

β -glucan and antioxidant activities of four edible mushroom extracts from Thailand

Chanida Kupradit^{1*}, Araya Ranok¹, Seksan Mangkalan¹, Chompoonuch Khongla¹, Sumalee Musika¹

¹Department of Applied Biology, Faculty of Sciences and Liberal Arts, Rajamangala University of Technology Isan, Nakhon Ratchasima, 30000, Thailand

Received:
July 19, 2021

Accepted:
July 16, 2022

Published Online:
September 19, 2022

Abstract

The commercial mushroom extracts from Thailand, *Lentinus squarrosulus*, *Pleurotus sajor-caju*, *Pleurotus ostreatus*, and *Volvariella volvacea*, could be sources of β -glucan and antioxidant. The objective of this research was to evaluate the β -glucan content and antioxidant activities of the mushroom extracts prepared from different extraction conditions. Various solvents, including water, ethanol, acid, and alkali were used for the mushroom extraction. The mushroom extracts were evaluated for their β -glucan content, total phenolic compounds, and antioxidant properties. Among all extraction conditions, the alkaline extracted (BE) *P. ostreatus* and *P. sajor-caju* showed high level of β -glucan content with $25.82 \pm 3.87\%$ w/w and $23.08 \pm 0.56\%$ w/w, respectively. Large amounts of total phenolic compounds were obtained from *V. volvacea* and *L. squarrosulus* extracted with water at 60°C (W60) as 38.07 ± 2.53 and 34.78 ± 5.69 mg gallic acid equivalent/g extract, respectively. Excellent scavenging of ABTS radicals and FRAP assay were also observed in W60 of *V. volvacea* as 67.12 ± 4.41 and 36.46 ± 3.44 mg trolox equivalent/g extract, respectively. The metal chelating effect of *P. ostreatus* was the highest at 66.13 ± 1.63 mg EDTA equivalent/g extract but was not significantly different from *V. volvacea* (63.76 ± 0.51 mg EDTA equivalent/g extract) ($p > 0.05$). In conclusion, alkaline extraction was the most suitable for β -glucan and metal chelating activity extracts whereas water extraction was suitable for antioxidant extracts. These simple extraction conditions could be applied in producing high bioactive compounds and antioxidant active ingredients from local mushrooms for further addition in food and health products.

Keywords: Antioxidant activity, β -glucan, Phenolic compound, Extraction, Mushroom

How to cite this:

Kupradit C, Ranok A, Mangkalan S, Khongla C, and Musika S. β -glucan and antioxidant activities of four edible mushroom extracts from Thailand. Asian J. Agric. Biol. 2023(1): 202107285. DOI: <https://doi.org/10.35495/ajab.2021.07.285>

*Corresponding author email:
lego7823@hotmail.com

This is an Open Access article distributed under the terms of the Creative Commons Attribution 3.0 License. (<https://creativecommons.org/licenses/by/3.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.



Introduction

Mushrooms are consumed worldwide because they are good sources of vitamins, minerals, proteins, carbohydrates and bioactive compounds with low level of lipids and caloric contents (Kosanić et al., 2011; Ruthes et al., 2015). Different functional compounds, including terpenes, sterols, ceramides and phenolic compounds have been found in mushrooms. The major compound groups in mushrooms are polysaccharides which are responsible for health properties including enhancing the immune system. Bioactive polysaccharides in mushroom are β - and α -glucan, hemicelluloses, chitin, xylans, galactans, and mannans (Synytsya et al., 2009; Villares et al., 2012). β -glucans are polysaccharides of D-glucose monomers linked by β -glycosidic bonds. Most of β -glucans are derived from the fruiting bodies of mushrooms (Zhu et al., 2015). This compound has bioactive carbohydrates which usually function as biological response modifiers and have been used to decrease cholesterol absorption (Villares et al., 2012; Bak et al., 2014). β -glucan content in mushroom ranges from 0.21 to 0.53 g/100 g (dry weight basis) and the composition depends on cultivation conditions and origin of cultivated mushroom (Khan et al., 2018). The importance of biological activities of crude natural extract is anti-oxidation. Oxidation is the biological process that produce energy in living organisms using biological processes as fuel. However, an unregulated production of oxygen-derived free radicals is involved in many diseases (Mau et al., 2002). Antioxidants can be effective in reducing oxidative damage by oxygen-derived free radicals. A past study showed that the intensity of antioxidant activity in mushroom depended on the species and the extraction solvents (Kosanić et al., 2011; Khan et al., 2018; Uddin et al., 2019; Barbosa et al., 2020a).

Commercial mushrooms including *Lentinus*, *Pleurotus* and *Volvariella* genus are widely cultivated in many parts of Thailand. In Thailand, *Lentinus squarrosulus* (Hed Khon Kao), *Pleurotus sajor-caju* (grey oyster mushroom), *Pleurotus ostreatus* (oyster mushroom) and *Volvariella volvacea* (straw mushroom) are widely cultivated because they require higher temperatures for rapid growth (Kupradit et al., 2017). Moreover, large amounts of these mushroom fruiting bodies are available year-round in Thailand. Therefore, the potential of these commercial mushrooms for use as bioactive compound sources

should be evaluated. However, bioactive compounds in mushroom vary according to cultivation regions, mushroom species, and extraction conditions. More recently, the extraction methods, structure, and bioactivities of polysaccharides from several mushroom species have been reviewed and reported in many publications (Hwang et al., 2018; Uddin et al., 2019; Barbosa et al., 2020a; Gong et al., 2020). Various extraction techniques, such as ultrasonic or microwave method, alkali or hot water extraction, have been applied for mushroom bioactive polysaccharide extraction including β -glucan (Xu et al., 2019). However, simple extraction conditions with safety and cost-effectiveness should be considered to obtain extracts with high bioactive compounds and antioxidant activities. For further application, the commercial mushrooms will be value-added and applied as β -glucan and antioxidant sources for food and health products. Thus, the effects of extraction process on β -glucan and antioxidant activities of these 4 different commercial mushroom species were evaluated.

The objectives of the present study were to evaluate the antioxidative properties including ABTS radical cation scavenging activity (ABTS), Ferric reducing antioxidant power (FRAP) and metal chelating activity of the extracted commercially cultivated fruiting bodies of mushrooms using different extraction conditions. The contents of bioactive compounds including β -glucan and the potential antioxidant components such as total phenolic compounds of these mushroom extracts were also determined.

Material and Methods

Sample preparations

The fruiting bodies of *L. squarrosulus*, *P. sajor-caju*, *P. ostreatus* and *V. volvacea* were collected from local mushroom farms in Nakhon Ratchasima, Thailand. The fruiting bodies of all mushrooms were cleaned and dried at 60°C for 72 hr. The dried fruiting bodies were then ground by blender and stored at 4°C for further extraction and analysis.

Extractions

Five different conditions were used to extract soluble compounds from the ground fruiting bodies of the mushrooms. The mushroom powder were weighed and transferred to Erlenmeyer flasks. The solvents, including water, ethanol, acid, and alkali were added to the mushroom powder and extracted using different



conditions as follows:

Water and ethanol extractions

For water and ethanol extractions, water or 70% ethanol solution were added into the mushroom powder at the ratio of 1:20 (w/v). For the first batch of water extraction at 100 and 60°C, 50 g of each mushroom powder were separately added to 475 mL of water. Then, the soluble fractions were extracted at 100°C for 20 min using autoclave (W100) and 60°C for 90 min using water bath (W60). A soluble fraction of the first batch were collected by filtration using Whatman No.1 filter paper then the mushroom residue were extracted again at 100°C for 20 min (W100) and 60°C for 90 min (W60) with 475 mL of water as the second batch. A soluble fraction of the second batch were collected by filtration through Whatman No.1 filter paper and separately pooled with the first batch of W100 and W60.

For ethanolic extraction method, ethanol (70% v/v) (Et) were used as the extraction solvent. Fifty gram of each mushroom powder were added to 475 mL of 70% ethanol solution. The first extracted batch were incubated at 60°C for 90 min using water bath. A soluble fraction of the first batch were collected by filtration through Whatman No.1 filter paper and the mushroom residue were extracted again at 60°C for 90 min with 475 mL 70% ethanol solution. A soluble fraction of the second batch were collected by filtration through Whatman No.1 filter paper and pooled with the first batch of Et60.

All filtrate of the total soluble fraction obtained from W100, W60 and Et60 were separately concentrated at 60°C using a rotary evaporator (Büchi Rotavapor R-144, Switzerland) to remove the solvents. The concentrated extracts were dried at 60°C to remove the residue solvent until a constant weight of crude extract was achieved. The dry weight of the mushroom crude extracts of W100, W60 and Et60 were obtained using a digital balance.

Acid extraction

For acid extraction (AE), 3.8% HCl were used as the extraction solution with some modifications from the procedure of Szwengiel and Stachowiak (2016). Briefly, 180 mL absolute methanol were added into the dry powdered fruiting bodies (20 g) of each mushroom and incubated at 80°C in water bath for 8 hr to remove undesired soluble materials such as vitamins, polyphenols, monosaccharides, disaccharides, and others. After methanol incubation,

the residue of mushroom was separated by centrifugation for 10 min at 3,000 rpm. The soluble fraction of methanolic extract were discarded and the mushroom residue was further extracted using 3.8% HCl. The acid solutions were added into the mushroom residue at the ratio of 1:10 (w/v) and incubated for 300 min at 30°C in water bath. Then, the extracts were neutralized with 4M NaOH and filtrated through Whatman No.1 filter paper. The crude extracts were precipitated using 95% ethanol at the ratio of 1:5 (v/v) and incubated at 4°C for 18 hr. The precipitate was harvested by centrifugation at 5,000 rpm for 15 min and dried at 60°C, to remove the residue ethanol, until a constant weight was achieved. The dry weight of the mushroom crude extracts of AE were obtained using a digital balance.

Alkaline extraction

For alkaline extraction (BE), the extraction method was performed following Maity et al. (2013) with some modifications. The mushroom powder (20 g) were added to 180 mL of 4% NaOH. The extractions were performed at 80°C in water bath for 60 min and the extracts kept overnight at 4°C. Then, the extracts were neutralized using 35% HCl and the soluble fractions were filtrated through Whatman No.1 filter paper. The crude extracts from alkaline extraction were precipitated using 95% ethanol at a ratio of 1:5 (v/v) at 4°C for 18 hr. The precipitate was harvested using centrifugation for 15 min at 5,000 rpm and dried at 60°C, to remove the residue ethanol, until a constant weight was achieved. The dry weight of the mushroom crude extracts of BE were obtained using a digital balance.

All extraction conditions are summarized in Table 1. The mushroom crude extracts were kept at 4°C for further analysis. Each extract was analyzed in four replicates to measure the β -glucan content, total phenolic compound content and antioxidant activity. The data results were reported as mean values \pm standard deviation.

Table-1. Mushroom extraction conditions.

Extraction solvent	Temperature (°C)	Total extraction time (min)	Crude extract code
Water	100°C	40	W100
Water	60°C	180	W60
70% Ethanol	60°C	180	Et60
3.8% HCl	30°C	300	AE
4% NaOH	80°C	60	BE



Determination of β -glucan content

The concentration of β -glucan in all crude extracts of each mushroom were measured using the Mushroom and Yeast β -glucan Assay kit (Megazyme International, Wicklow, Ireland). The total glucan content in crude extracts were measured using acid hydrolysis with ice-cold 12 M sulfuric acid for 1 hr. Then, samples were hydrolyzed with 2 M sulfuric acid for 2 hr at 100°C. After neutralization, a mixture of enzymes exo-1,3- β -glucanase and β -glucosidase were added in the reactions and incubated for 1 hr at 40°C for glucose hydrolysis. The content of total glucan was measured after GOPOD reagents were added and incubated for 20 min at 40°C. Finally, the absorbance of all reactions was determined by spectrophotometer at 510 nm. The contents of α -glucan were measured by alkaline hydrolysis with 2 M KOH and incubated on ice for 20 min. Then, a mixture of amyloglucosidase and invertase were added in the reactions. The glucose released in enzymatic hydrolysis reaction was determined by GOPOD reagent and incubated at the same condition as described above. The β -glucan concentration was calculated by subtracting the α -glucan from the total glucan content. The β -glucan concentration in mushroom extracts were reported in unit of g β -glucan /100 g extract (or % w/w).

Determination of total phenolic content

The level of total phenolic compounds in the various mushroom extracts were measured according to the method of Matthauss (2002) with some modifications. In brief, a 100 μ L of the crude extract samples were added to 2 mL of Na₂CO₃ solution (2% w/v) and incubated for 2 min at room temperature. Then, 100 μ L of Folin-Ciocalteu reagent (Merck, Darmstadt, Germany) was added and incubated for 30 min at room temperature under the dark condition. After a 30 min incubation, the absorbance was determined at 750 nm using a spectrophotometer. The concentration of total phenolic compounds was reported in unit of mg gallic acid equivalents per g extract (mg gallic acid eq./ g extract).

Antioxidant activity assay

ABTS radical cation scavenging activity

The antioxidant activity was carried out using ABTS assay, with minor alteration, as described by Wiriyaphan et al. (2012). At first, the production of ABTS radicals (ABTS^{•+}) were performed by mixing 7.4 mM ABTS solution and 2.6 mM potassium persulfate solution (in 10 mM phosphate buffer, pH

7.4). The ABTS radical (ABTS^{•+}) were incubated under a dark condition at room temperature for 16 hr. Then, the ABTS^{•+} stock solution was diluted with 10 mM phosphate buffer (pH 7.4) to adjust the absorbance value to 0.700 \pm 0.02 at 734 nm. For sample analysis, antioxidant activity of the mushroom crude extracts was performed by mixing 20 μ L of crude extracts with 1.980 mL of ABTS working solution and incubating at room temperature for 5 min in dark condition. The decrease in absorbance was determined at 734 nm. Results were reported as mg trolox equivalent per g extract (mg trolox eq./g extract).

Ferric reducing antioxidant power (FRAP)

FRAP assay was measured as described by Benzie and Strain (1996), with some modifications. The FRAP reagent was freshly prepared from mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ solution in 40 mM HCl, and 20 mM FeCl₃•6H₂O solution at ratio of 10 : 1 : 1 (v/v). For mushroom crude extract analysis, 1 mL of FRAP reagent was added to 100 μ L of crude extract sample. The reactions were incubated at 37°C for 15 min. Then, all samples were measured for the absorbance at 593 nm. Results were reported as mg trolox equivalents per g extract (mg trolox eq./ g extract).

Metal chelating activity

Metal chelating activity assay were performed, with slight modifications, as described by Decker and Welch (1990). Fifty μ L of 2 mM FeCl₂ and 100 μ L of 5 mM ferrozine were added into 100 μ L of crude extract samples. The reactions were incubated at room temperature for 20 min. Then, the absorbance were determined at 562 nm. The metal chelating activity of the analyzed samples were reported in unit of mg EDTA equivalents per g extract. (mg EDTA eq./g extract).

Statistical analysis

All experiments were performed in four replicates. The data were reported as mean \pm standard deviations and shown as error bars. Statistical analysis was performed by using PASW Statistics 18 Release 18.0.0 software. Data were determined to be significantly different ($p < 0.05$) by one-way analysis of variance (one-way ANOVA).

Results and Discussion

Extraction yield

The yield of crude extracts from each mushroom using different extraction conditions is shown in Table 2.



The yield of the extracts varied from 45.39 ± 0.50 to 446.35 ± 11.62 mg/g dry weight according to mushroom species and extraction conditions. The crude extract yield from water extraction (W) of all 4 edible mushrooms showed significantly higher values than those from ethanol (Et), acid (AE), and alkaline (BE) extraction. The results indicated that major component (20% – 45%) in the 4 mushrooms could be solubilized in water (Table 2). This might indicate that the large amount of soluble component in mushroom has high polarity as similar to previous reports (Cheung et al., 2003; Cheung and Cheung, 2005; Sudha et al., 2012). Most functional polysaccharides from mushrooms are polar molecules and highly soluble in water or alkaline solution. Thus, it can be solubilized by water (Gong et al., 2020; Leong et al., 2021). Large amount of water crude extracts were found in *V. volvacea* at 100°C (446.35 ± 11.62 mg/g dry weight) and 60°C (428.51 ± 69.51 mg/g dry weight) or 43% – 45% dry weight of mushroom which were slightly higher than the report from previous works (17% – 21% dry weight of mushroom) (Cheung et al., 2003; Cheung and Cheung, 2005). These results indicated that the cultivation regions and cultivation processes might have had an effect on the mushroom compositions resulting in obtaining different extraction yields.

Table-2. Yield of mushroom crude extracts by different extraction conditions.

Extraction condition	Yield of crude extract (mg/g dry weight)*			
	<i>P. ostreatus</i>	<i>P. sajor-caju</i>	<i>V. volvacea</i>	<i>L. squarrosulus</i>
W100	198.27 $\pm 1.71^{cd}$	318.62 $\pm 15.17^d$	446.35 $\pm 11.62^c$	353.10 $\pm 24.35^c$
W60	228.12 $\pm 26.05^d$	286.84 $\pm 0.77^c$	428.51 $\pm 69.51^c$	351.80 $\pm 5.09^c$
Et60	172.50 $\pm 6.38^c$	181.85 $\pm 12.51^b$	256.85 $\pm 18.39^b$	207.9 8 ± 10.46^b
AE	45.39 $\pm 0.50^a$	50.21 $\pm 6.75^a$	80.71 $\pm 13.93^a$	71.49 $\pm 12.08^a$
BE	85.88 $\pm 15.32^b$	53.91 $\pm 2.98^a$	92.85 $\pm 13.98^a$	101.56 $\pm 8.02^a$

*Values are reported as means \pm S.D. of duplicate measurements. Data with different letters in the column were significantly different between the same mushroom species ($p < 0.05$).

β -glucan content

The β -glucan content of the various crude extracts from different extraction conditions of the 4 edible mushrooms were measured as shown in Figure 1. In *P. ostreatus* and *P. sajor-caju*, the β -glucan content from

BE was significantly higher than those of other extraction conditions with $25.82 \pm 3.87\%$ and $23.08 \pm 0.56\%$ w/w, respectively ($p < 0.05$). However, the β -glucan content of Et60 from *V. volvacea* ($20.69 \pm 2.50\%$ w/w) and *L. squarrosulus* ($13.34 \pm 1.40\%$ w/w) were not significantly ($p > 0.05$) different from BE with values at $16.63 \pm 8.50\%$ and $12.13 \pm 1.38\%$ w/w, respectively (Figure 1). Although the extraction yield of mushroom fruiting bodies was high in W100, large amounts of β -glucan content was observed in BE of *P. ostreatus* and *P. sajor-caju* and in Et60 of *V. volvacea* and *L. squarrosulus*. In our investigation, only 8-11% w/w β -glucan were obtained from W100 and W60 of *P. ostreatus*, *P. sajor-caju* and *V. volvacea*. These results indicated that the most suitable extraction condition for β -glucan preparation of *P. ostreatus* and *P. sajor-caju* was alkaline extraction and ethanolic extraction for *V. volvacea* and *L. squarrosulus*. The β -glucan contents from various mushroom extracts have been investigated and reported. In 2008, the amount of crude β -glucan extracted from Chaga using alkaline extraction method was 13.7 % w/w. It was suggested that large amounts of soluble β -glucan may have been the cause of high dietary fiber content in Chaga (Rhee et al., 2008). In 2013, an alkaline extraction yield of 1.5 g of crude polysaccharide / 1 kg of mushroom fruiting body were obtained from somatic hybrid mushroom, *Pleurotus florida* and *V. volvacea* strains. After the fractionation of crude polysaccharide extract through Sepharose 6B column, the water-soluble polysaccharide was identified as a β -glucan. The polysaccharide extracts from the alkaline extraction showed the activities of immunoactivation of macrophages, splenocytes, and thymocytes (Maity et al., 2013). These results indicated that the concentration of β -glucan content and biological activities varied according to mushroom species and extraction condition. Moreover, a number of research studies have reported some biological properties of mushroom β -glucans including their antitumor, antioxidative, immunomodulatory, anti-inflammatory and recurrent respiratory tract infections (Khan et al., 2018; Barbosa et al., 2020b). Among the extraction conditions, alkaline extraction was the most suitable for β -glucan extraction in all of the 4 edible mushrooms in this research. The mushroom extraction using alkaline treatment caused a degradation of the fiber structure and destruction of cell walls. The linkage of cell wall protein and glucan are hydrolyzed. This resulted in intracellular polysaccharides and the alkali-soluble fraction being released (Leong et al.,

2021). However, the biological activity of β -glucan also varied with different molecular weight which was related to the extraction conditions and mushroom species.

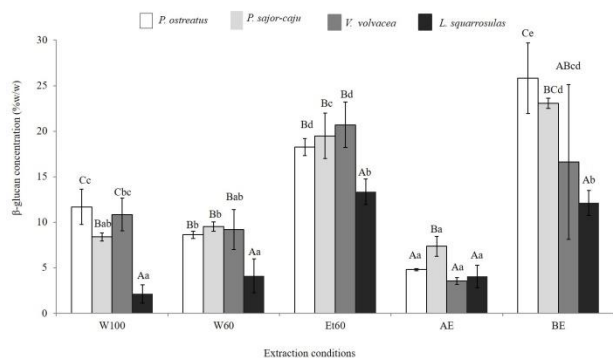


Figure-1. β -glucan concentration of the various crude extracts from different extraction conditions of the 4 edible mushrooms. Standard deviations are shown by the error bars. Bars with different capital letters determine significant difference between mushrooms in each extraction condition while different lower cases indicate significant difference between extraction conditions for each mushroom ($p < 0.05$)

Total phenolic content

In this research, the total phenolic content from the various mushroom extracts were determined and results shown in Figure 2. Total phenolic compounds in the crude extract of W100 and W60 were higher than those of other extraction conditions in all 4 edible mushrooms. The total phenolic content from W60 of *V. volvacea* (38.07 ± 2.53 mg gallic acid eq./g extract) were significantly higher than those from *P. ostreatus* and *P. sajor-caju* but not significantly different from *L. squarrosulus* (34.78 ± 5.69 mg gallic acid eq./g extract) at the same extraction condition. These results indicated that the total phenolic content in mushroom extracts contained high polarity compounds. Thus, the samples gave the highest amount of these compounds when water was used as the extraction solvent. Similar results were reported by Boonsong et al. (2016). Their results showed that a large amount of total phenolic compounds were obtained from *Lentinus edodes*, *Pleurotus eous*, *P. sajor-caju* and *Auricularia auricular* extracts when water was used as the extraction solvent (Boonsong et al., 2016).

A correlation between higher antioxidant activity and a large amount of total phenolics was found in mushroom water extract (Puttaraju et al., 2006). Thus, water-extracted samples were found to possess better antioxidant activities.

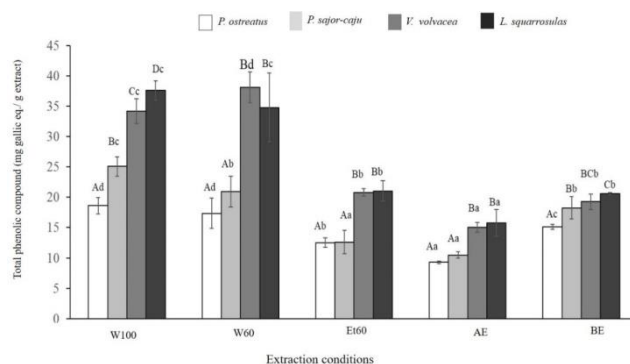


Figure- 2. Total phenolic concentration of the various crude extracts from different extraction conditions of the 4 edible mushroom. Standard deviations are shown by the error bars. Bars with different capital letters determine significant difference between mushrooms in each extraction condition while different lower cases indicate significant difference between extraction conditions for each mushroom ($p < 0.05$)

Antioxidant activity assay

ABTS radical cation scavenging activity

The ABTS assay is widely used to determine the antioxidant activity of hydrophilic and lipophilic compounds (Sudha et al., 2012). In Figure 3, the ABTS activity revealed that the W100 and W60 of all 4 edible mushrooms were significantly higher than those from other extraction conditions when compared in the same mushroom species. This result indicated that the extraction temperature (100 and 60°C) showed only minor effect on the ABTS assay of crude extracts when water was used as the extraction solvent. The high ABTS radical scavenging of mushroom extracts from water extraction might have been due to large amount of total phenolic content (Boonsong et al., 2016). The W60 of *V. volvacea* showed excellent scavenging of ABTS radicals at 67.12 ± 4.41 mg trolox eq./g extract which was significantly higher than those of other mushrooms at the same extraction condition ($p < 0.05$). These results indicated that the ABTS scavenging activity varied depending on the mushroom species and extraction condition.

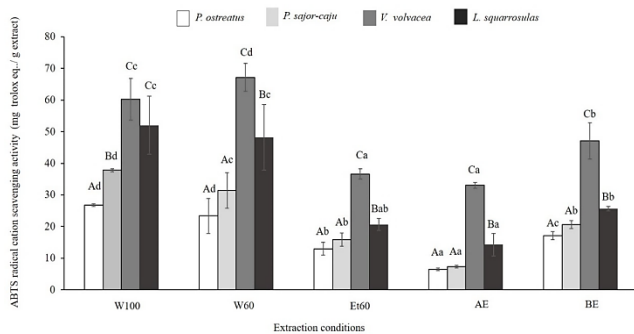


Figure-3. ABTS assay of the various crude extracts from different extraction conditions of the 4 edible mushrooms. Standard deviations are shown by the error bars. Bars with different capital letters determine significant difference between mushrooms in each extraction condition while different lower cases indicate significant difference between extraction conditions for each mushroom ($p < 0.05$)

Ferric reducing antioxidant power, FRAP

In the FRAP assay, on reduction of Fe III-TPTZ complex by antioxidant, Fe II-TPTZ is formed which can be measured by spectrophotometer at 595 nm (Lakshmi et al., 2004). The results of antioxidant from the 4 edible mushrooms using FRAP assays are shown in Figure 4. The results showed that the values from water- extracted *V. volvacea* and *L. squarrosulus* were significantly higher than those from ethanolic, acid and alkaline extractions. The scavenging effects of *V. volvacea* showed its highest value at 36.46 ± 3.44 mg trolox eq./g extract in the FRAP assay obtained from crude extract using water as the extraction solvent at 60°C . In *L. squarrosulus*, the excellent antioxidant activity by FRAP assay in W100 (30.27 ± 1.44 mg trolox eq./g extract) was not significantly different from W60 (28.14 ± 4.83 mg trolox eq./g extract) ($p > 0.05$). The result correlated with the total phenolic compounds content. Similar results were reported by (Attarat and Phermthai, 2015). Their results showed that the scavenging effects of *L. squarrosulus* extract correlated with the total phenolic compounds (Attarat and Phermthai, 2015). The antioxidant activity of mushroom extracts highly depends on the mushroom species, cultivation regions and extraction process. Bioactive compounds of cultivated mushrooms can be different from each other because of the differences in growth conditions, compost types, chemical types and quantities used for hygiene during the mushroom cultivations (Yildiz et al., 2017). The strong antioxidant properties in mushroom include the

components of ascorbic acid, tocopherols, polysaccharides, flavonoids, ergothioneine, carotenoids, phenolics and glycosides (Chun et al., 2021). Among the 4 commercial mushrooms in this research, the highest antioxidant activity using FRAP assay were found in W60 of *V. volvacea*.

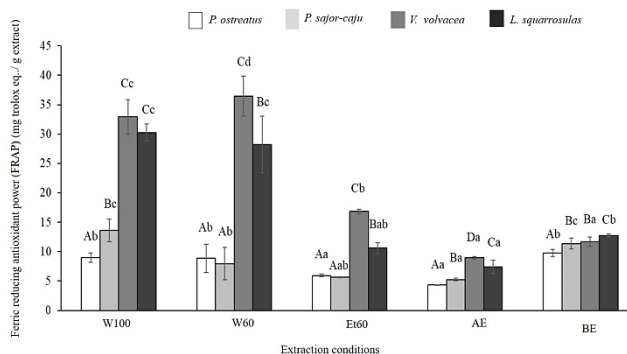


Figure-4. Ferric reducing antioxidant power (FRAP) of the various crude extracts from different extraction conditions of the 4 edible mushrooms. Standard deviations are shown by the error bars. Bars with different capital letters determine significant difference between mushrooms in each extraction condition while different lower cases indicate significant difference between extraction conditions for each mushroom ($p < 0.05$)

Metal chelating activity

In metal chelating activity assay, mushroom extracts can interfere with the formation of Fe^{2+} and the ferrozine complex. The chelating activity of mushroom extract can then capture Fe^{2+} before ferrozine resulting in the decrease of red color observed in the reaction (Boonsong et al., 2016). The results of metal chelating activity from various mushroom extracts are shown in Figure 5. The metal chelating activity of all the 4 edible mushroom extracts obtained from BE were significantly higher than those from the other extraction conditions ($p < 0.05$) (Figure 5). The highest chelating effect of 66.13 ± 1.63 mg EDTA eq./g extract were observed in BE of *P. ostreatus*. These results indicated that the metal chelating activity of BE from *P. ostreatus* was related to β -glucan contents in the crude extract. However, the chelating activity of BE from *P. ostreatus* was not significantly different from *V. volvacea* (63.76 ± 0.51 mg EDTA eq./g extract) but significantly higher than those from *P. sajor-caju* and *L. squarrosulus* when the same extraction method was compared. These results

indicated that alkaline extraction was the most suitable extraction method for β -glucan and metal chelating activity from *P. ostreatus*. The antioxidant activity of polysaccharide extracted from *P. ostreatus* was similar to recent research (Uddin et al., 2019; Barbosa et al., 2020a). The antioxidant activity of polysaccharide is mainly associated with monosaccharides components, chain conformation, molecular weight, and associations of proteins (Barbosa et al., 2020a).

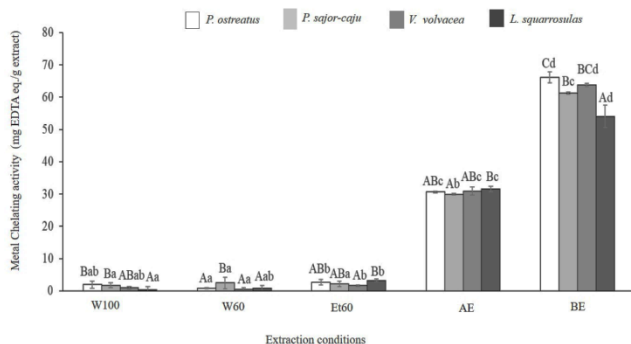


Figure-5. Metal chelating activity of the various crude extracts from different extraction conditions of the 4 edible mushrooms. Standard deviations are shown in the error bars. Bars with different capital letters determine significant difference between mushrooms in each extraction condition while different lower cases indicate significant difference between extraction conditions for each mushroom ($p < 0.05$)

Conclusions

All results in our research indicated that the extraction conditions and mushroom species clearly influenced β -glucan content, total phenolic compounds and antioxidant properties which led to different antioxidant effects. All of the 4 commercial mushrooms—*P. ostreatus*, *P. sajor-caju*, *V. volvacea* and *L. squarrosulus*—could be used as an alternative source of bioactive compounds and antioxidant from fungi. Among the 4 commercial mushrooms in this research, alkaline extraction was the most suitable extraction condition for β -glucan preparation from *P. ostreatus* ($25.82 \pm 3.87\%$ w/w) and *P. sajor-caju* ($23.08 \pm 0.56\%$ w/w). The highest antioxidant activity using ABTS and FRAP assay were found in the water extract of *V. volvacea*. The simple and easy extraction conditions with cost-effectiveness in this research could be applied to produce β -glucan and antioxidant additives for food and health products safe for consumers.

Acknowledgements

We acknowledge the financial support by a research grant from Rajamangala University of Technology Isan, Nakhon Ratchasima, Thailand.

Disclaimer: None

Conflict of Interest: None

Source of Funding: This research was funded by a research grant from Rajamangala University of Technology Isan (contract no. NKR2561REV014), Nakhon Ratchasima, Thailand.

References

- Attarat J and Phermthai T, 2015. Bioactive compounds in three edible *Lentinus* mushrooms. *Walailak J. Sci. & Tech.* 12(6): 491–504.
- Bak WC, Park JH, Park YA and Ka KH, 2014. Determination of glucan contents in the fruiting bodies and mycelia of *Lentinula edodes* cultivars. *Mycobiology.* 42(3): 301–304.
- Barbosa JR, Freitas S MM, Oliveira LC, Martins S LH, Almada-Vilhena AO, Oliveira RM and Carvalho Junior RN, 2020a. Obtaining extracts rich in antioxidant polysaccharides from the edible mushroom *Pleurotus ostreatus* using binary system with hot water and supercritical CO₂. *Food Chem.* 330: 127173. <https://doi.org/10.1016/j.foodchem.2020.127173>.
- Barbosa JR, dos Santos Freitas MM, da Silva Martins LH and de Carvalho Junior RN, 2020b. Polysaccharides of mushroom *Pleurotus* spp.: new extraction techniques, biological activities and development of new technologies. *Carbohydr. Polym.* 229: 115550. <https://doi.org/10.1016/j.carbpol.2019.115550>.
- Benzie IFF and Strain JJ, 1996. The ferric reducing ability of plasma as a measure of antioxidant power the FRAP assay. *Anal. Biochem.* 239: 70–76.
- Boonsong S, Klaypradit W and Wilaipun P, 2016. Antioxidant activities of extracts from five edible mushrooms using different extractants. *Agric. Nat. Resour.* 50: 89–97.
- Cheung LM, Cheung PCK and Ooi VEC, 2003. Antioxidant activity and total phenolics of edible mushroom extracts. *Food Chem.* 81: 249–255.
- Cheung LM and Cheung PCK, 2005. Mushroom extracts with antioxidant activity against lipid



- peroxidation. *Food Chem.* 89: 403–409.
- Chun S, Gopal J and Muthu M, 2021. Antioxidant activity of mushroom extracts/polysaccharides-their antiviral properties and plausible antiCOVID-19 properties. *Antioxidants (Basel)*.10(12): 1899. doi: 10.3390/antiox10121899.
- Decker EA and Welch B, 1990. Role of ferritin as a lipid oxidation catalyst in muscle food. *J. Agric. Food Chem.* 38: 674–677.
- Gong P, Wang S, Liu M, Chen F, Yang W, Chang X, Liu N, Zhao Y, Wang J and Chen X, 2020. Extraction methods, chemical characterizations and biological activities of mushroom polysaccharides: a mini-review. *Carbohydr. Res.* 494: 108037. doi.org/10.1016/j.carres.2020.108037.
- Hwang IW, Kim BM, Kim YC, Lee SH and Chung SK, 2018. Improvement in β -glucan extraction from *Ganoderma lucidum* with high-pressure steaming and enzymatic pre-treatment. *Appl Biol Chem.* 61: 235–242.
- Khan AA, Gani A, Khanday FA and Masoodi FA, 2018. Biological and pharmaceutical activities of mushroom β -glucan discussed as a potential functional food ingredient. *Carbohydr. Diet. Fibre.* 16: 1–13.
- Kosanić M, Ranković B and Dašić M, 2011. Mushrooms as possible antioxidant and antimicrobial agents. *Iran. J. Pharm. Sci.* 11(4): 1095–1102.
- Kupradit C, Khongla C, Musika S, Ranok A, Tamaruay K, Woraratphoka J and Mangkalan S, 2017. Cultivation of *Lentinus squarrosulus* and *Pleurotus ostreatus* on cassava bagasse based substrates. *IJAT.* 3(6): 883–892.
- Lakshmi B, Tilak JC, Adhikari S, Devasagayam TPA and Janardhanan KK, 2004. Evaluation of antioxidant activity of selected Indian mushrooms. *Pharm. Biol.* 42(3): 179–185.
- Leong YK, Yang FC and Chang JS, 2021. Extraction of polysaccharides from edible mushrooms: emerging technologies and recent advances. *Carbohydr. Polym.* 251: 117006. doi.org/10.1016/j.carbpol.2020.117006.
- Maity KK, Patra S, Dey B, Bhunia SK, Mandal S, Bahera B, Maiti TK, Sikdar SR and Islam SS, 2013. A β -glucan from the alkaline extract of a somatic hybrid (PfloVv5FB) of *Pleurotus florida* and *Volvariella volvacea*: structural characterization and study of immunoactivation. *Carbohydr. Res.* 370: 13–18.
- Matthaus B, 2002. Antioxidant activity of extract obtained from residues of different oil seeds. *J. Agric. Food Chem.* 50: 3444–3452.
- Mau JL, Lin HC and Song SF, 2002. Antioxidant properties of several specialty mushrooms. *Food Res. Int.* 35: 519–526a.
- Puttaraju NG, Venkateshaiah SU, Dharmesh SM, Urs SM and Somasundaram R, 2006. Antioxidant activity of indigenous edible mushrooms. *J. Agric. Food Chem.* 54(26): 9764–9772.
- Rhee SJ, Cho SY, Kim KM, Cha DS and Park HJ, 2008. A comparative study of analytical methods for alkali-soluble β -glucan in medicinal mushroom, Chaga (*Inonotus obliquus*). *LWT.* 41: 545–549.
- Ruthes AC, Smiderle FR and Iacomini M, 2015. β -glucans from edible mushrooms: a review on the extraction, purification and chemical characterization approaches. *Carbohydr. Polym.* 117: 753–761.
- Sudha G, Vadivukkarasi S, Shree RBI and Lakshmanan P, 2012. Antioxidant activity of various extracts from an edible mushroom *Pleurotus eous*. *Food Sci. Biotechnol.* 21: 661–668.
- Synytsya A, Mičková K, Synytsya A, Jablonský I, Spěvák J, Erban V, Kovářiková E and Čopíková J, 2009. Glucans from fruit bodies of cultivated mushrooms *Pleurotus ostreatus* and *Pleurotus eryngii*: structure and potential prebiotic activity. *Carbohydr. Polym.* 76(4): 548–556.
- Szwengiel A and Stachowiak B, 2016. Deproteinization of water-soluble β -glucan during acid extraction from fruiting bodies of *Pleurotus ostreatus* mushrooms. *Carbohydr. Polym.* 146: 310–319.
- Uddin Pk MM, Islam MS, Pervin R, Dutta S, Talukder RI and Rahman M, 2019. Optimization of extraction of antioxidant polysaccharide from *Pleurotus ostreatus* (Jacq.) P. Kumm and its cytotoxic activity against murine lymphoid cancer cell line. *PLoS ONE* 14(1): e0209371.
- Villares A, Laura MV and Guillamón E, 2012. Structural features and healthy properties of polysaccharides occurring in mushrooms. *Agriculture.* 2: 425–471.



- Wiriyaphan C, Chitsomboon B and Yongsawadigul J, 2012. Antioxidant activity of protein hydrolysates derived from threadfin bream surimi byproducts. *Food Chem.* 132: 104–111.
- Xu Z, Feng S, Qu J, Yuan M, Yang R, Zhou L, Chen T and Ding C, 2019. The effect of extraction methods on preliminary structural properties and antioxidant activities of polysaccharides from *Lactarius vividus*. *Processes.* 7: 482. <https://doi.org/10.3390/pr708048>.
- Yildiz S, Yilmaz A, Can Z, Tabbouche SA, Kiliç AO and Sesli E, 2017. Some bioactive properties of wild and commercial mushroom species. *J. Food Health Sci.* 3(4): 161–169.
- Zhu F, Du B, Bian Z and Xu B, 2015. Beta-glucans from edible and medicinal mushrooms: characteristics, physicochemical and biological activities. *J. Food Compos. Anal.* 41: 165–173.

Contribution of Authors

Kupradit C: Designed research and performed experiment in part of beta-glucan analysis, and prepared draft of manuscript

Ranok A: Performed experiment in part of mushroom extraction

Mangkalanan S: Performed experiment in part of antioxidant assay

Khongla C: Data analysis and interpretation

Musika S: Edited and gave final approval of manuscript

