Journal of Applied Biology & Biotechnology Vol. 14(3), pp. 66-74, May-June, 2026
Available online at http://www.jabonline.in
DOI: 10.7324/JABB.2026.658059

Mycelium and exopolysaccharide production and bioactivities of select cup mushrooms (Sarcoscyphaceae, Pezizomycetes) from Central Luzon, Philippines

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ARTICLE INFO

Article history:
Received on: 19/01/2026
Accepted on: 06/03/2026
Available online: 05/04/2026

Key words:
Antidiabetic,
Antioxidant,
Cup mushrooms,
Neuroprotective,
Sarcoscypha spp.,
Submerged cultivation.

ABSTRACT

Mushrooms are recognized as important sources of bioactive compounds with antioxidant, antidiabetic, and neuroprotective properties. However, cup mushrooms remain relatively understudied despite their potential for producing unique secondary metabolites. This study evaluated the effects of culture medium, pH, temperature, agitation, and incubation period on the mycelial biomass production of *Sarcoscypha* spp., *Phillipsia domingensis,* and *Cookeina tricholoma*, and assessed their chemical profiles and bioactivities. Among the culture media tested, rice bran broth supported the highest biomass for *Sarcoscypha* spp. (326.3 mg/100 mL) and *P. domingensis* (342.3 mg/100 mL), whereas coconut water was optimal for *C. tricholoma* (312 mg/100 mL). Maximum growth occurred at pH 6.0, with optimal temperatures of 20°C for *Sarcoscypha* spp. and *P. domingensis* and 30°C for *C. tricholoma*, and agitation at 150 rpm significantly enhanced biomass accumulation. Peak biomass was achieved after 20 days for *Sarcoscypha* spp. (1585 mg/100 mL) and *P. domingensis* (1293 mg/100 mL), and 10 days for *C. tricholoma* (1314 mg/100 mL). Thin-layer chromatography analysis revealed essential oils, coumarins, and anthrones in all species; triterpenes and sterols in *P. domingensis* and *C. tricholoma*; alkaloids in *Sarcoscypha* spp. and *P. domingensis*; steroids in *Sarcoscypha* spp. and *C. tricholoma*; and phenols, tannins, and flavonoids exclusively in *P. domingensis*. 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity ranged from 17.17–57.42% for mycelial extracts and 40.96–53.20% for exopolysaccharides. Mycelial extract of *Sarcoscypha* spp. at 10 µg/mL exhibited the highest α-glucosidase inhibition (23.03%) and acetylcholinesterase inhibition (12.74%), indicating its promising antidiabetic and neuroprotective potential. These findings underscore the biotechnological significance of cup mushrooms as sources of antioxidant, antidiabetic, and neuroprotective metabolites.

1. INTRODUCTION

Mushrooms are macrofungi that have long been recognized as valuable food resources due to their unique flavors, rich nutritional profiles, and functional properties. They are low in calories and fats but contain significant amounts of proteins, dietary fibers, vitamins, and minerals [1,2]. In addition, mushrooms are enriched with a wide range of bioactive compounds such as polysaccharides, terpenoids, phenolic compounds, and sterols, which contribute to their health-promoting effects [3,4]. Beyond their nutritional value, mushrooms have gained economic importance globally, not only as food and nutraceuticals but also as valuable raw materials for pharmaceutical, cosmetic, and biotechnological industries [5-7]. Their dual role as functional food and source of therapeutic compounds underscores their relevance in both local and international markets. The biological properties of mushrooms have been extensively studied, with particular attention to their antioxidant, antidiabetic, and neuroprotective activities. Mushroom-derived antioxidants, including phenolics and polysaccharides, play a crucial role in mitigating oxidative stress by neutralizing free radicals, thereby protecting cells from damage linked to aging and chronic diseases [8,9]. Several mushroom extracts have also demonstrated the ability to regulate glucose metabolism, inhibit α-glucosidase activity, and improve insulin sensitivity, making them promising agents for diabetes management [10,11]. Furthermore, some mushrooms exhibit acetylcholinesterase (AChE) inhibitory activity, which has implications in the prevention and management of neurodegenerative disorders such as Alzheimer's disease [12]. These multifaceted bioactivities make mushrooms attractive candidates for the development of functional foods and therapeutic agents.

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Recognizing the promising nutraceutical and pharmacological potential of mushrooms, it is essential to optimize its cultivation parameters to achieve maximal biomass yield. Submerged fermentation (SmF) has emerged as a powerful tool in mushroom biotechnology, offering controlled and efficient production of mycelial biomass and extracellular metabolites [13]. Unlike solid-state cultivation, SmF provides a reproducible and scalable system for the production of bioactive compounds under optimized conditions. Mycelial biomass produced through this method is a rich source of primary and secondary metabolites, whereas exopolysaccharides (EPSs) are secreted into the culture medium [14-17]. Submerged production of mycelium of *Ganoderma lucidum, Pleurotus cystidiosus, Volvariella volvacea,* and *Schizophyllum commune* has been investigated as a potential source of bioactive lipids [13]. A critical review has shown that plenty of basidiomycetes can grow in submerged cultures and produce bioactive compounds helpful in various applications [18].

The Family Sarcoscyphaceae (order Pezizales, class Pezizomycetes) comprises brightly pigmented, cup- to disc-shaped ascomycetes, commonly referred to as "cup fungi", which are primarily saprotrophic on decaying wood, plant litter, or soil. Among its well-known genera are *Sarcoscypha*, *Phillipsia*, and *Cookeina*, which are distributed across temperate and tropical regions. *Cookeina* mushrooms have attracted increasing attention due to their ethnomedicinal relevance and biotechnological potential. Ethnomycological records indicate that in Cameroon, *Cookeina* species are traditionally employed in the treatment of ear infections [19], whereas in Indonesia, *C. speciosa*, *C. sulcipes*, and *C. tricholoma* are commonly consumed and integrated into indigenous medicinal practices [20]. However, Jiménez-Zárate *et al.* [21] reported that *Phillipsia domingensis* exhibited strong antioxidant activity, limited antifungal effects, and showed promising potential as an anthelmintic agent. Moreover, Piljac-Zegarac *et al.* [22] demonstrated the promising antioxidant potential of *Sarcoscypha austriaca* water extract, with a reducing power of 17.22 mmol $Fe2+$/kg in the FRAP assay and an EC_{50} value of 15.8 in the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay.

Despite the increasing recognition of mushrooms as sources of functional and therapeutic compounds, cup fungi remain poorly studied in terms of cultivation potential and biological activities. Addressing this knowledge gap is essential for expanding the pool of underutilized mushroom resources with potential health benefits. Therefore, this study aims to investigate the mycelial biomass production and EPS yield of three species under Sarcoscyphaceae in SmF, alongside the analysis of biochemical compositions and evaluation of their antioxidant, antidiabetic, and AChE inhibitory activities. This study not only contributes to the scientific understanding of these underexplored fungi but also paves the way for their possible applications in functional food and pharmaceutical industries.

2. MATERIALS AND METHODS

2.1. Basidiocarp Collection and Tissue Culture

Wild basidiocarps of cup fungi [Figure 1] were collected from Gabaldon, Nueva Ecija, Philippines. Each mushroom species was placed in a properly labeled brown paper bag and brought to the laboratory for processing. A small tissue from the internal part of the basidiocarp was cut using a scalpel and inoculated onto a potato dextrose agar plate using an inoculating needle and incubated at 28°C for 7 days.

2.2. Optimization of Submerged Culture Requirements

Four liquid culture media (potato sucrose broth [PSB], corn grit broth [CGB], rice bran broth [RBB], and coconut water [CW]) in triplicate were prepared, sterilized, inoculated with a 10-mm-diameter mycelial disc, and incubated at 28°C. Mycelia from the 10-day-old cultures were harvested, oven-dried, and weighed to determine the most favorable medium. The best medium at varying pH levels from pH 4.0 to 8.0 was evaluated in order to identify the optimum pH. For temperature evaluation, cultures with the best medium and pH were incubated in refrigerated (10°C), air-conditioned (20°C), and room temperature (30°C). For agitation, cultures were placed in static or shaking incubators at 100 rpm and 150 rpm. The effect of incubation periods on the mycelial biomass production was also investigated. All treatments were replicated 3 times. The mycelial biomass yield was recorded. The best culture requirement and condition for each optimization parameter were based on the mycelial biomass yield of mushrooms.

2.3. Biomass Production and Extraction

Fifty culture bottles for each cup of mushrooms were prepared for the mass production of mycelia and EPS. The 15- or 20-day-old mycelia (depending on the mushroom) were harvested, air-dried, and extracted following the ethanol extraction protocol of Dulay *et al.* [23]. Culture spent was used to extract the EPS following the protocol of El-Mahdy *et al.* [24].

2.4. Mycochemical Screening

Mycochemical screening of ethanol extracts from 15- and 20-day-old mycelial cultures was performed following the protocol of Guevara [25]. Extracts were redissolved with ethanol and were then subjected to preparative silica gel thin-layer chromatography (TLC). Extracts were spotted onto the same 250 µm-silica plate. TLC was performed in a vertical glass chamber with ethyl acetate-chloroform

Figure 1: Fruiting body/ies of (a) *Sarcoscypha* spp., (b) *Phillipsia domingensis*, and (c) *Cookeina tricholoma* growing in their natural habitat.

(7:3) solvent system. The compounds were visualized under ultraviolet (UV) light and/or using specific spray reagents such as vanillin-sulfuric acid for phenols, sterols, fatty acids, triterpenes, and essential oils; methanolic KOH for anthraquinones, coumarins, and anthrones; potassium ferricyanide–ferric chloride for phenolics and tannins; Dragendorff's reagent for alkaloids; and antimony (III) chloride for flavonoids.

2.5. DPPH Radical Scavenging Assay

The method of Baliyan *et al.* [26] on the DPPH radical scavenging activity determination was followed with modifications. A stock solution was prepared by dissolving 24 mg of DPPH in 100 mL of ethanol. In each test tube, 3 mL of DPPH in 100 solutions was mixed with 100 mL of mushroom extract. A 3 mL solution containing DPPH in 100 mL of ethanol was used as a standard. The tubes were kept in total darkness for 30 min. The absorbance was then measured at 517 nm. Each extract was tested in triplicate. The percentage radical scavenging activity (%RSA) was calculated using the formula:

$$\% \text{ RSA} = ([Ac - As]/Ac) \times 100,$$

where Ac represents the absorbance of the control and As denotes the absorbance of the sample.

2.6. Alpha-Glucosidase Inhibition Assay

The antidiabetic activity of the mycelial extracts was evaluated using an α-glucosidase inhibition assay, which depends on the enzymatic hydrolysis of the substrate to release p-nitrophenol, correlated with the activity of the sample in comparison to the negative control. Stock substrate solution, p-nitrophenyl-α-D-glucopyranoside, with a concentration of 1.86 mM, and stock enzyme solution with a concentration of 120 mU/mL were prepared. Samples at 100 μg/mL final concentration were also prepared. The samples were homogenized in DMSO using a vortex mixer, followed by centrifugation. In separate wells of a 96-well microtiter plate, 190 μL of 50 mM sodium phosphate buffer solution (pH 6.8) containing NaCl (0.1 M) was mixed with 10 μL of the sample to make a sample concentration of 15 μg/mL. This was followed by the addition of 50 μL of the enzyme solution to the mixture, which was incubated for 10 min at 37°C. After which, 50 μL of the substrate was added, making a total volume of 300 μL in each well. Each test sample solution has two trials and two replicates ($n = 2$, $t = 2$). Absorbance of the liberated p-nitrophenol was measured every 30 sec for 30 min at 405 nm using UV VIS Spectrophotometer. The inhibitory activity of the samples and the positive control was determined based on the average slope of each replicate using the equation:

$$\% \text{ Inhibitory activity} = ([\text{Slope}_{\text{uninhibited}} - \text{Slope}_{\text{inhibited}}]/\text{Slope}_{\text{uninhibited}}) \times 100$$

2.7. AChE Inhibition Assay

For the AChE inhibition assay, a sample stock solution of 10,000 μg/mL and a working solution of 2,000 μg/mL were prepared. Samples were homogenized using a vortex mixer, sonicated, and centrifuged. In a 96-well plate, the reaction mixture consists of 180 μL of 100 mM phosphate buffer at pH 8.0, 10 μL of 1.0 U/mL Electrophorus electricus AChE enzyme solution, 80 μL of buffered Ellman's reagent (10.0 mM DTNB and 17.9 mM sodium bicarbonate ($NaHCO_3$) at pH 7.0), and 15 μL of each working solution sample/100 μg/mL positive control/negative control (methanol). Each test sample solution has two trials and two replicates ($n = 2$, $t = 2$). The sample, positive, and negative controls have a solvent well concentration of 5% methanol. After incubation for 15 min at 25°C, 15 μL of 2.87 mM acetylthiocholine iodide (AChI) was added, and the absorbance was monitored at 420 nm using Multiskan (Thermo Scientific) for 31 readings with 25-s intervals. The inhibitory activity of the sample and the positive control was determined based on the average slope of each replicate using the equation:

$$\% \text{ Inhibitory activity} = ([\text{Slope}_{\text{uninhibited}} - \text{Slope}_{\text{inhibited}}]/\text{Slope}_{\text{uninhibited}}) \times 100$$

2.8. Statistical Analysis

One-factor-at-a-time optimization study was laid out in a completely randomized design with three replicates per treatment. Data were analyzed using analysis of variance (ANOVA) in a one-way classification analysis and compared using Tukey's HSD at 5% significance level. A t-test was used to compare the two treatments in the evaluation of illumination.

3. RESULTS AND DISCUSSION

3.1. Optimal Submerged Biomass Production

In recognizing the promising nutraceutical and pharmacological potential of unexplored and underutilized mushrooms, it is essential to optimize their cultivation requirements, such as culture media, pH, temperature, illumination, agitation, and period of incubation, in order to achieve maximal biomass yield. SmF represents a viable approach for producing mushroom biomass in the form of mycelia. Although less commonly employed in mushroom cultivation, SmF offers a practical and innovative method for the production of mycelial biomass and bioactive metabolites of mushrooms.

The most important cultivation requirement of mushrooms is the culture media, which serve as the sole source of nutrients for their growth and development. Four culture media were evaluated in this study, and the mycelial biomass yields of the three cup fungi are presented in Table 1. RBB significantly produced the highest mycelial biomass yield for *Sarcoscypha* spp. and *P. domingensis*, whereas CW yielded the highest mycelial biomass of *C. tricholoma*. PSB and CGB showed lower mycelial biomass production of the three mushrooms. This indicates that mushrooms exhibit species-specific responses to different culture media. Similarly, the maximum mycelial biomass of *Lentinus sajor-caju* and *Lentinus tigrinus* was significantly achieved in a RBB [14]. However, CW produced the luxuriant mycelial growth of *P. cystidiosus* and *Coprinopsis cinerea* [27]. In addition, CW and RBB were identified as the most suitable culture media for mycelial biomass production of *V. volvacea* and *S. commune*, respectively [15].

The superiority of RBB and CW could be accounted for by their nutritional attributes. According to USDA FoodData Central [28], rice bran is rich in carbohydrates (49.7 g), dietary fiber (21 g), total sugars (0.9 g), sucrose (0.5 g), glucose (0.2 g), fructose (0.2 g), minerals such as potassium (1480 mg), magnesium (781 mg), calcium (57 mg), iron (18.5 mg), zinc (6.04 mg), manganese (14.2 mg), and copper, and vitamins. CW, on the other hand, contains carbohydrates (3.71 g), dietary fiber (1.1 g), total sugars (2.61 g), minerals such as potassium (250 mg), magnesium (25 mg), calcium (24 mg), iron (0.29 mg), zinc (0.1 mg), manganese (0.142 mg), and copper, and vitamins. These nutrient compositions play a critical role in the morphogenesis, growth, development, and physiology of fungal cells. Among sugars, fructose, sucrose, and starch have been identified as the preferred carbon sources for the mycelia of *Lentinus strigosus*, *L. tigrinus*, and *L. swartzii*, respectively [29]. Sugars serve not only as primary

Table 1: Mycelial biomass yields of *Sarcoscypha* spp., *P. domingensis*, and *C. tricholoma* as influenced by liquid culture media, pH, temperature, agitation, and incubation period.

Factors	Treatments	Mycelial biomass yield (mg d.w. 100/mL)		
		***Sarcoscypha* spp.**	***P. domingensis***	***C. tricholoma***
Culture media	PSB	28.0±10.03^{d}	144.8±48.98^{c}	146.5±13.30^{c}
	CGB	161.0±14.76^{c}	69.5±38.00^{d}	155.8±46.13^{c}
	RBB	326.3±29.34^{a}	342.3±34.94^{a}	205.5±39.69^{b}
	CW	206.8±59.12^{b}	222.3±21.64^{b}	312.0±37.16^{a}
pH	pH 4	160.3±24.21^{c}	177.3±9.57^{c}	142.0±25.07^{c}
	pH 5	235.0±30.50^{b}	242.3±29.50^{b}	242.8±11.67^{b}
	pH 6	368.0±20.99^{a}	316.0±42.78^{a}	367.8±20.14^{a}
	pH 7	155.3±20.17^{c}	104.3±12.01^{d}	136.0±23.76^{c}
	pH 8	38.3±4.27^{d}	25.7±5.85^{e}	120.8±26.70^{c}
Temperature	10°C	0.0±0.00^{c}	0.0±0.00^{c}	0.0±0.0^{c}
	20°C	434.5±29.65^{a}	408.5±28.90^{a}	242.3±36.06^{b}
	30°C	224.0±62.67^{b}	170.5±22.16^{b}	416.8±52.58^{a}
Agitation	Static	522.0±36.94^{c}	600.8±8.50^{c}	243.8±38.10^{c}
	100 rpm	844.8±54.66^{b}	796.0±59.89^{b}	639.5±57.75^{b}
	150 rpm	916.5±49.82^{a}	996.3±13.48^{a}	950.0±50.60^{a}
Incubation period	10 days	916.5±49.8^{d}	996.3±13.5^{c}	960.0±50.6^{d}
	15 days	1249.0±45.7^{c}	1228.0±19.2^{b}	1314.0±53.1^{a}
	20 days	1585.0±20.6^{a}	1293.0±36.4^{a}	1179.0±62.4^{b}
	25 days	1473.0±61.7^{b}	1225.0±68.8^{b}	1096.1±26.6^{c}

Values are expressed as mean±SD. In each column and factor, means with different letters of superscript indicate a significant difference ($P<0.05$). *P. domingensis: Phillipsia domingensis, C. tricholoma: Cookeina tricholoma,* PSB: Potato sucrose broth, CGB: Corn grit broth, RBB: Rice bran broth, CW: Coconut water

energy sources but also as precursors for the synthesis of structural biomolecules, being converted into polysaccharides, lipids, and the carbon skeletons of amino acids in vegetative mycelium [30,31].

Potassium is the most abundant mineral in the composition of rice bran and CW. Potassium regulates osmotic balance, maintains cellular hydration, and activates enzymes involved in protein and carbohydrate metabolism, thereby promoting vigorous mycelial expansion [32,33]. Magnesium acts as an enzymatic cofactor and supports polysaccharide biosynthesis, whereas calcium contributes to pH stabilization, enhances extracellular enzyme activities, and stimulates polysaccharide metabolism in species such as *Pleurotus* and *Flammulina* [34,35]. Trace elements, including copper, zinc, iron, and manganese, though required in smaller amounts, are also critical as cofactors in metabolic pathways; for example, copper induces laccase activity in *Pleurotus ostreatus*, facilitating lignin degradation and substrate colonization [34]. Vitamins are also important components of the two media. Supplementation of vitamins in culture media has been shown to enhance mycelial growth, enzymatic activity, and overall metabolic efficiency in species such as *G. lucidum* and *Pleurotus ostreatus* [36,37].

Mushroom species have different pH requirements; thus, the appropriate pH level of culture medium should be considered for the optimal growth and production of their mycelia [38]. pH regulates the uptake of sodium ions and essential molecules from the culture medium into individual cells, directly influencing their nutrient absorption capacity, upon which mycelial growth performance depends [39]. In the present study, the mycelial biomass yield of cup mushrooms cultivated in their best medium at varying pH levels was evaluated. The three mushrooms produced the highest biomass yield in the medium with an initial pH of 6.0 [Table 1]. In contrast, the medium at pH 4.0 demonstrated the lowest yield of mycelia. When pH is too acidic or too basic, mushroom mycelia experience physical stress that disrupts cell wall integrity, membrane stability, and hyphal turgor, resulting in reduced hyphal extension, abnormal branching, or growth inhibition. The findings of this study are in agreement with those of Fabros *et al.* [40], who reported that *Lentinus* species can tolerate a broad pH range from 4.5 to 8.0, but exhibit optimal mycelial growth in slightly acidic conditions, particularly at pH 5.0 to 6.0. In addition, the results also align with those of Kalaw *et al.* [41], who observed that pH levels between 5.0 and 8.0 are generally favorable for the growth of *Lentinus* species, with *L. strigosus* demonstrating a distinct preference for pH values around 6.0 to 7.5. Moreover, *Cordyceps militaris* favors pH 6 for maximum biomass production, which suggests the species-specific nature of pH preference in fungal cultivation [42].

In the evaluation of temperature, the most suitable medium with the best pH was used. As shown in Table 1, the highest biomass yield was achieved at 20°C for both *Sarcoscypha* spp. and *P. domingensis*, and 30°C for *C. tricholoma*. Contrastingly, no mycelial growth was observed at 10°C. These results suggest that temperature requirements are species-specific, reflecting the conditions that best support their metabolic and developmental processes. The temperature preference of the first two species is comparable to *Lentinula edodes*, which grows best at 23°C but exhibits reduced growth under refrigeration (4°C) [43]. Meanwhile, *C. tricholoma* aligns more closely with *Lentinus* species, which are generally classified as tropical mushrooms [38]. Similarly, Aguilar *et al.* [44] reported comparable temperature preferences for *Cyathus striatus* and *Xylaria hongkongensis*. The absence of growth at the lowest temperature tested in this study could be attributed to reduced

enzyme activity and impaired metabolic processes at extremely low temperatures, which cause hyphal deformation, reduced extension, cellular shrinkage, or mycelial death [45].

Agitation is another physical factor that significantly impacts the overall yield of mushrooms in SmF. The effect of agitation was evaluated by incubating the culture bottles in agitated (100 and 150 rpm) and static conditions. Agitation at 150 rpm yielded significantly the highest mycelial biomass yield, followed by those at 100 rpm [Table 1]. The results of the present study showed that agitation is a crucial factor that improves the growth of the mycelia of the three cup fungi. The observed increase in biomass production under 150 rpm can be attributed to improved oxygen transfer and uniform nutrient distribution, which promote more efficient mycelial growth [46]. Similar favorable responses to agitation have been reported in *G. lucidum*, *Polyporus tricholoma*, and *L. strigosus* [47,48]. In contrast, certain mushroom species, including *P. cystidiosus, V. volvacea*, and *S. commune*, exhibit better growth under static conditions [13]. However, high agitation physically affects mushroom mycelia by imposing mechanical stress that can fragment hyphae, alter branching patterns, disrupt mycelial networks, and reduce pellet or mat formation, depending on agitation intensity.

After establishing the optimal nutritional and physical conditions for biomass production, the effect of varying periods of incubation was evaluated [Table 1]. It can be seen that the maximum biomass yield was achieved after 20 days of fermentation for both *Sarcoscypha* spp. and *P. domingensis*, and after 10 days of fermentation for *C. tricholoma*. These results indicate that extending the fermentation for a certain period can be beneficial for maximizing fungal biomass. This progressive increase in biomass can be attributed to the continuous accumulation of fungal cells as the mycelia efficiently utilize available nutrients for growth. Similarly, *Auricularia polytricha* exhibited enhanced cell mass when fermentation was prolonged to 20 days [49]. In addition, Devi *et al.* [50] reported that the optimum incubation period for mycelial biomass production of three *Auricularia* species (*A. delicata, A. polytricha,* and *A. auricula*) was 20–25 days in SmF; however, a longer incubation period of 30–40 days resulted in a decrease in the biomass yield. The mycelial biomass of the three mushrooms was mass-produced using the optimal submerged culture conditions. The EPS of each mushroom was also extracted from the culture spent.

3.2. Biochemical Composition of Cup Mushrooms

Mushrooms are well-recognized producers of secondary metabolites that contribute to their diverse biological and functional activities. TLC analysis revealed the presence of essential oils, coumarins, and anthrones in all three mushroom species analyzed [Table 2]. Both *P. domingensis* and *C. tricholoma* contained triterpenes and sterols, whereas alkaloids were detected only in *Sarcoscypha* spp. and *P. domingensis*. Steroids were detected in *Sarcoscypha* spp. and *C. tricholoma* but were absent in *P. domingensis*. Interestingly, *P. domingensis* was the only species that tested positive for phenols, tannins, and flavonoids. Anthraquinones were not detected in any of the samples. Collectively, nine distinct mycochemicals were detected in *P. domingensis*, whereas *Sarcoscypha* spp. and *C. tricholoma* contained five and six, respectively. Bioactive compounds derived from the mycelia and fruiting bodies of mushrooms, including coumarins, anthrones, triterpenes, sterols, steroids, alkaloids, phenols, tannins, and flavonoids, contribute to their wide-ranging health benefits [18,10]. Coumarins are a natural class of occurring compounds with benzopyrone structure found in fungi, plants, and microorganisms that exhibit

Table 2: Biochemical constituents of the ethanolic extract of mycelia of *S. minuta*, *P. domingensis*, and *C. tricholoma.*

Mycochemical	*S. minuta*	*P. domingensis*	*C. tricholoma*
Alkaloids	Present	Present	Not detected
Anthraquinones	Not detected	Not detected	Not detected
Anthrones	Present	Present	Present
Coumarins	Present	Present	Present
Essential oil	Present	Present	Present
Flavonoids	Not detected	Present	Not detected
Phenols	Not detected	Present	Not detected
Steroids	Present	Not detected	Present
Sterols	Not detected	Present	Present
Tannins	Not detected	Present	Not detected
Triterpenes	Not detected	Present	Present

S. minuta: Sarcoscypha minuta, P. domingensis: Phillipsia domingensis, C. tricholoma: Cookeina tricholoma

different bioactivities such as antioxidants, anti-inflammatory, antimicrobial, and antiviral properties [51]. Terpenes derived from Basidiomycetes are referred to as terpenoids or isoprenoids, and they have been associated with various biological activities such as antioxidant, anticancer, and anti-inflammatory effects [52]. Phenolic compounds, though commonly found in plants, are also abundant in mushrooms [52], and their presence is closely associated with the antioxidant activity of these fungi. *Polyporus pinicola* and *P. volvatus* have been found to contain significant phenolic content and hence display antioxidant capabilities [53]. Compounds from mushrooms, such as alkaloids, terpenoids, flavonoids, polysaccharides, tannins, steroids, glycoproteins, phenols, ergosterols, sesquiterpenes, and lactones [54] have been associated with many pharmacological properties, including antioxidant, antibacterial, anticancer, anti-inflammatory, and antidiabetic effects [55,56]. Moreover, polysaccharides, phenols, tocopherols, flavonoids, glycosides, and organic acids have often been described as contributors to antioxidant properties [57]. The presence of key metabolites underscores the potential therapeutic significance of cup mushrooms. Given the well-documented pharmacological properties of these compounds, the findings support the growing evidence that mushrooms serve as promising sources of natural agents with antioxidant, antimicrobial, anti-inflammatory, and other health-promoting activities. This emphasizes the importance of further biochemical and pharmacological investigations to explore their functional and medicinal applications fully.

3.3. Antioxidant Activity of Cup Mushrooms

The antioxidant activities of mycelial extracts and EPS of the three cup mushrooms were evaluated using the DPPH radical scavenging assay. Table 3 presents the percentage of DPPH radical scavenging activities (RSA) of the tested samples. The mycelial extracts and EPS exhibited RSA values ranging from 17.17% to 57.42% and 40.96% to 53.20%, respectively. Among these, the mycelial extract of *Sarcoscypha* spp. showed higher RSA than its EPS, whereas the EPS of *P. domingensis* and *C. tricholoma* demonstrated greater activity than their respective mycelial extracts. Although all RSA values were lower than that of ascorbic acid, the results still indicate promising antioxidant potential, particularly for *Sarcoscypha* spp. mycelial extract and *P. domingensis* EPS, both exceeding 50% RSA. Notably, the maximum RSA values observed in this study were higher than

those reported for the acetonitrile extracts of *L. tigrinus* (39.2%) and *P. djamour* (32.8%) [58]. Similar findings have been documented in other mushroom species. For instance, Acharya *et al.* [59] reported that the methanolic extract of *O. canarii* basidiocarp exhibited strong antioxidant potential (EC_{50} = 0.912 μg), primarily attributed to phenols, flavonoids, ascorbic acid, β-carotene, and lycopene. Likewise, among five *Pleurotus* species, *P. flabellatus* 5013 showed the highest antioxidant activity, with its methanolic extract exhibiting 24.9 mg TE/g in the DPPH assay and 63.9 mg TE/g in the ORAC assay, a property attributed in part to ergothioneine (ERG), a water-soluble thiol derivative of histidine with well-documented antioxidant effects *in vitro* and *in vivo* [60,61]. Furthermore, the methanolic extract of *Dictyophora indusiata* at 6.4 mg/mL exhibited 92.1% DPPH radical scavenging activity [62]. Collectively, the present results, together with previous studies, underscore that medicinal and edible mushrooms are valuable sources of natural antioxidants. During oxidative stress, excessive free radicals can damage cellular components such as DNA, RNA, and proteins, leading to apoptosis and diseases including cancer, diabetes, cardiovascular disorders, and age-related conditions [63]. Consequently, antioxidant interventions that neutralize free radicals hold significant potential in mitigating these health risks.

3.4. Alpha-glucosidase Inhibitory Activity of Cup Mushrooms

Several mushroom species have been reported to alleviate diabetes-related symptoms, including *G. lucidum*, *Cordyceps sinensis*, *Agaricus subrufescens*, *Coprinus comatus*, *Agaricus bisporus*, *Phellinus linteus*, *Poria cocos*, *Inonotus obliquus*, *Pleurotus* species, and *Sparassis crispa* [64]. In the present study, the α-glucosidase inhibitory activities of the mycelial extracts and EPSs of the three cup mushrooms were evaluated to assess their antidiabetic potential [Table 4]. Among the tested samples, the mycelial extract of *Sarcoscypha* spp. exhibited the highest inhibitory activity against α-glucosidase, whereas the other samples demonstrated none to very low inhibition. The limited α-glucosidase inhibitory activities observed in the mycelial extracts and EPSs of the three mushrooms could be attributed to the concentration of the samples tested and the extraction solvent used. The concentration employed in this study may not have been sufficient to elicit strong enzyme inhibition. Furthermore, the bioactive compounds responsible for α-glucosidase inhibition may not have been efficiently extracted using ethanol. Supporting this, Deveci *et al.* [65] reported that hexane extracts of *P. ostreatus*, *M. procera*, *P. schweinitzii*, *L. gentianeus*, and *P. pini* exhibited higher α-glucosidase inhibitory activities than their corresponding methanol extracts, whereas the methanol extracts of *T. pubescens* and *G. adspersum* showed greater activity than their hexane counterparts. Moreover, Ji *et al.* [66] revealed that the α-glucosidase inhibitory activity of mushroom polysaccharides depends on their monosaccharide composition, molecular weight, higher-order structure, and the type and position of glycosidic bonds. To establish more conclusive findings, further investigations should evaluate varying extract concentrations, different extraction solvents and methods, and the structural characteristics of the bioactive compounds involved.

Table 3: DPPH radical scavenging activity of mycelial extract and EPS of the three mushrooms.

Mushroom isolate	DPPH radical scavenging (%)	
	Mycelial extract	EPS
S. minuta	57.42±6.78[b]	40.96±5.56[d]
P. domingensis	30.30±6.48[c]	53.20±6.01[b]
C. tricholoma	17.17±9.17[d]	47.96±9.94[c]
Ascorbic acid	90.71±0.79[a]	

Values are expressed as mean±SD. Means with different letters of superscript indicate significant difference ($p<0.05$). Mycelial extract (1000 μg/mL) and EPS (1000 μg/mL) of each mushroom and ascorbic acid (1000 μg/mL) were compared using Tukey's HSD. *S. minuta: Sarcoscypha* spp., *P. domingensis: Phillipsia domingensis, C. tricholoma: Cookeina tricholoma*, EPS: Exopolysaccharide, DPPH: 2,2-diphenyl-1-picrylhydrazyl

3.5. AChE Inhibitory Activity of Cup Mushrooms

AChE inhibition is a key therapeutic strategy in neuroprotection, particularly in the management of neurodegenerative disorders such as Alzheimer's and Parkinson's diseases [67]. AChE is responsible for hydrolyzing acetylcholine, a neurotransmitter essential for learning, memory, and cognitive processing. Overactivity of this enzyme leads to decreased acetylcholine levels, contributing to impaired synaptic transmission and cognitive decline. Thus, compounds that inhibit AChE are of significant interest for their potential to preserve neurotransmitter function and mitigate the progression of neurodegeneration. In this study, the AChE inhibitory activities of the mycelial extracts and EPS of three cup mushroom isolates were assessed [Table 5]. The mycelial extracts of *Sarcoscypha* spp. and *P. domingensis* exhibited higher inhibitory activity than their corresponding EPS, suggesting that AChE-active metabolites in these species are more abundant or more potent in the mycelial matrix. In contrast, the EPS of *C. tricholoma* demonstrated greater AChE inhibition than its mycelial extract, indicating that its bioactive components may be concentrated in the EPS. Among the three species, *P. domingensis* showed consistently low inhibitory effects in both extract types. Although the mushroom isolates exhibited low to mild AChE inhibition compared to the positive control, the activity observed in *Sarcoscypha* spp. and *C. tricholoma* highlights their

Table 4: α-glucosidase inhibitory activity of mycelial extract and EPS of the three mushrooms.

Mushroom isolate	α-glucosidase inhibition (%)	
	Mycelial extract	EPS
Sarcoscypha spp.	23.03±2.77[b]	2.36±0.93[b]
P. domingensis	0.00±0.00[c]	0.16±1.77[c]
C. tricholoma	2.70±1.78[c]	0.90±0.88[c]
Acarbose	91.92±0.53[a]	

Values are expressed as mean±SD. Means with different letters of superscript indicate significant difference ($P<0.05$). Mycelial extract (10 μg/mL) and EPS (10 μg/mL) of each mushroom and acarbose (1000 μg/mL) were compared using Tukey's HSD. *P. domingensis: Phillipsia domingensis, C. tricholoma: Cookeina tricholoma*, EPS: Exopolysaccharide

Table 5: Acetylcholinesterase inhibitory activity of mycelial extract and EPS of the three mushrooms.

Mushroom isolate	Acetylcholinesterase inhibition (%)	
	Mycelial extract	EPS
Sarcoscypha spp.	12.74±3.99[b]	8.35±1.42[c]
P. domingensis	6.38±1.50[b]	4.51±1.24[c]
C. tricholoma	5.91±0.83[c]	10.98±2.06[b]
Galantamine	98.29±0.12[a]	

Values are expressed as mean±SD. Means with different letters of superscript indicate significant difference ($P<0.05$). Mycelial extract (10 μg/mL) and EPS (10 μg/mL) of each mushroom and galantamine (10 μg/mL) were compared using Tukey's HSD. *P. domingensis: Phillipsia domingensis, C. tricholoma: Cookeina tricholoma*, EPS: Exopolysaccharide

potential as sources of neuroprotective compounds. These findings support further purification, structural characterization, and in vivo evaluation to explore their possible applications in preventing or managing neurodegenerative diseases.

4. CONCLUSION

This study highlights the biotechnological potential of cup mushrooms as valuable sources of functionally important bioactive compounds, underscoring their relevance in the development of natural antioxidants, antidiabetics, and neuroprotective agents. By establishing the optimal cultural conditions for efficient biomass and metabolite production, the findings provide a practical foundation for sustainable cultivation and future utilization of these species in health-promoting products. The demonstrated biological activities suggest that these mushrooms can serve as promising candidates for nutraceutical and pharmaceutical research, particularly for addressing lifestyle and neurodegenerative diseases that continue to pose major public health challenges. Moving forward, it is recommended that large-scale fermentation and controlled bioprocessing be pursued to increase yield and consistency, followed by the targeted isolation and characterization of the active compounds to identify those responsible for the observed therapeutic effects. The specific type and molecular size of EPS responsible for enzyme inhibition activities should also be thoroughly characterized. In addition, further mechanistic, toxicological, and in vivo studies are essential to validate stability, efficacy, and safety, which will help support the eventual formulation of functional food supplements or drug leads derived from these cup fungi.

5. AUTHORS' CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agree to submit to the current journal; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work. All the authors are eligible to be author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

6. FUNDING

There is no funding to report.

7. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

8. ETHICAL APPROVALS

The study protocol was approved by the Institutional Ethics Review Committee of College of Science, Central Luzon State University, Science City of Muñoz, Philippines (Approval No.: ERC 2025-1131).

9. DATA AVAILABILITY

The data that was used in this study will be given to the corresponding author upon a fair request.

10. PUBLISHER'S NOTE

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11. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declare that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

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How to cite this article:
Alfonso ED, Dulay RMR, Kalaw SP. Mycelium and exopolysaccharide production and bioactivities of select cup mushrooms (Sarcoscyphaceae, Pezizomycetes) from Central Luzon, Philippines. J Appl Biol Biotech 2026;14(3):66-74. DOI: 10.7324/JABB.2026.658059