RESEARCH ARTICLE

Comparative Evaluation of Chemical Compositions and Antioxidant Activity in Fruiting Bodies and Mycelia of *Volvariella volvacea* and *Pleurotus sajor-caju* Extracts

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ABSTRACT

Mushrooms, recognized for their rich medicinal and nutritional content, have garnered significant interest due to their potent antioxidant properties. This study investigates the fruiting bodies and mycelia of straw mushrooms (*Volvariella volvacea*) and grey oyster mushrooms (*Pleurotus sajor-caju*). The mushrooms were extracted using distilled water at ratios of 1:25, 1:30, and 1:35 g/mL by soaking in a temperature-regulated shaking bath set at 50°C. The aqueous extracts were analyzed for their bioactive components, including polysaccharides, proteins, GABA, total phenolic content (TPC), and total flavonoid content (TFC), along with their antioxidant capacities using DPPH and ABTS assays. The results indicated that the straw mushroom exhibited higher levels of polysaccharides, GABA, TPC, and TFC compared to the grey oyster mushroom, which contained higher protein levels. Specifically, the fruiting bodies extract of the straw mushroom demonstrated the highest levels of polysaccharides (416.94 mg/g extract), GABA (12.86 mg/g extract), TPC (33.27 mg GAE/g extract), and TFC (9.39 mg CE/g extract). Furthermore, the fruiting bodies extract showed a greater capacity to scavenge free radicals than the mycelia extract of both straw mushroom and grey oyster mushroom, with DPPH assay values of 0.65 and 0.47 mg TE/g extract and ABTS assay values of 2.26 and 1.74 mg TE/g extract, respectively. These results suggest that the fruiting bodies possess higher free radical scavenging potential as an outcome of their high levels of bioactive compounds. Consequently, these extracts indicate promise as natural antioxidative agents and could be effectively utilized as ingredients in the culinary industry.

Keywords: *Volvariella volvacea*, *Pleurotus sajor-caju*, Fruiting bodies, Mycelia, Antioxidants.

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INTRODUCTION

When the generation of reactive oxygen species (ROS) and antioxidant defense mechanisms are out of balance, oxidative stress results. Environmental factors such as pollution, smoking, ultraviolet exposure, ionizing radiation, organic solvents, and pesticides contribute to the production of ROS, specifically hydrogen peroxide, hydroxyl radicals, singlet oxygen, superoxide anion radicals, nitric oxide radicals, and hypochlorite radicals. When ROS levels become excessive, the antioxidant system is overwhelmed and its efficacy is compromised, leading to oxidative stress and subsequent cellular damage. This ROS-induced damage affects proteins, nucleic acids, lipids, carbohydrates, and other small molecules, thereby impairing cellular structure and activity, homeostasis, and metabolic processes.[1] Excessive ROS levels and metabolic imbalances are implicated in numerous diseases, including cardiovascular diseases, metabolic conditions, neurodegenerative disorders like Parkinson's and Alzheimer's diseases, cancers, and aging.[2] The global rise in these conditions is primarily attributed to poor nutrition and increased life expectancy. While a balance exists between the production of free radicals and antioxidant defenses in

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the human body[3], it is essential to enhance the diet with antioxidant-rich foods to mitigate oxidative damage and maintain overall health.[4]

Edible mushrooms present a promising alternative source of natural antioxidants, characterized by a balanced nutritional profile low in calories, fats, and cholesterol while remaining high in proteins, carbohydrates, dietary fiber, vital vitamins, and minerals.[5,6] They demonstrate potential applications in dietary supplements for protecting the body against oxidative stress.[7] Findings from recent studies highlight the high antioxidant content of mushrooms and their potential use in a range of commercial food products.[8] Both the fruiting bodies and mycelia of mushrooms are nutrient-dense and possess desirable flavor and aroma profiles suitable for food processing applications.[9] The fruiting bodies of mushrooms are particularly noted for their antioxidant content, including phenols, polysaccharides, vitamins, and minerals, while their mycelia also exhibit significant antioxidant properties.[10] Mushrooms have shown efficacy as a nutritional intervention for cardiovascular patients due to their low sodium and high potassium content, which can help reduce blood pressure.[11] Moreover, selenium in the form of selenoprotein functions as a crucial cofactor in antioxidant enzymes, further enhancing the nutritional value of mushrooms.[12]

Polysaccharides extracted from mushrooms are widely acknowledged for their roles as biological response modifiers and their associated therapeutic benefits. These bioactive polysaccharides typically consist of β-glucans, characterized by linear backbones of β-(1,3)-glucan chains branched with β-(1,6)-side chains. β-glucans exhibit immunomodulatory effects, including antitumor properties, enhanced wound healing, wrinkle reduction, and alleviation of immune system disorders.[13,14] Beyond their prebiotic properties, which improve gastrointestinal health by regulating intestinal microbiota, β-glucans also demonstrate free radical scavenging abilities, reduce infections, promote regeneration of tissue in both animal and human studies, and exhibit hypoglycemic, hypocholesterolemic, and anti-inflammatory activities.[15,16] Additionally, certain mushrooms synthesize specialized metabolites such as γ-aminobutyric acid (GABA), lovastatin, flavonoids, ergothioneine, glycoproteins, sesquiterpenes, carotenoids, and phenolics. These metabolites function as potent antioxidants, safeguarding cells from damage caused by free radicals and lowering the risk of chronic illnesses, thereby enhancing overall health.[6,17] The extensive range of biological activities associated with mushrooms has garnered considerable interest, given their diverse health benefits. These include immunomodulation, anticancer activity, anti-inflammatory effects, hypoglycemic and antithrombotic properties, reduction of blood lipid concentrations, prevention of hypertension and atherosclerosis, and antiviral and antimicrobial actions.[18-20]

Volvariella volvacea (Bull. ex Fr.) Sing., also referred to as the edible straw mushroom, is regarded as a highly nutritious food source extensively cultivated in East and Southeast Asia, utilizing agricultural waste as growth substrates.[21] The fruiting bodies of *V. volvacea* are enriched with bioavailable metabolites, including phenolics, terpenoids, polysaccharides, lectins, ergosterols, antioxidants, and ribosome-inactivating proteins, which are enzymes that inhibit protein synthesis.[22,23] Previous studies have highlighted the pharmaceutical potential of *V. volvacea* due to the presence of polysaccharides with antitumor activities, proteins with immunosuppressive properties, and lectins with immunomodulatory capacity.[24] Moreover, aqueous extracts of *V. volvacea* have demonstrated various bioactivities in vitro, including stimulation of collagen biosynthesis in human skin fibroblast cell lines, as well as anti-coagulant, anti-inflammatory, anti-hypertensive, and antioxidant properties.[25,26]

Pleurotus sajor-caju (Fr.) Sing., frequently referred to as the grey oyster mushroom, is found predominantly in tropical and subtropical regions. It is acknowledged for its dietary and therapeutic properties, largely because of the existence of mycochemicals such as saponins, terpenoids, alkaloids, and cardiac glycosides.[27] The antioxidants present in *P. sajor-caju* are potential protective agents that help mitigate oxidative damage in the human body, offering defense against a range of illnesses, including cancer, cardiovascular, and cerebrovascular disorders.[28,29] Additionally, *P. sajor-caju* exhibits antirheumatic, anti-inflammatory, and antitumor properties and has demonstrated inhibitory effects on human laryngeal carcinoma cell lines.[30,31] Consequently, the bioactive compounds and antioxidants present in edible mushrooms garnered considerable interest for their potential nutritional and medicinal benefits. In light of these attributes, the primary objectives of the current study were to examine and contrast the chemical compositions and antioxidant properties of the fruiting bodies and mycelia of two commercially significant mushrooms, *V. volvacea* and *P. sajor-caju*, thereby contributing valuable insights to the pharmaceutical field.

MATERIALS AND METHODS

Reagents and Chemicals

Reagents used in this study, including gallic acid, catechin Folin-Ciocalteu's, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), and 2,2-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) were procured from Sigma Chemical Co. (St. Louis, MO, USA). Analytical grade solvents and other chemicals were all employed in this investigation.

Samples Preparation

Fruiting bodies and mycelia of the two commercially cultivated mushrooms, *V. volvacea* and *P. sajor-caju*, were sourced from a local market in Nonthaburi province, Thailand, during the period of February to April 2022. Fresh samples were thoroughly cleaned, sliced into tiny pieces, and dried for 48 hours at 50°C in a hot air oven. After being dried, the samples were then pulverized using a blender and kept in zip-locked bags at 4°C for subsequent examination.

Preparation of Mushroom Extracts

The extraction procedure was conducted using previously approved methods, with minor adjustments.[32] The powdered samples were macerated in purified water at ratios of 1:25, 1:30, and 1:35 g of dry sample per mL of purified water and agitated in a shaking water bath at 50 °C for 48 hours. The resulting mixtures were filtered through Whatman No.1 filter paper, with the residues undergoing two additional extraction cycles following the same procedure. To obtain crude extracts, a rotary evaporator was used to condense the filtrates obtained under reduced pressure at every extraction. For subsequent analysis, the unrefined extracts were redissolved in 5% dimethyl sulfoxide (DMSO) to a final concentration of 100 mg/mL, and the solutions were kept in glass vials at 4°C until analysis. The crude extract yield from the mushrooms was quantified and presented as a percentage of the dry sample weight (% w/w).

Determination of Polysaccharide Content

The mushroom extracts' polysaccharide content was assessed using the phenol–sulfuric acid assay, following a modified procedure as outlined by Chaiyarat *et al*. (2022)[33]. The fruiting bodies and mycelia extracts were concentrated to a final level of 28.57 mg/mL (1:35 w/v) using distilled water. Initially, 25 µL of the crude extracts were mixed with 125 µL of sulfuric acid (H_2SO_4) and 25 µL of 5% (v/v) phenol solution. The mixture of reactions was vortexed and incubated at 50°C for 20 minutes before the absorbance was measured at 490 nm using a microplate reader (SpectraMax M2), with a blank serving as the reference. The results were presented as milligrams per gram of extract (mg/g extract). This quantification was calculated using a calibration curve generated from glucose standards ranging from 5 to 500 mg/L.

Determination of Total Protein Content

The Bradford protein assay (BSA) was used to determine the total protein content, with minor modifications.[33] Extracts of mushrooms were prepared in distilled water at a 28.57 mg/mL concentration (1:35 w/v). A 10 µL aliquot of each extract was combined with 190 µL of Coomassie Brilliant Blue G-250 solution and vortexed. After that, the mixture was then incubated for 5 minutes at room temperature. Then, using a microplate reader (SpectraMax M2), the absorbance of the reaction mixtures was subsequently determined at 595 nm. The total protein content was determined using a calibration curve produced from standards of bovine serum albumin (BSA) at values between 1.25 to 25 µg/mL. Every sample was examined three times, with the findings presented as milligrams per gram of extract (mg/g extract).

Determination of GABA Content

The γ-aminobutyric acid (GABA) content was determined using a UV-vis spectrophotometer.[34] To prepare the borate buffer, 2.54 g of borax was dissolved in 4.7 g of boric acid and diluted to a final volume of 1000 mL. To prepare 6% (v/v) phenol and 6% sodium hypochlorite (NaClO) solutions, 100 mL of distilled water was used to dissolve 6 mL of phenol and 6 g of NaClO, respectively. For the assay, 1.5 mL of 6% sodium hypochlorite, 0.5 mL of borate buffer, and 0.5 mL of 6% phenol were combined with each 0.5 mL of mushroom extracts. Then, the reaction mixture was subsequently boiled for 10 minutes and left to equilibrate to room temperature before the absorbance at 630 nm was measured. To achieve a standard GABA (γ-$C_4H_9NO_2$) solution containing 1000 µg/mL, it was obtained by dissolving 0.1 g of GABA in 100 mL of distilled water. Standard solutions with concentrations ranging from 200 to 1000 µg/mL were examined to generate a calibration curve. The content of GABA was expressed as milligrams per gram of extract (mg/g extract).

Determination of Total Phenolic Content

Using a modified Folin-Ciocalteu assay, the total phenolic content (TPC) of the mushroom extracts was evaluated.[35] Briefly, 20 µL of 28.57 mg/mL extracts (1:35 w/v) were added with 100 µL of freshly prepared 10% Folin-Ciocalteu reagent and placed in a 96-well microplate.

The reaction mixture was left to settle at room temperature of approximately 25°C for 5 minutes. After adding 80 µL of 7.5% (w/v) sodium carbonate (Na_2CO_3) solution, the mixture was incubated for 30 minutes at 25 °C. A microplate reader (SpectraMax M2) was used to measure absorbance at 765 nm. Each assay was performed three times. Based on a calibration curve constructed from a series of gallic acid dilutions ranging from 5 to 100 mg/L, the TPC was quantified and expressed as milligrams of gallic acid equivalent per gram of extract (mg GAE/g extract).

Determination of Total Flavonoid Content

The total flavonoid content (TFC) of the mushroom extracts was assessed with an adjusted aluminum-chloride colorimetric technique.[35] In order to perform the procedure involved, 0.5 mL of 28.57 mg/mL (1:35 w/v) extracts were added to 0.15 mL of 5% sodium nitrite ($NaNO_2$) and 2 mL of distilled water. The mixture was incubated for 6 minutes, after which 0.15 mL of 10% aluminum chloride ($AlCl_3$) was added. A 1-mL (1 M) of sodium hydroxide (NaOH) was introduced after an additional 6-minute incubation, and the volume was then adjusted to 5 mL with distilled water. After vortexing the resulting solution, 200 µL of the mixture was transferred to a 96-well microplate. The absorbance was measured at 510 nm using a microplate reader (SpectraMax M2). The findings were presented as milligrams of catechin equivalent per gram of extract (mg CE/g extract) using a calibration curve generated from catechin standards ranging from 30 to 300 mg/L. Every sample was analyzed in triplicate.

DPPH Radical Scavenging Activity

The antioxidant activity of the mushroom extracts was evaluated using the DPPH assay, following a modified traditional procedure.[35] The 0.4 mg of DPPH powder was dissolved in 9.6 mL of 80% ethanol to create the 0.1 mM of DPPH stock solution. For the assay, 20 µL of 28.57 mg/mL (1:35 w/v) mushroom extracts were added with 180 µL of 0.1 mM DPPH solution in a 96-well microplate. The mixture was vigorously shaken and incubated under dark conditions at approximately 25°C for 30 minutes. Using a microplate reader (SpectraMax M2), absorbance was measured at 515 nm with the blank reference (ethanol). The activity of scavenging DPPH radicals was measured as a percentage of inhibition using the formula below:

$$\% \text{ inhibition of DPPH radicals} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

in which A_{control} = Absorbance of DPPH without samples
A_{sample} = Absorbance of DPPH with samples

A Trolox standard curve, produced with concentrations ranging from 1 to 10 mg/mL, was used to assess relevant values. The results were reported as milligrams of Trolox equivalents per gram of extract (mg TE/g extract). The average of three measurements is shown for each set of data.

ABTS Radical Cation Scavenging Activity

The scavenging of ABTS radical cations was conducted following a methodology explained by González-Palma *et al.* (2016)[35], with slight modifications. By reacting a 4 mM aqueous ABTS solution with 2.45 mM potassium persulfate ($K_2S_2O_8$) at a 1:1 (v/v) ratio, the stable ABTS radical cation stock solution was created. Before being used, this reaction mixture was incubated at room temperature for 12 to 16 hours in the dark. After that, distilled water was added to the ABTS solution to dilute it until the absorbance at 734 nm was 1.0 ± 0.02. For the assay, 20 µL of mushroom extracts (28.57 mg/mL) was mixed with 150 µL of the working ABTS solution in a 96-well microplate. Following a 15-minute incubation under dark conditions at approximately 25°C, the absorbance was recorded with a microplate reader (SpectraMax M2) at 734 nm. Using the formula below, the percentage inhibition of ABTS radical cation scavenging was calculated:

$$\% \text{ inhibition of ABTS radical cations} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

in which A_{control} = Absorbance of ABTS without samples
A_{sample} = Absorbance of ABTS with samples

All outcomes were presented as mg TE/g extract and calculated using a Trolox calibration curve at concentrations between 5 to 100 mg/mL. All procedures were carried out in triplicate.

Statistical Analysis

There were three duplicates of each analysis (n = 3). The data were analyzed using the Statistical Package for Social Sciences (SPSS, version 21) and presented as mean ± standard deviation. A one-way ANOVA was utilized to evaluate significant differences between means, and Duncan's new multiple-range test was then employed to determine the least significant differences at a 95% confidence level ($p < 0.05$).

RESULTS AND DISCUSSION

Percentage Yields of Mushroom Extracts

The fruiting bodies and mycelia of dried mushroom powder samples obtained from straw mushroom (*V. volvacea*) and grey oyster mushroom (*P. sajor-caju*) (Figure 1) were extracted using distilled water at ratios of 1:25, 1:30, and 1:35 grams of dry sample per milliliter of distilled water. Samples were subjected to agitation in a shaking water bath at 50 °C. The resulting extracts exhibited a viscous appearance and brown color, characteristic of their respective mushroom species. Notably, the extraction ratio of 1:35 yielded the highest percentage for both mushroom species, indicating that the extraction yield is influenced by the water to raw material ratio.[36] This finding corroborates with the Preedalikit *et al.* (2020)[37] investigation, which examined three species of mushrooms: *Pleurotus sajor-caju* (Fr.) Sing, *Pleurotus ostreatus* (Fr.) Kummer, and *Auricularia auricular-judae* (Bull.). Their study reported that the extraction ratio of 1:35 produced the highest percentage yields of 9.28, 28.20, and 22.20% w/w, respectively. Additionally, solvents containing 20 to 40% water content proved to extract bioactive compounds more efficient than pure solvents.[38] The fruiting bodies and mycelia of *V. volvacea* and *P. sajor-caju* yielded extracts at percentages of 20.19, 13.24, 23.54, and 15.18% w/w, respectively, in the current investigation. According to Table 1, these results suggested that the fruiting bodies extracts of both mushroom species yielded higher percentages compared to the mycelia extracts. The difference in percentage yields between the fruiting bodies and mycelia extracts can be ascribed to variations in their chemical composition and the effects of the solvent on the bioactive compounds in mushrooms.[39] The use of distilled water, owing to its high polarity, resulted in relatively high yields, as mushroom extracts predominantly consist of water-soluble polar molecules, particularly polysaccharides that constitute nearly 50% of the mushroom cell wall.[40] The similarity in polarity between the solvent and solute facilitates the extraction of polar compounds, leading to higher percentage yields.[41] However, it is crucial to acknowledge that high percentage yields do not always correlate with high antioxidant concentrations in the extracted compounds. The solvent may dissolve various groups of compounds, some of which may not exhibit antioxidant properties.[42]

Chemical Composition of Fruiting bodies and Mycelia of *V. volvacea* and *P. sajor-caju*

It is widely acknowledged that mushrooms are a significant source of polysaccharides, proteins, and phytochemicals such as gamma-aminobutyric acid (GABA), lovastatin, and ergothioneine. They also contain a variety of antioxidants that offer substantial health benefits. The chemical composition of mushroom extracts was evaluated by measuring the polysaccharide, protein, GABA, total phenolic, and total flavonoid content in the fruiting bodies (stipe and cap) and mycelia (filament or hyphae) of *V. volvacea* and *P. sajor-caju*. Table 2 presents an illustration of the analysis' results. The

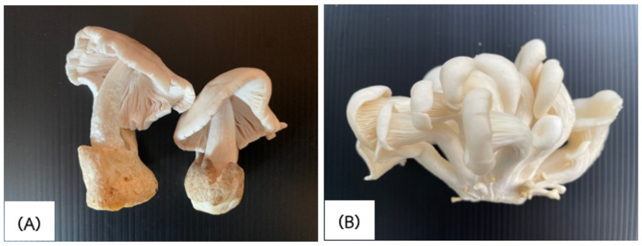

Figure 1: Physical appearances of the fruiting bodies (stipe and cap) and mycelia (filament or hyphae) of (A) *Volvariella volvacea* (Bull. ex Fr.) Sing. and (B) *Pleurotus sajor-caju* (Fr.) Sing

Table 1: Percentage yields of extracts obtained from *V. volvacea* and *P. sajor-caju* using distilled water at different extraction ratios (w/v

Sample	*Appearance of extract*	*Yields (% w/w)*		
		1:25	*1:30*	*1:35*
V. volvacea				
- Fruiting bodies	viscous brown	6.89	12.64	20.19
- Mycelia		2.54	5.35	13.24
P. sajor-caju				
- Fruiting bodies	viscous brown	8.27	14.72	23.54
- Mycelia		3.16	8.19	15.18

chemical composition of crude extracts obtained from the fruiting bodies and mycelia of *V. volvacea* at an extraction ratio of 1 gram of dry sample to 35 milliliters of distilled water showed significant differences. The fruiting bodies exhibited higher concentrations of polysaccharides, proteins, GABA, total phenolics, and total flavonoids, with values of 416.94 ± 8.26 mg/g extract, 19.56 ± 0.14 mg/g extract, 12.86 ± 0.11 mg/g extract, 33.27 ± 0.11 mg GAE/g extract, and 9.39 ± 0.10 mg CE/g extract, respectively, compared to the mycelia, which had 287.71 ± 8.77 mg/g extract, 11.95 ± 0.16 mg/g extract, 3.11 ± 0.09 mg/g extract, 6.78 ± 0.05 mg GAE/g extract, and 2.65 ± 0.07 mg CE/g extract, respectively. There were statistically significant differences as observed ($p < 0.05$). Similarly, for *P. sajor-caju*, the fruiting bodies exhibited higher concentrations of polysaccharides, proteins, GABA, total phenolics, and total flavonoids, with values of 372.53 ± 7.49 mg/g extract, 28.27 ± 0.11 mg/g extract, 10.25 ± 0.10 mg/g extract, 21.50 ± 0.06 mg GAE/g extract, and 4.63 ± 0.06 mg CE/g extract, respectively, compared to the mycelia, which had 243.37 ± 12.60 mg/g extract, 13.48 ± 0.10 mg/g extract, 2.56 ± 0.06 mg/g extract, 3.65 ± 0.07 mg GAE/g extract, and 1.29 ± 0.09 mg CE/g extract, respectively, with statistical significance ($p < 0.05$). These variations can be ascribed to the fruiting bodies of mushrooms having higher concentrations of essential nutrients than the mycelia, which primarily consist of filamentous structures embedded in the substrate. Unlike plants, mushrooms do not photosynthesize and must rely on absorbing organic matter from other organisms or substrates. Under suitable environmental conditions, mushroom spores germinate and develop into thin mycelial threads, which eventually combine to form a mycelial network. As the mycelia continue to grow, they form a pinhead that matures into a stipe and cap, constituting the fruiting bodies.[27] The mycelia, typically found within the substrate, function as the nutrient-absorbing structures of the mushroom. Accordingly, it can be inferred that both *V. volvacea* and *P. sajor-caju* fruiting bodies contain higher levels of nutrients than their mycelia, leading to higher concentrations of polysaccharides, proteins, GABA, total phenolics, and total flavonoids. The biochemical composition of the fruiting bodies is determined by factors such as growth substrate, strain, developmental stage, and the beneficial microbial community.[43,44]

The various mushroom species have different fruiting bodies and mycelia that contain a diverse array of polysaccharides with distinct biological activities. Variations in polysaccharide content among different mushrooms can be attributed to differences in their polysaccharide structures, which affect water solubility. Research has shown that polysaccharides in the fruiting bodies are predominantly composed of mannose, whereas those in the mycelia are primarily glucose-based.[45] Several factors influence these differences, including the structural characteristics, chemical properties, and composition of each mushroom species, as well as the polarity of the solvents and bioactive compounds, sample size, and extraction methods such as solvent types and volumes, extraction duration, temperature, and other disturbances encountered during the process.[46,47] This study demonstrated that the volume of distilled water used during extraction significantly influenced the polysaccharide content. Higher volumes of distilled water led to a higher concentration of soluble polysaccharides being extracted because the polysaccharides contained hydroxyl groups. Furthermore, the mushroom hyphae expanded and softened more easily when hot water was used as a solvent, thereby enhancing polysaccharide extraction.[48] The protein content in mushrooms varies significantly among species, substrate composition, harvest time, environmental factors, and the fruiting bodies' stage of maturity.[49] These findings align with the study conducted by Cheung (2003)[50], which reported that the cap of the oyster mushroom contained higher protein content than the mycelia, which comprise the stipe and hyphae. Additionally, the protein content in the stipe and hyphae was found to be similar.

The enzyme glutamate decarboxylase facilitates the process of removing the carboxyl group from glutamic acid, which results in the synthesis of gamma-aminobutyric acid (GABA). This process is particularly pronounced during germination, as evidenced by Milon *et al.* (2024)[51], who reported higher GABA content in the tips or shoots of plants compared to other structures. This increased GABA content is attributable to the biochemical changes during germination, which stimulate the degradation of glutamic acid, leading to a higher accumulation of GABA in germinating plant tissues. A study by Chen *et al.* (2012)[52] discovered that the fruiting bodies of *Boletus edulis* and *Flammulina velutipes* possessed the highest levels of GABA, with 0.229 and 0.202 mg/g dry weight, respectively. In contrast, the mycelia of *Cordyceps sinensis*, *Cordyceps cicadae*, and *Agaricus blazei* exhibited the highest GABA content, with values of 254.9, 220.5, and

200.4 mg/100 g, respectively. The present result indicated that although the fruiting bodies of *V. volvacea* and *P. sajor-caju* showed higher concentrations of bioactive compounds, the mycelia of these two species of mushrooms had similar levels of polysaccharides, proteins, GABA, total phenolics, total flavonoids, and antioxidant capacity. This suggests that these mushrooms' mycelia still hold significant potential for value addition and utilization.

One of the main contributing factors to mushrooms' antioxidant activity is their phenolic contents.[53] The predominant phenolic compounds found in mushrooms are flavonoids and gallic acid, both of which are recognized for their antioxidant activities.[46] This study demonstrated a direct correlation between the total phenolic content of mushroom extracts and their antioxidant ability.[54,55] Phenolic compounds can be extracted from various mushroom structures, including both fruiting bodies and mycelia. According to this discovery, the total phenolic content of *V. volvacea* and *P. sajor-caju* ranged from 6.78 to 33.27 mg GAE/g extract and 3.65–21.50 mg GAE/g extract, respectively, with the higher values observed in *V. volvacea*. This result is in line with that of Kalava *et al*. (2012) [56], who proved that the aqueous extract of *V. volvacea* mycelia had a total phenolic content of 19.08 mg/g. Similarly, Yim *et al*. (2009)[57] reported that the *Pleurotus* species' aqueous extract had a total phenolic content of 9.01 mg GAE/g extract, which is less than the amounts found in our investigation for the fruiting bodies. These variations in total phenolic content are attributable to various factors, including mushroom species and structures, hyphal growth rate, and the materials used in mushroom cultivation.[58] The accumulation of phenolic chemicals in fruiting bodies of mushrooms is probably caused by the absorption of these compounds from the substrate during development.[59] Additionally, the polarity of solvent affects the efficiency of polyphenol extracts, with polar organic solvents such as methanol being more effective than water. The extraction method also plays a significant role in determining total phenolic content.[38] For instance, using a shaking water bath has been shown to result in the highest total phenolic content, as it promotes increased contact between the solvent and solute. In comparison, ultrasound-assisted extraction, which applies ultrasound waves to disrupt cellular structures and enhance solvent penetration and mass transfer, yields the second-highest total phenolic content and requires the shortest extraction time.[60]

Flavonoids, a class of phenolic compounds, are extensively present in plants and fungi. They encompass various subclasses, including anthocyanins, flavanols, flavonols, flavanones, flavones, and isoflavones, all of which have been recognized for their capacity as antioxidants and their ability to inhibit lipid oxidation and chelate ferrous ions.[61] In this study, the total flavonoid content in the crude extracts of *V. volvacea* and *P. sajor-caju* ranged from 2.65–9.39 mg CE/g extract and 1.29 to 4.63 mg CE/g extract, respectively, with *V. volvacea* exhibiting higher total flavonoid content. Specifically, the fruiting bodies of *V. volvacea* possessed the greatest flavonoid content, measuring 9.39 ± 0.10 mg CE/g extract. These findings are consistent with the results published by Boonsong *et al*. (2016)[62], who reported a total flavonoid content of 7.29 ± 0.21 mg QE/g dry weight in *V. volvacea* extracted with distilled water. Additionally, the aqueous extract of *V. volvacea* mycelia contained 8.23 mg/g of total flavonoids, according to Kalava *et al*. (2012).[56] Variations in total flavonoid content among different mushroom species are further demonstrated by Palacios *et al*. (2011)[63], who suggested that the red pine mushroom (*Lactarius deliciosus*) exhibited the highest quantity of total flavonoid content out of the eight species examined. Similarly, Barros *et al*. (2008)[54] found that the scaly wood mushroom (*Agaricus silvaticus*) showed the greatest level of total flavonoids among the five species studied. Variations in the total flavonoid content are highly dependent on the extraction solvent, solvent ratio, and extraction time.[64] Flavonoids exhibit higher solubility in polar solvents owing to their hydroxyl groups, which are polar in nature.[65]

Evaluation of Antioxidant Capacity of Mushroom Extracts by DPPH and ABTS Assays

The antioxidant potential of crude extracts from the fruiting bodies and mycelia of *V. volvacea* and *P. sajor-caju* was evaluated using the DPPH assay at a concentration of 28.57 mg/mL. This assay measures the compounds' ability to eliminate DPPH free radicals (2,2-diphenyl-1-picrylhydrazyl) through

Table 2: Chemical composition of crude extracts from the fruiting bodies and mycelia of *V. volvacea* and *P. sajor-caju* using distilled water with a concentration of 28.57 mg/mL (1:35 w/v)

Chemical compositions	*V. volvacea*		*P. sajor-caju*	
	Fruiting bodies	*Mycelia*	*Fruiting bodies*	*Mycelia*
Polysaccharide (mg/g extract)	416.94 ± 8.26^{d}	287.71 ± 8.77^{b}	372.53 ± 7.49^{c}	243.37 ± 12.60^{a}
Protein (mg/g extract)	19.56 ± 0.14^{c}	11.95 ± 0.16^{a}	28.27 ± 0.11^{d}	13.48 ± 0.10^{b}
GABA (mg/g extract)	12.86 ± 0.11^{d}	3.11 ± 0.09^{b}	10.25 ± 0.10^{c}	2.56 ± 0.06^{a}
Total phenolic content (mg GAE/g extract)	33.27 ± 0.11^{d}	6.78 ± 0.05^{b}	21.50 ± 0.06^{c}	3.65 ± 0.07^{a}
Total flavonoid content (mg CE/g extract)	9.39 ± 0.10^{d}	2.65 ± 0.07^{b}	4.63 ± 0.06^{c}	1.29 ± 0.09^{a}

Note: The mean ± standard deviation is used to express the values (n = 3).
Distinct superscript lowercase characters within a row signify noteworthy variations at $p < 0.05$, determined using one-way ANOVA with Duncan's new multiple range test (DMRT).
GAE = gallic acid equivalent; CE = catechin equivalent

a change in color from purple to yellow, indicating a reaction between DPPH and antioxidant compounds. The results, reported as Trolox equivalent antioxidant capacity (TEAC), revealed differences in the fruiting bodies and mycelia of both mushroom species's potential to scavenge DPPH free radicals. Specifically, the fruiting body and mycelia extracts from *V. volvacea* had TEAC values of 0.65 ± 0.06 and 0.24 ± 0.06 mg TE/g extract, respectively. These values were greater than those of *P. sajor-caju*, which exhibited TEAC values of 0.47 ± 0.05 and 0.11 ± 0.04 mg TE/g extract, respectively. Statistical analysis indicated that the species, structures, and chemical compositions of the mushrooms significantly influenced the extracts' capacity to remove free radicals using DPPH.[58] The fruiting bodies and mycelia extracts from *V. volvacea* displayed higher antioxidant capacity compared to those from *P. sajor-caju*. Furthermore, the fruiting bodies of both mushroom species demonstrated significantly increased antioxidant ability compared to the mycelia ($p < 0.05$), although both were lower than vitamin C with statistical significance (Table 3). These findings correlate with the fruiting bodies' higher total phenolic content than the mycelia of both *V. volvacea* and *P. sajor-caju*. Reduced amounts of phenolics, polyunsaturated fatty acids, indole compounds, and variations in hypogeal oxygen and temperature have been associated to the mycelia's reduced antioxidant capacity.[66]

Alternatively, extracts from the fruiting bodies and mycelia of *V. volvacea* and *P. sajor-caju* were assessed for their antioxidant ability using the ABTS assay at a concentration of 28.57 mg/mL. Trolox equivalent antioxidant capacity (TEAC) was employed to convey the compounds' capability to decolorize ABTS free radicals ($ABTS^{\bullet+}$; 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid radical)). The findings revealed that the ABTS radical scavenging activity of the fruiting bodies and mycelia of both mushroom species differed significantly. In particular, the extracts from the fruiting bodies and mycelia of *V. volvacea* had TEAC values of 2.26 ± 0.08 and 0.89 ± 0.08 mg TE/g extract, respectively, whereas *P. sajor-caju* exhibited TEAC values of 1.74 ± 0.07 and 0.58 ± 0.07 mg TE/g extract, respectively. A statistical analysis verified that both the species and the structural composition of mushrooms significantly influenced the extracts' ABTS free radical scavenging ability. The extracts from the fruiting bodies and mycelia of *V. volvacea* exhibited higher antioxidant capacity compared to those from *P. sajor-caju*. Additionally, the fruiting bodies of both mushroom species displayed a significantly higher antioxidant capacity ($p < 0.05$) in comparison to the mycelia; however, both were significantly lower than vitamin C (Table 3). These results agree with investigations of total phenolic content, total flavonoid content, and DPPH free radical scavenging activity, which revealed higher total phenolic content in the fruiting bodies than in the mycelia of both species. Nonetheless, the chemical composition of mycelia from other mushroom species indicates that the mycelia of the poplar fieldcap mushroom (*Agrocybe cylindracea*) possess notable antioxidant properties, with an inhibition range of 47.7–76.1%, surpassing that of the fruiting bodies.[67] This highlights the potential of mycelia as valuable ingredients in food and medicinal applications.

The fruiting bodies and mycelia extracts from both mushroom species were subjected to antioxidant assays, which demonstrated their ability to scavenge free radicals, including DPPH and ABTS, indicating strong antioxidant properties. The high content of flavonoids and total phenolics in *V. volvacea* is responsible for its antioxidant activity.[68] In similar lines, it has been reported that *V. volvacea*-free phenolic extracts possessed the highest level of antioxidant activity at 82.9%.[69] These findings correspond with those of Zhang *et al*. (2012)[70], who reported that the fruiting bodies of *P. ostreatus* showed greater abilities to scavenge DPPH and superoxide anion radicals. The water-soluble crude polysaccharides from *P. ostreatus* demonstrated greater antioxidant activity in their ability to scavenge free radicals. This is probably because they contained carbohydrate components, particularly β-glucan, which is known to enhance antioxidant activity (Mitra *et al*., 2013)[71]. The study by Bhatia *et al*. (2014)[72], which proved that extracts from selenium-enriched *P. sajor-caju* and *V. volvacea* displayed stronger reducing and scavenging capabilities, further supports the current findings.

The antioxidant capacity and α-glucosidase inhibitory action of 12 edible mushroom species were examined by Rochanakit *et al*. (2021).[73] It was found that bioactive compounds extracted with polar and semipolar solvents, like distilled water and ethanol, exhibited higher antioxidant capacity compared to those extracted with hexane. This result concurs with the research conducted by Seephonkai *et al*. (2011)[74], who observed no antioxidant activity in 10 species of *Phellinus* mushrooms (*Phellinus* spp.) when extracted with hexane. Previous research has demonstrated that various species of edible mushrooms can synthesize antioxidants, including polysaccharides, amino acids, polyphenols, vitamins, and carotenoids while inhibiting oxidation processes.[75] These bioactive compounds are typically soluble in polar

Table 3: Antioxidant capacity of extracts from the fruiting bodies and mycelia of *V. volvacea* and *P. sajor-caju* using distilled water at a concentration of 28.57 mg/mL, evaluated by DPPH and ABTS assays, compared to ascorbic acid at a concentration of 1 mg/mL.

Sample	*DPPH scavenging activity (mg TE/g extract)*	*ABTS scavenging activity (mg TE/g extract)*
V. volvacea		
- Fruiting bodies	0.65 ± 0.06^{d}	2.26 ± 0.08^{d}
- Mycelia	0.24 ± 0.06^{b}	0.89 ± 0.08^{b}
P. sajor-caju		
- Fruiting bodies	0.47 ± 0.05^{c}	1.74 ± 0.07^{c}
- Mycelia	0.11 ± 0.04^{a}	0.58 ± 0.07^{a}
Ascorbic acid	0.87 ± 0.02^{e}	3.69 ± 0.05^{e}

Note: The mean ± standard deviation is used to express the values (n = 3).
Distinct superscript lowercase characters within a row signify noteworthy variations at $p < 0.05$, determined using one-way ANOVA with Duncan's new multiple range test (DMRT).
TE = trolox equivalent

and semipolar solvents such as water, methanol, and ethanol. Additionally, it has been noted that mushroom extracts obtained using distilled water and ethanol have a greater total phenolic content than those extracted with hexane. Phenolic chemicals are synthesized by living organisms as a means of growth since they are antioxidants. They are essential in preventing or postponing oxidation because they provide free radicals with hydrogen atoms or electrons.[76,77]

CONCLUSION

To the best of our knowledge, this work is the first to compare the phytochemicals and antioxidant capacities found in the fruiting bodies and mycelia of *V. volvacea* and *P. sajor-caju*. The results indicate that the aqueous extracts of the fruiting bodies and mycelia of *V. volvacea* and *P. sajor-caju* exhibit significant antioxidant activities, which can be correlated with their phytochemical contents. However, the extracts yielded varying amounts of diverse phytochemicals and exhibited different levels of antioxidant activities. The fruiting bodies demonstrated greater phytochemical contents and antioxidant properties compared to the mycelia. Consequently, both mushrooms have been identified to be abundant in natural antioxidants and nutraceuticals, offering potential protection against various oxidative stress-mediated disorders.

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