polyphenols compounds from insoluble portion of mushroom, that increases the accumulation of bioaccessible antioxidant compounds (Choi et al., 2006). These bioactive compounds are highly soluble in semi-polar solvents such as ethyl acetate. Therefore, ethyl acetate fractions displayed the highest antioxidant activities in DPPH and FRAP assays among water-soluble fractions and the crude extracts. To identify the exact type of compounds, further work is required which involve complete purification of the components and further analysis using chromatographic and spectroscopic techniques.

In general, according to our results, the functional groups of organic compounds from the analysed water extracts of *S. commune* are mainly phenolic compounds. Therefore, organic fractions were more active as antioxidants than water-soluble fractions and the crude extracts. In accordance with the present results, Tepsongkroh et al. (2019) and Choi et al. (2006) both suggested that hot water extraction might cause disruption of the cell walls resulting in easier release of bound polyphenolic compounds which, in turn, increases the antioxidant activities of the extracts. Data were published indicating that the presence of phenolic compounds in the water extracts/fractions was highly involved in the antioxidant activities of the extracts (Cheung et al., 2003; Ramírez-Anguiano et al., 2007). These results are supported by Pearson's correlation analysis as shown in Table 6, where the total phenolic and flavonoid contents of the fractions showed significant correlations with their ion reducing activities/capacities. The presence of low molecular weight compounds, possibly phenolic compounds, in the fractions/crude extracts are the main contributors to the antioxidant activity of *S. commune* crude hot water extracts.

Conclusion

In the present study, the antioxidant activities of the various strains of *S. commune* were investigated and compared with two commonly eaten mushrooms, *P. pulmonarius* and *A. bisporus*. Chemical composition of the extracts and fractions of selected strains were determined and correlated to their radical scavenging and reducing capacities. Our results indicated that, in general, the antioxidant activities of the crude hot water extracts of *S. commune* strains were higher than those of *P. pulmonarius*. The crude hot water extracts of *S. commune* strains W and the gamma-irradiated strain W4000 displayed the highest antioxidant activities. Further fractionation of strains W and W4000 yielded several water-soluble and organic fractions. It was observed that the organic fractions, in particular the ethyl acetate fractions exhibited the highest scavenging activities towards DPPH radicals and ion reducing activities in FRAP assay among all tested fractions. Correlation analysis showed that phenolic and flavonoid compounds present in both crude hot water extracts and their fractions are the main contributors to the antioxidant activities of the extracts and fractions of *S. commune* strains, suggesting that these low-molecular-weight compounds played a bigger role in the overall antioxidant activities of the hot water extracts of *S. commune*.

There are several recommendations for further study. First of all, strains improvement in this study via irradiation and hybridisation needs to be further investigated. An increase in the antioxidant activities of certain gamma-irradiated strains was observed in this study. Thus, further research regarding the effect of gamma-irradiation on the levels of bioactive components in mushrooms would be worthwhile. Also, identification and characterization of the polar low-molecular weight compounds would help us to understand better the antioxidant activities of the hot water extracts of *S. commune*.

References

Abidin, M. H., Abdullah, N., & Abidin, N. Z. (2016). Protective effect of antioxidant extracts from grey oyster mushroom: *Pleurotus pulmonarius* (Agaricomycetes), against human low density lipoprotein oxidation and aortic endothelial cell damage. *International Journal of Medicinal Mushrooms*, *18*(2), 109-121.

Al Joudi, F. S. (2013). Adverse effects of excessive antioxidant supplements and their underlying mechanisms. *The Journal of Aging Research and Clinical Practice*, *2*(4), 339-345.

Apak, R., Guclu, K., Demirata, B., Ozyurek, M., Celik, S. E., Bektasoglu, B., ... & Ozyurt, D. (2007). Comparative evaluation of various total antioxidant capacity assays applied to phenolic compounds with the CUPRAC assay. *Molecules*, *12*(7), 1496-1547.

Apak, R., Guçlu, K., Ozyurek, M., Karademi, E. N., & Altun, M. (2005). Total antioxidant capacity assay of human serum using copper (II)-neocuproine as chromogenic oxidant: The CUPRAC method. *Free Radical Research*, *39*(9), 949-961.

Appels, F. V., Dijksterhuis, J., Lukasiewicz, C. E., Jansen, K. M., Wösten, H. A., & Krijgsheld, P. (2018). Hydrophobin gene deletion and environmental growth conditions impact mechanical properties of mycelium by affecting the density of the material. *Scientific Reports*, *8*(1), 1-7.

Bellettini, M. B., Fiorda, F. A., Maieves, H. A., Teixeira, G. L., Ávila, S., Hornung, P. S., ... & Ribani, R. H. (2019). Factors affecting mushroom *Pleurotus* spp. *Saudi Journal of Biological Sciences*, *26*(4), 633-646.

Benzie, I. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Analytical Biochemistry, 239*(1), 70–76.

Bozdoğan, A., Ulukanlı, Z., Bozok, F., Eker, T., Dogan, H. H., & Buyukalaca, S. (2018). Antioxidant potential of *Lactarius deliciosus* and *Pleurotus ostreatus* from Amanos Mountains. *International Quarterly Journal of Life Sciences, 5*(3), 113-120.

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT- Journal of Food Science and Technology, 28*(1), 25–30.

Cardoso, R. V., Fernandes, Â., Barreira, J. C., Verde, S. C., Antonio, A. L., Gonzaléz-Paramás, A. M., ... & Ferreira, I. C. (2019). Effectiveness of gamma and electron beam irradiation as preserving technologies of fresh *Agaricus bisporus* Portobello: A comparative study. *Food Chemistry*, *278*, 760-766.

Chandrawanshi, N. K., Tandia, D. K., & Jadhav, S. K. (2017). Nutraceutical properties evaluation of *Schizophyllum commune*. *Indian Journal of Scientific Research, 13*(2), 57-62.

Cheung, L. M., Cheung, P. C., & Ooi, V. E. (2003). Antioxidant activity and total phenolics of edible mushroom extracts. *Food Chemistry*, *81*(2), 249-255.

Choi, Y., Lee, S. M., Chun, J., Lee, H. B., & Lee, J. (2006). Influence of heat treatment on the antioxidant activities and polyphenolic compounds of Shiitake (*Lentinus edodes*) mushroom. *Food Chemistry*, *99*(2), 381-387.

Chowdhary, A., Randhawa, H. S., Gaur, S. N., Agarwal, K., Kathuria, S., Roy, P., ... & Meis, J. F. (2013). *Schizophyllum commune* as an emerging fungal pathogen: A review and report of two cases. *Mycoses*, *56*(1), 1-10.

De Silva, D. D., Rapior, S., Fons, F., Bahkali, A. H., & Hyde, K. D. (2012). Medicinal mushrooms in supportive cancer therapies: An approach to anti-cancer effects and putative mechanisms of action. *Fungal Diversity*, *55*(1), 1-35.

Devi, L. S., Dasgupta, A., Chakraborty, M., Borthakur, S. K., & Singh, N. I. (2014). Chemical composition and antioxidant activity of *Schizophyllum commune*. *International Journal of Pharmaceutical Sciences Review and Research*, *27*(2), 173-177.

Di Meo, S., Reed, T. T., Venditti, P., & Victor, V. M. (2016). Role of ROS and RNS sources in physiological and pathological conditions. *Oxidative Medicine and Cellular Longevity, 2016, 1-44*.

Djajanegara, I. (2008). White oyster mushroom (*Pleurotus florida*) mutant with altered antioxidant contents. *Biotrofla: The Southeast Asian Journal of Tropical Biology*, *15*(1), 65-73.

Emsen, B., Kocabas, A., Kaya, A., Cinar, S., Aasim, M., & Sadi, G. (2017). *In vitro* cytotoxicity, antibacterial and antioxidant properties of various extracts from *Schizophyllum Commune* Fr. *Fresenius Environmental Bulletin, 26*, 1144-1153.

Feeney, M. J., Dwyer, J., Hasler-Lewis, C. M., Milner, J. A., Noakes, M., Rowe, S., ... & Castlebury, L. A. (2014). Mushrooms and health summit proceedings. *The Journal of Nutrition*, *144*(7), 1128-1136.

Gąsecka, M., Siwulski, M., & Mleczek, M. (2018). Evaluation of bioactive compounds content and antioxidant properties of soil-growing and wood-growing edible mushrooms. *Journal of Food Processing and Preservation*, *42*(1), 1-10.

Halliwell, B. (2006). Reactive species and antioxidants: Redox biology is a fundamental theme of aerobic life. *Plant Physiology*, *141*(2), 312-322.

Huang, S. J., & Mau, J. L. (2007). Antioxidant properties of methanolic extracts from *Antrodia camphorata* with various doses of γ-irradiation. *Food Chemistry*, *105*(4), 1702-1710.

Jeong, S. M., Kim, S. Y., Kim, D. R., Jo, S. C., Nam, K. C., Ahn, D. U., & Lee, S. C. (2004). Effect of heat treatment on the antioxidant activity of extracts from citrus peels. *Journal of Agricultural and Food Chemistry*, *52*(11), 3389-3393.

Jyothi, K. R., & Thara, S. S. (2021). Development of improved strain in species of *Pleurotus* by gamma irradiation. *Journal of Food Science and Technology*, 1-8.

Kaur, J., Sodhi, H. S., & Kapoor, S. (2008). Breeding of *Pleurotus florida* (oyster mushroom) for phenotypic pigmentation and high yield potential. *Journal of the Science of Food and Agriculture*, *88*(15), 2676-2681.

Kim, J. H. (2011). Physiological activities of hot water extract and solvent fractions of *Pleurotus ferulea*. *The Korean Journal of Mycology*, *39*(3), 189-193.

Kim, J. H. (2012). Biological activities of water extract and solvent fractions of an edible mushroom, *Hericium erinaceus*. *The Korean Journal of Mycology*, *40*(3), 159-163.

Kumar, A., Ali, S., Lal, S. B., & Sinha, M. P. (2018). Mycochemical screening and determination of nutritive potency and antioxidant activity of edible macrofungi *Dacryopinax spathularia* and *Schizophyllum commune*. *World Journal of Pharmaceutical Research*, *7*(16), 1311-1321.

Lee, D. H., Yang, M., Giovannucci, E. L., Sun, Q., & Chavarro, J. E. (2019). Mushroom consumption, biomarkers, and risk of cardiovascular disease and type 2 diabetes: A prospective cohort study of US women and men. *The American Journal of Clinical Nutrition, 110*(3), 666-674.

Liu, H., Qiu, N., Ding, H., & Yao, R. (2008). Polyphenols contents and antioxidant capacity of 68 Chinese herbals suitable for medical or food uses. *Food Research International, 41*(4), 363–370.

Liu, J., Sandahl, M., Sjöberg, P. J., & Turner, C. (2014). Pressurised hot water extraction in continuous flow mode for thermolabile compounds: Extraction of polyphenols in red onions. *Analytical and Bioanalytical Chemistry*, *406*(2), 441-445.

Liu, S. R., Ke, B. R., Zhang, W. R., Liu, X. R., & Wu, X. P. (2017). Breeding of new *Ganoderma lucidum* strains simultaneously rich in polysaccharides and triterpenes by mating basidiospore-derived monokaryons of two commercial cultivars. *Scientia Horticulturae*, *216*, 58-65.

Mahmood, I., Mohamad, A., Daud, F., Othman, B. A., Law, D., Hoong, C. Y., ... & Fazry, S. (2019). Potential mutant of *Lentinula edodes* with high yield of (1-3), (1-6-), β-D-glucan. *Malaysian Journal of Fundamental and Applied Sciences*, *15*(2-1), 336-340.

Masuko T, Minami A, Iwasaki N, Majima T, & Mishimura. (2005). Carbohydrate analysis by a phenol-sulphuric acid method in microplate format. *Analytical Biochemistry Journal, 339*, 69–72.

Miles, P. G., & Chang, S. T. (2004). Mushrooms: Cultivation, nutritional value, medicinal effect, and environmental impact. (pp. 27- 47). *Boca Raton, Florida: CRC Press.*

Minatel, I. O., Borges, C. V., Ferreira, M. I., Gomez, H. A., Chen, C. Y., & Lima, G. P. P. (2017). Phenolic compounds: Functional properties, impact of processing and bioavailability. *Phenolic Compounds Biological Activity.* (pp. 1-24). *London, UK: IntechOpen.*

Mirfat, A. H., Noorlidah, A., & Vikineswary, S. (2010). Scavenging activity of *Schizophyllum commune* extracts and its correlation to total phenolic content. *Journal of Tropical Agriculture and Food Science*, *38*(2), 231-238.

Mishra, J., Joshi, A., Rajput, R., Singh, K., Bansal, A., & Misra, K. (2018). Phenolic rich fractions from mycelium and fruiting body of *Ganoderma lucidum* inhibit bacterial pathogens mediated by generation of reactive oxygen species and protein leakage and modulate hypoxic stress in HEK 293 cell line. *Advances in Pharmacological Sciences*, *2018*, 1-10.

Mittal, M., Siddiqui, M. R., Tran, K., Reddy, S. P., & Malik, A. B. (2014). Reactive oxygen species in inflammation and tissue injury. *Antioxidants and Redox Signaling*, *20*(7), 1126-1167.

Mleczek, M., Gąsecka, M., Budka, A., Niedzielski, P., Siwulski, M., Kalač, P., ... & Rzymski, P. (2020). Changes in mineral composition of six strains of *Pleurotus* after substrate modifications with different share of nitrogen forms. *European Food Research and Technology*, 1-13.

Mohajer, S., Mat Taha, R., Lay, M. M., Khorasani Esmaeili, A., & Khalili, M. (2014). Stimulatory effects of gamma irradiation on phytochemical properties,

mitotic behaviour, and nutritional composition of Sainfoin (*Onobrychis viciifolia* Scop.). *The Scientific World Journal*, *2014,* 1-9.

Oropeza-Guerrero, M. P., Santos-Sánchez, N. F., Salas-Coronado, R., Valadez-Blanco, R., Hernández-Carlos, B., & Guadarrama-Mendoza, P. C. (2018). Productivity and Antioxidant Activity of Wild, Reconstituted, and Hybrid Strains of the Pink Oyster Mushroom, *Pleurotus djamor* (Agaricomycetes), from Mexico. *International Journal of Medicinal Mushrooms*, *20*(7), 607-621.

Phaniendra, A., Jestadi, D. B., & Periyasamy, L. (2015). Free radicals: properties, sources, targets, and their implication in various diseases. *Indian Journal of Clinical Biochemistry*, *30*(1), 11-26.

Rahal, A., Kumar, A., Singh, V., Yadav, B., Tiwari, R., Chakraborty, S., & Dhama, K. (2014). Oxidative stress, prooxidants, and antioxidants: The interplay. *BioMed Research International, 2014,* 1-19.

Rajput, R., Mishra, J., Sharma, P., Bhardwaj, A., Sharma, R. K., Singh, K., ... & Misra, K. (2020). Bioactive fractions from the Chinese caterpillar mushroom, *Ophiocordyceps sinensis* (Ascomycetes): Elucidate adaptogenic role against hypoxia stress. *International Journal of Medicinal Mushrooms*, *22*(11), 1121-1133.

Raman, J., Jang, K. Y., Oh, Y. L., Oh, M., Im, J. H., Lakshmanan, H., & Kong, W. S. (2021). Interspecific hybridization between *Ganoderma lingzhi* and *Ganoderma applanatum* through protoplast fusion. *World Journal of Microbiology and Biotechnology, 37*(7), 1-17.

Ramírez-Anguiano, A. C., Santoyo, S., Reglero, G., & Soler-Rivas, C. (2007). Radical scavenging activities, endogenous oxidative enzymes and total phenols in edible mushrooms commonly consumed in Europe. *Journal of the Science of Food and Agriculture*, *87*(12), 2272-2278.

Rashid, R. A., Daud, F., Awang, M. R., Mutaat, H. H., Senafi, S., Mohamad, A., ... & Rahim, K. A. (2015). Evaluation of mycelia growth, morphology and yield for low dose gamma irradiated grey oyster mushroom *Pleurotus sajor-caju*. *International Journal of Innovation and Scientific Research, 24*, 332-336.

Rathee, S., Rathee, D., Rathee, D., Kumar, V., & Rathee, P. (2012). Mushrooms as therapeutic agents. *Revista Brasileira de Farmacognosia*, *22*(2), 459-474.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine, 26*(9–10), 1231–1237.

San, T., Thant, K. C., & Than, H. H. (2019). Impact of gamma radiation on the productivity and quality of first generation mutant oyster mushroom: *Pleurotus osteratus. Myanmar Korea Conference Research Journal,* 259-266.

Shah, S. R., Ukaegbu, C. I., Hamid, H. A., & Alara, O. R. (2018). Evaluation of antioxidant and antibacterial activities of the stems of *Flammulina velutipes* and *Hypsizygus tessellatus* (white and brown var.) extracted with different solvents. *Journal of Food Measurement and Characterization*, *12*(3), 1947-1961.

Sim, C. Y. (2014). Molecular identification of *Schizophyllum commune* and its production of schizophyllan. *Doctoral Dissertation*, 5-10.

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture, 16*(3), 144–158.

Tepsongkroh, B., Jangchud, K., & Trakoontivakorn, G. (2019). Antioxidant properties and selected phenolic acids of five different tray dried and freeze

dried mushrooms using methanol and hot water extraction. *Journal of Food Measurement and Characterization*, *13*(4), 3097-3105.

Tsai, S. Y., Mau, J. L., & Huang, S. J. (2014). Enhancement of antioxidant properties and increase of content of vitamin D2 and non-volatile components in fresh button mushroom: *Agaricus bisporus* (higher Basidiomycetes) by γ-irradiation. *International Journal of Medicinal Mushrooms*, *16*(2), 137-147.

Wang, B. N., Yang, Z. Q., & Yang, X. H. (2007). Breeding of a new strain of *Flammulina velutipes* by γ-rays induced mutation. *Henan Science*, 25 (*3),* 413-415.

Wang, N., Ren, D. M., Gong, T., Guan, Y. L., & Yang, Y. (2005). Screening of high polysaccharide yield strain of *Hericium erinaceus* by (60) Co-gamma irradiation. *Edible Fungi of China*, 1-6.

Wang, X. M., Zhang, J., Wu, L. H., Zhao, Y. L., Li, T., Li, J. Q., & Liu, H. G. (2014). A mini review of chemical composition and nutritional value of edible wild grown mushroom from China. *Food Chemistry, 151*, 279-285.

Wei, W., Liu, Q., Tan, Y., Liu, L., Li, X., & Cai, L. (2009). Oxidative stress, diabetes, and diabetic complications. *Hemoglobin*, *33*(5), 370-377.

Zhao, L., Liu, L., Wang, J., Guo, H., Gu, J., Zhao, S., ... & Xie, Y. (2015). Development of a new wheat germplasm with high anther culture ability by using a combination of gamma-ray irradiation and anther culture. *Journal of the Science of Food and Agriculture*, *95*(1), 120-125.