Antifungal Activity of Medicinal Mushrooms and Optimization of Submerged Culture Conditions for Schizophyllum commune (Agaricomycetes)

V. Berikashvili,[a] T. Khardziani,[a] Aza Kobakhidze,[a] M. Kulp,[b] M. Kuhtinskaja,[b] T. Lukk,[b] M.L. Gargano,[c] G. Venturella,[d] E. Kachlishvili,[a] E. Metreveli,[a] V. Elisashvili,[a] & M. Asatiani[a,]

[a]The Institute of Microbial Biotechnology, Agricultural University of Georgia, 0131 Tbilisi, Georgia; [b]Department of Chemistry and Biotechnology, Tallinn University of Technology, 12618 Tallinn, Estonia; [c]Department of Soil, Plant, and Food Sciences, University of Bari, 70126 Bari, Italy; [d]Department of Agricultural, Food and Forest Science, University of Palermo, 90128 Palermo, Italy

ABSTRACT: The main goal of the present study was the exploration of the antifungal properties of Agaricomycetes mushrooms. Among twenty-three tested mushrooms against *A. niger*, *B. cinerea*, *F. oxysporum*, and *G. bidwellii*, *Schizophyllum commune* demonstrated highest inhibition rates and showed 35.7%, 6.5%, 50.4%, and 66.0% of growth inhibition, respectively. To reveal culture conditions enhancing the antifungal potential of *Sch. commune*, several car- bon (lignocellulosic substrates among them) and nitrogen sources and their optimal concentrations were investigated. Presence of 6% mandarin juice production waste (MJPW) and 6% of peptone in nutrient medium promoted antifungal activity of selected mushroom. It was determined that, extracts obtained in the presence of MJPW effectively inhibited the grow of pathogenic fungi. Moreover, the content of phenolic compounds in the extracts obtained from *Sch. commune* grown on MJPW was several times higher (0.87 ± 0.05 GAE/g to 2.38 ± 0.08 GAE/g) than the extracts obtained from the mushroom grown on the synthetic (glycerol contained) nutrient medium (0.21 ± 0.03 GAE/g to 0.88 ± 0.05 GAE/g). Flavonoid contents in the extracts from *Sch. commune* varied from 0.58 ± 0.03 to 27.2 ± 0.8 mg QE/g. Identification of phenolic compounds composition in water and ethanol extracts were provided by mass spectrometry analysis. Extracts demonstrate considerable free radical scavenging activities and the IC50 values were generally low for the extracts, rang- ing from 1.9 mg/ml to 6.7 mg/ml. All the samples displayed a positive correlation between their concentration (0.05–15.0 mg/ml) and DPPH radical scavenging activity. This investigation revealed that *Sch. commune* mushroom has great po- tential to be used as a source of antifungal and antioxidant substances.

KEY WORDS: medicinal mushrooms, *Schizophyllum commune*, antifungal properties, sub-merged mycelium, mushroom extracts, radical scavenging, flavonoids

I. INTRODUCTION

Medicinal mushrooms (MMs) mainly belong to the Agaricomycetes. Many species of them are edible with great nutritional significance, due to their unique flavor, and great organoleptic properties. Never- theless, there are nonedible (based on their texture and consistency) species, however very valuable from medicinal point of view and all of them can synthesize and accumulate variety of pharmacologically active molecules and biologically active compounds extremely beneficial for humans. Although almost all mushroom species are abundantly rich in bioactive compounds, they remain mostly an unexplored and untapped source of valuable natural compounds. Fruiting bodies, submerged mycelia and culture broth of MMs provide wide range of primary and secondary metabolites including proteins, amino ac- ids, polysaccharides (mainly glucans), vitamins, dietary fiber, micro-macro elements, sterols, terpenes,

phenolic compounds and many others.[1–5] Due to the fact that mushrooms have the ability to synthesize medicinally important unique bioactive substances, they comprise a wide range of biological activities such as antioxidant, free radical scavenging, anticancer, immunomodulation, antidiabetic, antibacterial, antifungal and many other effects.[2,3,6–11] Therefore, in recent years, much attention has been paid to the use of pharmaceutically significant biologically active substances obtained from MMs. It should be noted that most studies have been conducted on the fruiting bodies of wild or commercially cultivated mush- rooms, while numerous studies have identified the greatest pharmaceutical potential of submerged cul- tivated mushroom biomass or culture liquid.[1] Moreover, it should be considered that it is very difficult (almost impossible) to maintain the quantitative stability of bioactive compounds in mushrooms under solid-state cultivation. Besides this, the cultivation of mushrooms is longterm process. Therefore, for the accumulation of bioactive compounds for pharmaceutical/biotechnological purposes and for phys- iological regulation of bioactive compounds synthesis the best alternative is submerged cultivation of mushrooms under controlled conditions.

Among many positive medicinal properties and biological activities, one of the noteworthy is the antifungal potential of MMs. Several fungal genera, such as *Aspergillus*, *Botrytis*, *Cryptococcus*, *Candida*, *Fusarium*, and *Guignardia* are known particularly significant as pathogens that can infect both plants and humans. These pathogens may cause human diseases such as allergic bronchopulmonary aspergillosis (by *Aspergillus* spp.); damage of central nervous system (by *Criptococcus* spp.); skin, oral and vaginal infections (by *Candida* spp.); plant diseases such as rusts, blasts, smuts, blotches, blights, and mildews (by *Aspergillus* spp., *Botrytis* spp., *Fusarium* spp., and *Guignardia* spp.).[12–17] It is known that various phytopathogenic fungi synthesize secondary metabolites that are harmful to humans and can successfully cause various diseases in the body.[18] In addition, given the circumstance that harmful microorganisms can damage wide spectrum of industrial plants and products, they (especially their spores) can often cause diseases such as diarrhea, vomiting, headaches, dizziness, and chills. Although there are various antifungal treatments available today, the severity of infections caused by fungal pathogens is associated with a concerted interplay between antifungal drug resistance, virulence and immune system evasion features.[19] Besides this, in the past few decades, synthetic fungicides played a fundamental function in suppressing plant diseases and enhancing crop yield to reduce food insecurity in today's world.[20] However, recent studies reported that the prolonged use of chemicals could cause harmful effects, including environmental and health hazards, residual toxicity and resistance of patho- genic fungi to the broad class of chemicals.[20–23] It should be noted that although there are number of synthetic antifungal drugs and fungicides, a universal strategy/remedy does not appear to exist which ensures inhibition of pathogenic fungi without causing harm to humans. In addition, in recent years it has been confirmed that chemical fungicides have a negative/toxic effect on the human health.[24–26] Therefore, obtaining bioactive compounds from natural sources and their further use is a significant and perspective challenge.

Although higher Basidiomycetes MMs are one of the richest sources of bioactive compounds and their antifungal properties have been actively studied in the last few decades, the data are still limited and there are many gaps in the peculiarities of the physiological regulation mechanisms of antifungal bioactive com- pounds synthesis. Most mushroom species used in the present research (except *Lentinus* spp.) are isolated in different ecological niches of Georgia and belonging to different taxonomic groups. The antifungal ac- tivity of all tested submerged cultivated higher Basidiomycetes MMs was tested for the first time. During the study of physiological factors, beside to different carbon sources, the effect of different lignocellulosic substrates on antifungal activity was evaluated. In addition, the free radical scavenging properties of the obtained extracts and the content of bioactive compounds were investigated. Therefore, enhanced under- standing of capabilities of MMs could be of significant to reveal new compounds that possess antioxidant and antimicrobial properties.

II. MATERIALS AND METHODS

A. Microorganisms and Inoculum Preparation

Twenty-three Agarocimycetes mushroom species belonging to 14 genera (*Armillaria*, *Coprinus*, *Daedalea*, *Daedaleopsis*, *Fomitipsis*, *Flammulina*, *Fomes*, *Ganoderma*, *Hericium*, *Lenzites*, *Lentinus*, *Schizophyllum*, *Stereum*, *Trametes*) were evaluated for their antifungal potential. Pure cultures of tested mushrooms were obtained from the culture collection of the Institute of Microbial Biotechnology, agricultural University of Georgia. Four phytopathogenic fungi, *Aspergillus niger*, *Botrytis cinerea*, *Fusarium oxysporum*, and *Guignardia bidwellii* were used for evaluation of higher Basidiomycetes mushrooms antifungal potential. Pathogenic fungal cultures were obtained from CBS-KNAW Collection (Netherlands). All mushroom cultures and pathogenic fungi were maintained on the malt extract agar slants contained tubes and Petri dishes and storage at 5°C.

Mushroom inoculums were grown in 250 mL Erlenmeyer flasks (contained 100 mL nutrient medium), on a rotary shaker (New Brunswick Scientific, USA) at 150 rpm and 27°C for 7–10 d (depended on mushroom species) in synthetic medium containing (g/L of distilled water): KH_2PO_4, 0.8; K_2HPO_4, 0.6; $MgSO_4 \cdot 7H_2O$, 0.5; yeast extract, 3.0, glucose, 15.0; peptone, 3.0. Initial pH was adjusted to 6.0 prior to sterilization. The final fungal biomass was homogenized in a Waring laboratory blender (Waring Commercial, Torrington, CT, USA) two times for 20 seconds and used as an inoculum (10%) for shake-flask cultures.

B. Carbon and Nitrogen Sources and Shake-Flask Cultivation Conditions

For screening of mushrooms for their antifungal activity it was provided submerged cultivation on synthetic nutrient medium (g/L of distilled water): KH_2PO_4, 0.8; K_2HPO_4, 0.6; $MgSO_4 \cdot 7H_2O$, 0.5; Peptone, 3.0; yeast extract, 3.0; glucose, 10; pH 6.0, at 150 rpm and 27°C. To study the effect of lignocellulosic materials on antifungal activity 40 g/L of mandarin juice production waste (MJPW), wheat straw (WS), banana peels (BP), corn cobs (CC), ethanol production waste (EPR), and wheat bran (WB) were used. All plant residues were collected from the local facilities (Georgia), oven-dried at 50°C and ground to the powder in a laboratory mill KM-1500 (MRC, Israel) prior to addition to the nutrient medium. Besides this, glycerol, maltose, glucose, xylose and mannitol were tested as carbon sources in concentration 10 g/L. To evaluate the effect of nitrogen sources ($(NH_4)_2SO_4$, NH_4NO_3, peptone, casein hydrolysate, and soy protein were used in the concentration 3%. All above mentioned carbon and nitrogen sources were supplemented to the synthetic medium (g/L: KH_2PO_4, 0.8; K_2HPO_4, 0.6; $MgSO_4 \cdot 7H_2O$, 1.5; yeast extract, 3.0) as growth substrates. The pH of all media was adjusted to 6.0 prior to inoculation. Submerged cultivations were carried out for 8–12 d. At predetermined time intervals, the contents of each flask were filtered, and solids were separated by centrifugation (Eppendorf 5417R, Hamburg, Germany) at 10,000× g for 5 min at 4°C. The supernatants were analyzed for pH and pasteurized for 1 hour at 70°C. The sterile cultural liquids (10 mL) were used for analysis. For evaluation of the antifungal properties on Petri dishes the following agar medium was used (g/L of distilled water): KH_2PO_4, 0.8; K_2HPO_4, 1.6; $MgSO_4 \cdot 7H_2O$, 0.5; peptone, 3.0; yeast extract, 3.0; malt extract, 3.0, glucose, 15; agar–agar, 15; pH–6.0. All nutrient mediums sterilized at 121°C, for 35 min. For obtaining of mushroom mycelial biomass and culture liquid for preparation of extracts, cultivation procedure was conducted in 2 L Erlenmeyer flasks contained 1 L of optimized nutrient medium in presence of optimal concentrations of lignocellulosic/carbon and nitrogen sources, and incubation was provided on 130 rpm, at 27°C for 8 d on a rotary shaker (refrigerated shaker laboratory incubator, MRC, Israel).

C. Preparation of Extracts

After 8 d of mushroom submerged cultivation, mycelial biomasses were harvested, filtered, centrifuged at 6000 rpm for 15 min at 4°C and dried at 45°C to a constant weight and milled to fine powder in a laboratory

mill KM-1500 (MRC, Israel). The dried and milled biomasses were used for the extraction of biologically active compounds with distilled water and organic solvents with different polarities.

- Hot water extracts: hot water extractions of mycelial biomasses were performed for 3 h with dH_2O (1 g/10 mL ratio) at 80°C (using a water bath).
- Ethanol, and ethyl acetate extracts: mycelial biomasses were extracted on a rotary shaker at 150 rpm with ethanol (80%) or ethyl acetate (1 g/10 mL ratio) at 27°C for 3 h.

After extraction, insoluble compounds were separated by centrifugation (Eppendorf 5417R, Hamburg, Germany) at 6000 rpm at 4°C for 15 min and filtrated through the Whatman® filter paper N 1.

- Extraction from culture liquid: after biomass separation, pH of the culture liquid was decreased to 2.5 using hydrochloric acid. One liter of culture liquid was extracted 3 times with 500 mL of ethyl acetate, and the extract-solvent mixture was washed once with 500 mL distilled water using a glass chemical separator.

All extracts were evaporated using vacuum evaporator till a resin (or powder) will be formed, which represented the actual crude extract. All crude extracts were collected in previously weighed tubes and kept at –20°C. Stock solutions were prepared in 99.9% dimethyl sulfoxide (DMSO) (Sigma-Aldrich, USA).

D. Antifungal Activity

The antifungal activity of tested samples (mushroom culture liquids and crude extracts) were determined by the growth rate method.[27] The tested mushrooms' culture liquid (10 mL) or extracts (up to final concentra- tions 2.5 mg/mL, 5.0 mg/mL, 10.0 mg/mL) were added to each Petri dish, followed by the required quantity (in mL) of malt extract agar to adjust the total volume to 20 mL, and mixed carefully. To ensure sterility, the culture liquids were thermally treated at 70°C for 1 hour in a water bath, and all extracts that were diluted in DMSO were filtered using 0.2 µm membrane filter before use. The malt extract agar medium with no extract or culture liquid was used as the blank control. All the treatment and control groups were inoculated in triplicate with a mycelium plug (in diameter 5 mm) of each fungal pathogenic strain in the center of the plate. As a positive control 5 mM propamocarb hydrochloride (fungicide) was used. Inoculated plates were incubated at 27°C for a period until blank control plates completely covered the plate. Measurement of fun- gal colony diameter of each plate was started on the second day after inoculation. Antifungal activity was evaluated as the percentage of growth inhibition of mycelium according to the equation:

$$\%\ \text{of inhibition} = 100 - [GS_{(\text{treated})} \times 100]/GC_{(\text{control})},$$

where GS and GC are the mycelium growth rate (mm) of treated agar plates (samples) and controls. All results were expressed as the mean ± SD.

E. Determination of Total Phenols and Flavonoid Content

Total phenols of extracts were determined according to the Folin-Ciocalteu's assay.[28] Each extract (500 mg) was dissolved in DMSO (6 mL) using an ultrasonic bath with 40 kHz for 15 min, and then the volume was adjusted to 10 mL by ethanol. Folin-Ciocalteu's reagent (0.4 mL) was added to each sample (0.2 mL), and stood for 1 min. Then, 6 mL of 5 g/100 mL sodium carbonate solution was added to the mixture and held for 1 h in dark place for stabilization of the blue color. The absorbance was measured spectrophotometrically at

760 nm. Gallic acid used as a standard. The content of total phenols was calculated based on the calibration curve of gallic acid. Results were expressed as milligrams of gallic acid equivalents (GAE) per gram of mushroom extract. All determinations were carried out in triplicate.

The quantification of total flavonoid content was conducted following the methodology with minor changes.[29] One milliliter of the mushroom extract, diluted in DMSO, was mixed with 4 mL of distilled water, and then 0.3 mL of 5% sodium nitrite was added. After 5 min, 0.3 mL of 10% aluminum chloride was added to the mixture. The reaction mixture was incubated at room temperature for 15 min. Absorbance was measured at 415 nm. The standard curve of quercetin was used to calculate the total flavonoid content in the samples. The total flavonoid contents were calculated using a calibration curve of quercetin (QE) equivalents and results were expressed as mg QE equivalents per gram of mushroom extract.

F. Scavenging Activity on 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) Radicals

Free radical scavenging activity of the mushroom extract was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay.[30] DPPH solution was prepared by dissolving 5.9 mg of DPPH in 100 mL of ethanol. 1.9 mL of freshly prepared DPPH ethanolic solution was added to 0.1 mL of mushroom extract with different concentrations (0.05–15.0 mg/mL). The mixture was vortexed vigorously for 10 seconds and left to stand at room temperature in the dark for 30 min. Absorbance was measured against the blank at 517 nm. BHA, TBHQ, α-tocopherol, and ascorbic acid were used as a positive control (standard). Triplicate analyses were carried out for all determinations. The radical scavenging activity (RSA) was calculated according to the equation and presented as

$$\text{blanc}\quad \%\ \text{of inhibition:}\%\ \text{of inhibition} = [(A \quad - A_{\text{sample}})/A_{\text{blanc}}] \times 100,$$

where A_{blank} is the absorbance of the control (containing all reagents except the test sample) and A_{sample} is the absorbance of sample or
standard.

G. Chromatographic Conditions

Chromatographic separation was carried out on an Agilent 1200 Infitity system (Agilent Technologies, Santa Clara, CA) equipped with an Agilent Poroshell 120 EC-C18 column (100 mm × 4.6 mm i.d.) with 2.7 µm particle size, operating at a temperature 40°C. A binary mobile phase with a gradient elution was used. Mobile phase A was water/0.1% formic acid and mobile phase B was ACN/0.1% formic acid. The flow rate was set at 0.6 mL/min. The elution gradient was as follows: 0% B–40% B in 20 min, 90% B in 5 min and then 5 min at 90% B and afterwards the analytical column was re-equilibrated to initial gradient settings. The sample injection volume was 5 µL.

H. Mass Spectrometry

MSD Trap XCT mass spectrometer (Agilent Technologies, USA), with an ESI-ion source was used for experiments. The following mass spectrometer parameters were applied: capillary voltage +3500V; nebulizer (N2) 60 psi; drying gas (N2) temperature and flowrate 350°C and 12 L/min; trap accumulation time 200 ms; trap smart target value 50,000; mass range 100–1500 m/z. MS^2 experiments were applied for the identifica- tion of compounds using helium as collision gas. Data analysis was performed using Agilent ChemStation software.

III. RESULTS AND DISCUSSION

A. Screening of Agaricomycetes for Antifungal Activity

Fungal diseases are one of the crucial problems and revealing of new approaches of their inhibition is very urgent. Fungal infections are managed through the application of appropriate fungicidal agents. Fungicides are a class of pesticides used for killing/inhibiting the growth of fungi. They are extensively used and find widespread application in pharmaceutical industry and in agriculture.[31] In recent years, fungicides gained increased attention due to the resistance mechanisms developed by pathogens towards antifungal agents/ drugs. Besides this, synthetic fungicides can lead to pose different health hazards to humans and other living organisms because, in addition to the fact that they can disrupt the ecological balance by inducing resistance, fungicides are frequently toxic and their residues persist for extended periods.[32,33] The patho- genic fungi evaluated in present research serve as a source for numerous infections of plant and/or human. *Aspergillus niger* is generally regarded as a micromycete with low virulence, but it was shown that it may cause a disease such as invasive aspergillosis, non-dermatophyte mold onychomycosis, and cerebral vas- culitis.[34–36] In addition, fungi are a major contributor to the occurrence of plant diseases as they are one of the most dominant causal agents. Fungi belonging to the genera *Aspergillus*, *Botrytis*, *Fusarium*, and *Guig- nardia* are a source of infections of many agro-industrially important plants such as wheat, groundnut crop, grapes, tomato, strawberry, potato, sunflower, citrus and many others during the growth phase and/or after harvest.[37–41] Moreover, *Fusarium* genus is known for its remarkable adaptability and versatility within the fungal kingdom Eumycota. Specifically, the *Fusarium* spp. can cause diseases in a wide range of hosts, in- cluding plants, animals, and humans.[42] Due to the relevance of different diseases caused by these particular pathogenic fungal species, they were chosen as test organisms.

In the present study, at the initial stage, to reveal the MMs with the best antifungal potential, a screening of 23 Agaricomycetes was carried out according to the procedure described in the method. For this reason, culture liquids obtained after submerged cultivation of investigated mushrooms were tested for their anti- fungal activity against pathogenic fungi such as *A. niger*, *B. cinerea*, *F. oxysporum*, and *G. bidwelii* by my- celial growth inhibition and results are presented in Table 1. Nearly all the MMs exhibited growth inhibition and levels of sensitivity of tested pathogens to them were completely different. All tested MMs were culti- vated for 7–10 d (depending on their growth intensity). Only the cultivation of *Lentinus* spp. was long term and lasted for 19 d. As described in Methods, the antifungal tests were performed on Petri dishes containing MMs submerged culture liquids (10 mL) and malt extract agar medium in ratio 1:1. The intensity of growth of pathogenic fungi was measured daily until the untreated control of each of them covered the entire area of the Petri dish, which was carried out for 5–6 d. The table shows the peak levels of antifungal activity over a period of incubation. *A. niger*, *F. oxysporum* and *G. bidwelii* were most susceptible to inhibition. *A. mellea* 1041, *D. quercina* 459, *G. adspersum* 83, *G. applanatum* 258, *L. edodes* 3776, *Sch. commune* 64,
T. versicolor 137 demonstrate highest inhibition rates and varied from 32.7% to 45.3% at different d of incubation of *A. niger* in comparison to untreated control (Table 1). All tested mushrooms possess *F. oxys- porum* inhibitory effect that varied from 6.3% to 50.4% (50.4% by *Sch. commune* 64). Also, *Sch. commune* 64 showed the highest rate of growth inhibition of *G. bidwelii*, which was consisted 66.0%. The antifungal effectiveness of the tested mushrooms against *B. cinerea* was found to be considerably weak in comparison

TABLE 1:

Mushrooms	*A. niger*	*B. cinerea*	*F. oxysporum*	*G. bidwelii*
A. mellea 1041	38.7 ± 1.7	2.2 ± 0.2	18.3 ± 1.0	7.8 ± 0.7
C. comatus 802	29.0 ± 1.6	4.3 ± 0.8	16.3 ± 1.1	2.9 ± 1.0
D. quercina 459	45.3 ± 2.5	3.1 ± 0.4	19.3 ± 0.5	7.9 ± 0.6
D. tricolor 180	27.3 ± 1.8	8.3 ± 0.4	24.1 ± 1.7	7.4 ± 0.4
F. pinicola 58	22.6 ± 1.2	11.4 ± 0.7	41.6 ± 1.9	43.4 ± 3.6
F. velutipes 966	15.2 ± 1.1	< 1	6.3 ± 0.2	9.2 ± 0.3
F. fomentarius 38	28.0 ± 0.6	4.3 ± 0.3	48.3 ± 1.3	51.8 ± 1.3
G. abietinum 89	31.0 ± 1.1	4.2 ± 0.2	44.3 ± 0.8	41.8 ± 1.1
G. adspersum 83	35.8 ± 0.8	5.3 ± 0.9	9.9 ± 1.7	24.3 ± 1.1
G. applanatum 258	38.0 ± 2.8	5.1 ± 1.0	44.4 ± 2.7	34.5 ± 1.7
H. erinaceus 819	11.6 ± 1.3	3.3 ± 0.8	43.2 ± 2.1	13.2 ± 1.2
L. betulina 559	16.9 ± 1.0	< 1	17.9 ± 1.1	26.2 ± 1.5
L. edodes 363	15.2 ± 1.0	2.4 ± 0.4	27.7 ± 2.1	47.0 ± 2.3
L. edodes 3776	32.7 ± 0.8	8.4 ± 0.7	43.6 ± 2.2	50.2 ± 3.4
Sch. commune 64	35.7 ± 1.4	6.5 ± 1.1	50.4 ± 2.4	66.0 ± 2.1
S. hirsutum 124	24.3 ± 2.8	< 1	18.0 ± 1.3	2.4 ± 0.2
T. pubescens 138	9.4 ± 2.2	< 1	15.5 ± 0.6	10.7 ± 1.0
T. gibbosa 135	28.6 ± 1.3	8 ± 0.8	42.7 ± 1.6	55.1 ± 1.8
T. gibbosa 19	21.1 ± 0.5	< 1	26.2 ± 1.5	9.1 ± 0.7
T. versicolor 113	26.3 ± 1.4	7.4 ± 0.2	11.7 ± 0.9	65.5 ± 2.6
T. versicolor 137	38.4 ± 2.6	6.3 ± 0.6	23.9 ± 1.0	32.1 ± 1.2
T. versicolor 123	29.4 ± 2.1	4.4 ± 0.5	40.3 ± 3.0	12.0 ± 1.0
T. ochracea 76	8.2 ± 1.3	< 1	11.8 ± 0.7	18.4 ± 0.5

to other pathogens. *F. pinicola* 58 demonstrated the most significant inhibition, reaching up to 11.4%. In contrast, some Basidiomycetes exhibited no inhibitory activity against *B. cinerea*. The tested MMs showed not only species-specific but even strain-specific antifungal properties. An example of this could be strains of genera *Lentinus*, *Ganoderma*, and *Trametes*, which showed significantly different activities against the same pathogen (Table 1). At the same time, it should be noted that growth inhibition of fungi may occur due to a factor of unsuitable pH, either excessively low or high. To investigate the impact of pH on growth inhibition of pathogens they were inoculated on malt extract agar medium with various pH and tested in separate experiment. The findings revealed that the pH range from 3.5 to 7.5 did not adversely effect on growth of used pathogenic fungi (data are not shown). This finding agrees with other previous studies.[43–45] In the present work, the pH of all culture liquids was measured before use and ranged from 3.4 to 7.2. since 10 mL of each culture liquid was mixed to 10 mL malt extract agar (pH 6.0), finally, in all testing cases, the pH of used agar mediums ranged from 4.6 to 6.7. Consequently, it is reasonable to hypothesize that the growth suppression of pathogens observed in this study was a result of the synthesis of bioactive com- pounds by the tested Basidiomycetes MMs and their presence in the culture liquids.

According to the results presented in Table 1, it can be seen that *Sch. commune* 64 demonstrate most promising antifungal activity among all tested Agaricomycetes. Therefore, this mushroom was selected for further studies.

B. Effect of Carbon and Nitrogen Sources and Their Concentrations on Antifungal Activity of *Sch. commune* 64

Sch. commune, commonly known as split gill mushroom is a wood-rotting higher Basidiomycete belonging to the class Agaricomycetes. It can successfully colonize a vast diversity of plant substrates and degrade all components of the lignocellulosic biomass, which is caused by its ability to synthesize all the necessary lignocellulose-decomposing enzymes.[46] Numerous reports indicate that *Sch. commune* has medicinal characteristics, including antimicrobial activity.[48–49] In previous study, it was shown that *Sch. commune* had remarkable antimicrobial activity that was strongly dependent on the growth substrate used.[50] In our study, two different type of substrates/carbon sources (10 g/L of maltose, glucose, glycerol, xylose, mannitol; and 40 g/L of MJPW-mandarin juice production waste, WS-wheat straw, BP-banana peels, CC-corn cobs, EPR-ethanol production waste, WB-wheat bran) were used in order to obtain the effect of on antifungal activity of *Sch. commune* 64 and results are presented in Table 2. The antifungal activity of *Sch. commune* 64 was evaluated after 8 and 12 d of submerged cultivation. It appeared that, in most cases, the synthetic medium was more favorable in terms of antifungal activity compared with the lignocellulosic substrates contained medium. Estimation of *Sch. commune* 64 antifungal activity toward *F. oxysporum* and *G. bidwelii* revealed that the mushroom cultivation in the presence of easily metabolized carbon sources such as malt- ose, glucose, glycerol, xylose, and mannitol no significant difference were observed. *Sch. commune* 64 after 8 d and 12 d of cultivation on these substrates showed antifungal activity against *F. oxysporum* that varied from 45.3% to 49.4% and from 54.0% to 57.4%, respectively. The inhibition rate of *G. bidwelii* reached the ranges 64.1% – 68.1%, and 29.2% – 40.3% using 8 and 12-d old culture liquids of *Sch. commune* 64 (Table 2). The antifungal activity of *Sch. commune* 64, cultivated on mandarin juice production waste (MJPW) for 8 and 12 d, exhibited a significant degree of antifungal efficacy against all tested pathogenic fungi. It should be noted that cultural characteristics have a great influence on mushrooms' biological efficiency. This is evidenced by other work that shows the influence of growth substrates on the accumulation of bioactive compounds of higher Basidiomycetes mushrooms and their antimicrobial activities.[51] It should be noted,

TABLE 2:

Growth substrate	*A. niger*		*B. cinerea*		*F. oxysporum*		*G. bidwelii*	
	8 d	12 d	8 d	12 d	8 d	12 d	8 d	12 d
Control	24.3 ± 3.3	26.3 ± 3.6	2.1 ± 0.2	1.4 ± 0.3	19.3 ± 3.2	18.2 ± 2.2	20.2 ± 1.3	25.4 ± 1.8
Glycerol	46.4 ± 1.2	37.1 ± 0.6	8.1 ± 0.3	6.2 ± 0.3	49.4 ± 1.2	54.3 ± 3.2	66.2 ± 0.5	33.1 ± 0.4
Maltose	42.2 ± 1.2	30.2 ± 0.0	7.1 ± 0.4	8.2 ± 0.4	46.3 ± 0.7	57.0 ± 4.2	64.1 ± 0.7	29.2 ± 0.5
Glucose	37.0 ± 2.1	38.1 ± 0.5	9.2 ± 0.3	6.3 ± 0.3	48.1 ± 0.8	54.2 ± 2.8	64.0 ± 0.8	40.3 ± 0.5
Xylose	40.3 ± 0.7	34.3 ± 0.6	7.0 ± 0.7	5.1 ± 0.7	49.2 ± 0.8	54.2 ± 2.3	65.3 ± 1.1	34.1 ± 0.6
Mannitol	46.2 ± 1.7	36.2 ± 0.7	6.1 ± 0.6	3.3 ± 0.6	45.3 ± 0.6	57.4 ± 3.1	68.1 ± 0.8	36.0 ± 0.7
MJPW	59.3 ± 1.4	65.1 ± 3.7	12.5 ± 0.7	12.4 ± 0.7	59.1 ± 1.1	45.3 ± 1.7	72.3 ± 0.3	66.4 ± 3.7
WS	23.2 ± 1.5	6.2 ± 0.7	8.4 ± 0.5	5.3 ± 0.5	41.2 ± 1.2	7.3 ± 0.1	39.4 ± 0.4	28.1 ± 0.6
BP	34.1 ± 2.1	28.3 ± 1.3	11.2 ± 0.9	13.2 ± 0.9	50.1 ± 1.0	32.2 ± 0.6	62.2 ± 1.1	30.4 ± 0.6
CC	11.2 ± 0.6	22.3 ± 0.6	6.3 ± 0.3	8.4 ± 0.3	33.0 ± 2.4	14.1 ± 0.8	17.2 ± 1.3	22.1 ± 0.6
EPR	32.4 ± 0.6	50.1 ± 0.2	9.2 ± 0.3	4.2 ± 0.3	23.4 ± 1.2	15.2 ± 1.0	33.3 ± 2.1	40.2 ± 0.5
WB	45.1 ± 1.1	41.0 ± 0.7	5.2 ± 0.6	2.1 ± 0.6	36.3 ± 1.8	30.3 ± 1.1	78.1 ± 2.1	41.3 ± 0.7

that among all tested lignocellulosic substrates only MJPW better was promote antifungal activity of *Sch. commune* 64. This may be due to the rich chemical composition of this substrate.[50,52,53] From the results shown in Table 2, it seems that antifungal activities of the control (where no carbon sources have been added), were significantly weak. Since MJPW appeared to be the best stimulator of antifungal activity of *Sch. commune* 64 compared with other carbon sources used, the effect of its different concentrations was tested. To determine the optimal concentration that stimulates the antifungal activity of *Sch. commune* 64, it was tested 2%, 4%, and 6% of MJPW and results are presented in Table 3. Among three different con- centrations, the use of 6% MJPW ensured a slight increase in antifungal activity. In this case, the 12-d old culture liquids were slightly more (2–6%) active than the 8-d old. Additionally, it was examined whether incorporation of 10% of MJPW substrate is feasible, nevertheless, it transpired that utilization of such quantity resulted in highly viscous nutrient medium, posing significant hindrance towards agitation, and consequently impeding adequate aeration of the growing mushroom culture. Accordingly, 6% MJPW was used as growth substrate in further experiments.

Various studies confirm that different nitrogen sources have significant effect on biomass yield, me- tabolite biosynthesis and antimicrobial activities of submerged cultivated mushrooms.[50,54,55] The type and concentration of the nitrogen source significantly impact to the accumulation of mushroom biomass and production of bioactive compounds, therefore, determining the optimal nitrogen source and its optimal
concentration is crucial. In the present study, 3% of different nitrogen sources, such as $(NH_4)_2SO_4$, NH_4NO_3, peptone, casein hydrolysate, and soy protein were tested and results are presented in Table 4. It was found
that the antifungal activity was well demonstrated in the nutrient medium containing peptone. Almost simi- lar activities were shown after 8 and 12 d of *Sch. commune* 64 cultivation. Among tested pathogenic fungi,
G. bidwelii was more susceptible to inhibition, 77.1% and 70.0% by 8 d and 12 d old *Sch. commune* 64 culture liquids, respectively. The degree of *A. niger* and *F. oxysporum* growth inhibition was almost similar and varied 67.2–72.3% and 65.1–67.2%, respectively. Similar to previous results, *B. cinerea* was the most resistant to inhibition and demonstrated only 9.2% and 13.4% of growth suppression. To reveal the optimal concentration of peptone, four different concentrations were used (Table 4). It appeared that *F. oxysporum* and *G. bidwelii* were slightly more susceptible to inhibition when the *Sch. commune* 64 was grown in the presence of 6% peptone. Therefore, an optimized nutrient medium containing 6% MJPW and 6% peptone was used to scale up *Sch. commune* 64 cultivation procedure in 2-liter shake-flasks to obtain mushroom extracts from submerged biomass and culture liquid.

C. Yield and Abbreviations of Extracts from *Schizophyllum commune* 64

To obtain submerged biomass and culture liquid, mushroom cultivation was carried out for 8 d in 2-liter flasks on a rotary shaker at 150 rpm, and 27°C. The resulting culture liquid was immediately used for

TABLE 3:

Substrate		*A. niger*		*B. cinerea*		*F. oxysporum*		*G. bidwellii*	
		8 day	**12 day**	**8 day**	**12 day**	**8 day**	**12 day**	**8 day**	**12 day**
Control		22.2 ± 2.4	23.1 ± 2.2	3.5 ± 0.3	2.2 ± 0.5	17.1 ± 2.1	19.4 ± 3.0	21.1 ± 1.8	23.2 ± 1.4
MJPW	2%	44.3 ± 0.9	62.3 ± 0.8	4.4 ± 0.7	9.1 ± 0.6	55.0 ± 1.3	40.4 ± 1.1	62.3 ± 1.4	24.3 ± 0.7
	4%	56.1 ± 1.0	67.2 ± 0.3	11.1 ± 1.3	11.3 ± 0.7	61.2 ± 1.4	47.1 ± 1.1	71.2 ± 1.0	68.1 ± 1.3
	6%	68.3 ± 1.3	70.2 ± 1.0	10.3 ± 0.9	14.2 ± 0.7	64.3 ± 1.4	70.2 ± 0.8	79.3 ± 1.1	72.2 ± 0.9

TABLE 4:

Nitrogen source	*A. niger*		*B. cinerea*		*F. oxysporum*		*G. bidwelii*	
	8 d	12 d	8 d	12 d	8 d	12 d	8 d	12 d
Nitrogen sources								
Control	21.2 ± 1.7	24.5 ± 1.6	3.3 ± 0.2	3.3 ± 0.3	18.2 ± 1.4	20.5 ± 2.6	22.2 ± 1.3	26.2 ± 1.4
Peptone	67.2 ± 2.1	72.3 ± 0.9	9.2 ± 0.4	13.4 ± 0.6	65.1 ± 2.8	67.2 ± 1.7	77.1 ± 1.8	70.2 ± 2.4
$(NH_4)_2SO_4$	48.3 ± 1.8	41.1 ± 2.2	6.5 ± 0.2	12.1 ± 0.3	62.2 ± 2.1	51.3 ± 2.8	58.3 ± 1.4	49.1 ± 0.9
NH_4NO_3	41.2 ± 2.0	46.2 ± 1.4	5.2 ± 0.4	7.2 ± 0.4	58.1 ± 2.4	52.3 ± 1.5	52.4 ± 2.2	57.3 ± 1.8
Casein hydrolysate	48.4 ± 1.1	47.3 ± 2.6	3.1 ± 0.2	5.2 ± 0.2	43.1 ± 2.7	56.2 ± 2.5	41.5 ± 1.3	48.4 ± 2.1
Soy protein	48.1 ± 2.3	71.3 ± 3.1	6.2 ± 0.2	13.4 ± 0.4	56.3 ± 2.4	61.3 ± 2.6	59.1 ± 2.0	58.2 ± 1.5
Peptone concentrations								
3%	55.5 ± 0.8	71.3 ± 0.8	9.4 ± 0.4	9.2 ± 0.4	65.1 ± 0.8	67.3 ± 2.1	76.2 ± 1.9	78.2 ± 1.3
6%	62.2 ± 1.2	75.2 ± 1.4	11.3 ± 0.3	11.4 ± 0.2	73.2 ± 1.0	72.1 ± 0.9	77.3 ± 1.7	81.1 ± 2.2
9%	57.2 ± 2.3	68.2 ± 0.4	6.4 ± 0.3	9.2 ± 0.2	67.2 ± 1.8	70.3 ± 1.3	70.1 ± 1.6	76.3 ± 1.4
12%	63.4 ± 2.5	70.2 ± 0.7	8.3 ± 0.2	13.1 ± 0.2	71.3 ± 2.0	73.2 ± 1.3	78.2 ± 1.8	81.3 ± 1.4

extraction. The biomass was dried at 45°C to constant weight and fine powdered. The yield and abbrevia- tions of extracts from submerged mycelia and culture liquid of *Sch. commune* 64 is presented in Table 5. Abbreviation S1, S2, S3, and S4 indicate extracts from mushroom submerged biomass and culture liquid obtained on glycerol contained synthetic medium. Abbreviation L1, L2, L3, and L4 indicate extracts from mushroom submerged biomass and culture liquid obtained on lignocellulose (MJPW) contained medium.

TABLE 5:

Extracts from mushroom submerged biomass and culture liquid obtained on glycerol contained synthetic medium		
Extracts	**Abbreviations**	**Yield**
Hot water (biomass)	S1	0.378 (g/g DW)
EtOH (biomass)	S2	0.337 (g/g DW)
EtAc (biomass)	S3	0.064 (g/g DW)
EtAc (culture liquid)	S4	0.146 (g/L)
Extracts from mushroom submerged biomass and culture liquid obtained on lignocellulose (MJPW) contained medium		
Extracts	**Abbreviations**	**Yield**
Hot water (biomass)	L1	0.384 (g/g DW)
EtOH (biomass)	L2	0.375 (g/g DW)
EtAc (biomass)	L3	0.073 (g/g DW)
EtAc (culture liquid)	L4	0.165 (g/L)

Dry powdered biomass of tested mushroom was extracted with different solvents such as, distilled water (hot extraction at 80°C), ethanol (80%), and ethyl acetate. Ethyl acetate was used to obtain extracts from culture liquid. Quantitative differences of yield were revealed among the extracts received from submerged cultivation of *Sch. commune* 64 on different growth substrates. Moreover, the yield of extracts received from mushroom biomasses significantly depended on the solvent used (Table 5).

D. Antifungal Activity of *Sch. commune* 64 Extracts

The discovery of antifungal nutraceutical compounds of natural origin and evaluation their potential to inhibit microbial growth represents an essential task. There are consistent reports indicating that many me- dicinal Agaricomycetes, including *Sch. commune*, possess antimicrobial properties. Mushrooms offer great potential as a source for natural compounds with anti-infective activity. Antifungal properties of mush- rooms have been described by various authors, where it was shown that extracts obtained from medicinal higher Basidiomycetes possess positive species and strain-specific activity.[56,57] Previous studies represent that *Sch. commune* is capable to accumulate wide range of bioactive compounds and possess antimicrobial and antioxidant properties.[50,58–60] In the present study, different concentrations of extracts obtained from *Sch. commune* 64, were evaluated for their antifungal activity and results are presented in Fig. 1. Tested extracts, according to their origin and solvent used, showed positive antifungal activity in dose dependent manner against one or more of the four pathogenic fungi. One-way ANOVA test was used to compare whether ex- tracts from mushroom biomass and culture liquid obtained on synthetic medium (S extracts) and extracts from mushroom biomass and culture liquid obtained on lignocellulose contained medium (L extracts) pro- vide significantly different antifungal effect or not. According to ANOVA results, L extracts inhibited more effectively *B. cinerea*, *F. oxysporum* and *G. bidwelii* grow (*P* value 0.003, 0.004, and 0.0003, respectively) which could be attributed to the presence of MJPW in the nutrient medium, which itself is a rich source of bioactive compounds. S extracts however were more active against *A. niger* with statistical significance of 0.004. For growth inhibition of *A. niger*, extracts S1 and S2 (water and ethanol extracts from *Sch. commune* 64 submerged biomass obtained on synthetic medium) have demonstrated more efficiency (44% and 58% of inhibition) at highest concentrations used (10 mg/mL), which was almost 2–2.5 fold higher to compare to L1 and L2 at the same concentrations. L1 and L2 had a weak activity against *A. niger* at all concentrations used and ranged 14%–26% and 14%–19%, respectively. Slightly preferred activities were shown by ethyl acetate extract (L4) obtained from lignocellulose (MJPW) contained culture liquid. Contrary outcomes were observed while inhibiting *B. cinerea*. The water and ethanol extracts L1 and L2 appeared more ac- tive and demonstrate 3–4-fold higher inhibitory effects to compare to the same extracts from submerged biomass obtained on synthetic medium (S1, S2). Surprisingly, at highest concentration 10 mg/mL were demonstrated significant and similar growth inhibitory effect by both extract (L1 and S1) 92% and 85%, respectively. It should be noted that in our previous experiments, an impressively weak inhibitory effect of submerged culture liquids from *Sch. commune* 64 was observed on *B. cinerea*, with the highest inhibitory activity reaching only 14% (Table 3). In contrast, extracts showed a highest inhibitory effect. Nevertheless, these data cannot be considered as comparable, due to the differing nature of the samples employed in the current and previous experiments. Specifically, while the previous experiments utilized culture liquid, the current study employed extracts derived from biomass. Notably, the observed heightened antifungal activ- ity of the extracts from culture liquids (L1 and S1) may be attributed to their concentrated bioactive com- pound composition relative to that of the culture liquid. The inhibitory effect on *F. oxysporum* was enhanced upon the application of extracts L1, L2, L3, and L4, while lower activities were observed with extracts obtained from the synthetic (glycerol contained) culture liquid (S1, S2, S3, and S4). In this case, it is worth highlighting the ethyl acetate extract (L4) obtained from lignocellulose (MJPW) containing culture liquid exhibited approximately 4–6 times higher activity in comparison to the analogue (S4), extract obtained

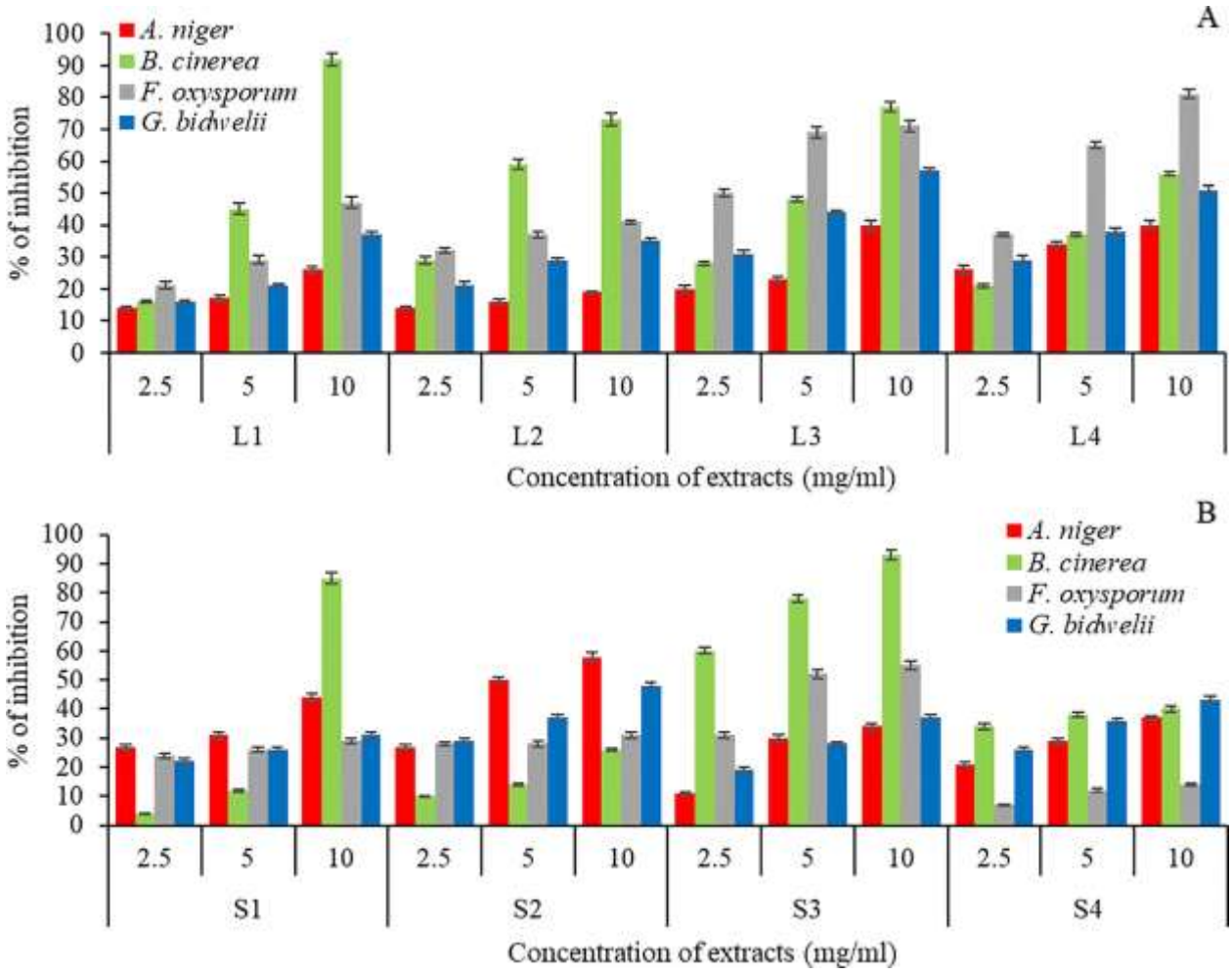

FIG. 1: (A) growth inhibition of pathogenic fungi by extracts from mushroom submerged biomass and culture liquid obtained on lignocellulose (MJPW) contained medium; (B) growth inhibition of pathogenic fungi by extracts from mushrooms submerged biomass and culture liquid obtained on glycerol contained synthetic medium

from synthetic culture liquid. It should be noted that, the presence of 5 mM of synthetic fungicide, propa- mocarb hydrochloride (which was used as a positive control) in the agar medium completely inhibited the growth of pathogens during incubation period. The biological activity of overall all tested extracts varied based on the solvent employed for the extraction process. This observation aligns with previous studies that indicates the biological properties of extracts can be significantly altered by the specific organic solvents used during the extraction process. Such research has shown that extracts derived by different solvents can exhibit widely varying levels of activity.[61,62]

E. Content of Bioactive Compounds and Free Radical Scavenging Activity of Mushroom Extracts

Agaricomycetes MMs high nutritional value, coupled with their diverse range of bioactive compounds that possess exceptional medicinal properties, makes them an excellent source of nutraceuticals aimed at pro- moting human health. Over the past years, mushrooms have gained a significant amount of recognition as

a functional food and have emerged as a potential source for expanding the production of pharmaceutical products and nutraceuticals. This is primarily due to the numerous health benefits they offer, including their antioxidant and antimicrobial properties. Mushrooms have the ability to produce bioactive substances such as phenolic compounds (flavonoids–low molecular weight phenolic compounds among them), which quite often provide their biological activities documented by various *in vitro* and *in vivo* investigations.[63,64] It has been proven that biological activity of mushrooms is directly related to antioxidant activity which is often correlated with high content of phenolic compounds and flavonoids in mushrooms fruiting bodies or myce- lial biomass.[65] In recent years, the search of antioxidant agents of natural origin that can protect against oxi- dative stress or stabilize or deactivate free radicals become a challenge. The reasons for these are increasing understanding of the harmful nature of reactive oxygen species (ROS) produced during oxidation processes and harmful nature of synthetic antioxidants.[66] In present study, the content of total phenols, and flavonoids were quantified in *Sch. commune* 64 extracts, and results are given in Table 6. Completely different contents of total phenols were detected in tested extracts. As it was expected, the content of phenolic compounds in the extracts obtained from the biomass and culture liquid of *Sch. commune* 64 grown on MJPW (L1, L2, L3, L4 ranged from 0.87 ± 0.05 GAE/g to 2.38 ± 0.08 GAE/g) was several times higher than the extracts obtained from the biomass and culture liquid grown on the synthetic (glycerol contained) nutrient medium (ranged from 0.21 ± 0.03 GAE/g to 0.88 ± 0.05 GAE/g). It is most likely caused by the MJPW substrate which is a rich source of bioactive compounds and phenolics among them. It is known that *Sch. commune* is white-rot mushroom and in addition to many metabolites, it successfully can synthesize several enzymes capable for lignocellulose degradation.[67] It was documented, that mostly, cell walls of plant substrates were digested with extracellular enzymes produced by mushrooms and then released the free phenol com- pounds.[68,69] Therefore, it is anticipated that the fermentation process of MJPW would entail enzymatic/ microbial degradation of lignocellulose components, and/or the generation of novel secondary bioactive substances, which could potentially enhance the biological activity of the obtained extracts. Although sev- eral studies have confirmed that different lignocellulosic residues/row materials are rich source of bioactive substances, limited research has been conducted to determine the potential impact of the lignocellulosic substrate on phenolic content and antioxidant activity in mushrooms.[70] In present study lignocellulose dom- inates as the growth substrate, therefore we can assume that the accumulation of phenolic compounds in the extracts is ensured by the content of phenolics in MJPW. Results obtained in the previous work proved that the content of bioactive compounds and biological efficiency of mushroom extracts directly depended on the growth substrate/type of lignocellulosic materials used.[70] It was shown that total phenolic content in the ethyl acetate extracts was significantly high (1.97 ± 0.09 – 2.38 ± 0.08 GAE/g, and 0.88 ± 0.05 – 0.79 ± 0.02 GAE/g, respectively), as well as the amount of total flavonoids (1.53 ± 0.03 – 1.91 ± 0.04 QE/g, and 21.2 ±

TABLE 6:

Extracts	TPC, GAE/g	FC, QE/g
S1	0.285 ± 0.06	0.58 ± 0.03
S2	0.214 ± 0.03	1.77 ± 0.08
S3	0.876 ± 0.05	21.20 ± 0.8
S4	0.790 ± 0.02	27.20 ± 0.8
L1	0.954 ± 0.04	1.72 ± 0.05
L2	0.870 ± 0.05	0.54 ± 0.03
L3	1.973 ± 0.09	1.53 ± 0.03
L4	2.382 ± 0.08	1.19 ± 0.04

0.8 – 27.2 ± 0.8 QE/g) to compare to water and ethanol extracts (Table 6). Moreover, in terms of phenolic compounds extraction, water and ethanol demonstrated comparable results, whereas ethyl acetate proved to be a more effective solvent (Table 6). This is in agreement with previous study where it was shown that, water extracts contain relatively less amount of phenols compared with extracts obtained by other organic solvents.[70] In opposite, considerable amount of total phenols was detected in ethanolic extracts obtained from fruiting bodies of 31 mushroom species belonging to different genera and ranged between 2.79 ± 0.04 and 53.13 ± 2.68 mg GAE/g.[71] Therefore, several crucial factors, including investigated mushroom species and strains, cultivation conditions, and growth substrate, extraction procedure, solvent type, polarity and percentage, significantly affect the content of bioactive compounds in the extracts.

Research of phenolic compounds of mushrooms has revealed the presence of flavonoids in a growing number of studies. Flavonoid contents in the different extracts from *Sch. commune* 64 varied significantly from 0.58 ± 0.03 to 27.2 ± 0.8 mg QE/g (Table 6). The results imply that flavonoids are probably the pre- dominant phenolic compounds present in samples S3 and S4. Interestingly, the highest content of total flavonoids was found in the extracts obtained when the mushroom was cultivated on a synthetic (glycerol contained) nutrient medium. Substantially, low amounts of flavonoids were determined in all samples, ex- cept ethyl acetate extracts S3 and S4 (21.2 ± 0.8 and 27.2 ± 0.8 QE/g, respectively).

The bioactive compounds from fruiting bodies, submerged mycelia and culture broth of mushrooms hold promising potential in the development of functional foods and dietary supplements for the application as antioxidants. Numerous mushroom species, and *Sch. commune* among them, are considered to be favor- able sources of antioxidant substances and various studies have shown that antioxidants, present in mush- rooms at high levels, are the compounds responsible for medicinal functionalities.[2,48,72] In the present work, the DPPH radical-scavenging assay was successfully used for the evaluation of antioxidant activity of the crude extracts, derived from *Sch. commune* 64 and results are presented in Fig. 2. Well known standard an- tioxidant compounds such as BHA, TBHQ, and α-tocopherol were used as positive control. The scavenging

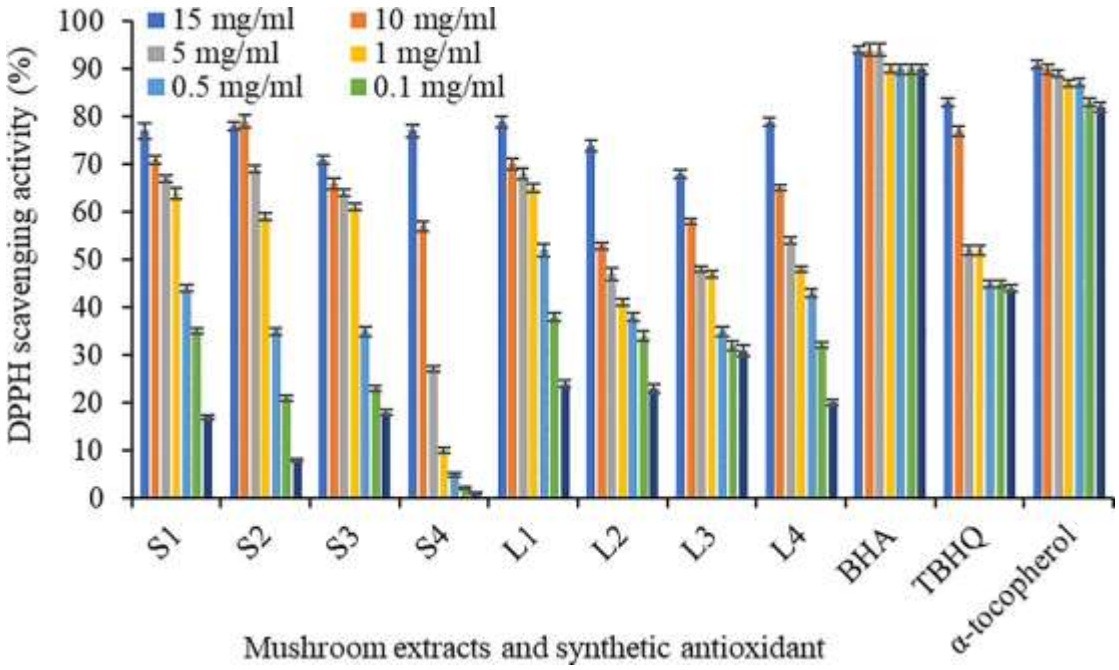

FIG. 2: DPPH free radical scavenging activity of *Sch. commune* 64 extracts

activity of tested extracts on DPPH radicals increased with increasing concentrations. Highest activities were revealed by high concentrations 5, 10, and 15 mg/mL used and demonstrate 27–69%, 53–79%, and68–79%, respectively. The IC values, which indicate the amount of antioxidant material needed to scav-enge 50% of free radicals in the assay system, were generally low for the extracts, ranging from 1.9 mg/mL to 6.7 mg/mL. However, extract S4 was the only exception, as it required a relatively higher concentration of 9.3 mg/mL. All the samples displayed a positive correlation between their concentration (0.05 – 15.0 mg/ mL) and DPPH radical scavenging activity. In the extracts L1, L2, L3, L4, significant scavenging activities were expected due to their high content of phenolic compounds (0.95 ± 0.04 – 2.38 ± 0.08 GAE/g), but extracts S1, S2, S3, S4 also showed significant activities although a relatively low content of phenolic com- pounds (0.29 ± 0.06 – 0.79 ± 0.02 GAE/g) observed in them. It is commonly known that mushroom extracts (*Sch. commune* among them) are rich in polysaccharide compounds.[73–76] Given that a synthetic nutrient medium containing an easily metabolizable carbon sources is a favorable environment for the accumulation of polysaccharides during the submerged cultivation of mushrooms, it is reasonable to assume the observed high scavenging activities in the tested extracts (S1–S4) can be attributed to their content of soluble poly- saccharides. Although the extracts showed different degrees of antioxidant capacity, activity levels did not reach those of the positive controls (BHA and α-tocopherol). BHA and α-tocopherol, exhibited remarkable antioxidant potential, as expected. Their scavenging activity was especially high, even at the lowest con- centration of 0.05 mg/mL, ranging from 90% to 82%, respectively, similarly to previous study (94.74% and 97.79%, respectively at 400 μg/mL).[77] However, relatively low activity was found for TBHQ, which ranged from 44% to 83% at different concentrations. Although the antioxidant activity is mostly correlated with the content of phenolic compounds, it has been documented that, apart from phenolic compounds, diverse bioactive substances, including polysaccharides, vitamins, and amino acids, that are emerge during the fermentation process, exert favorable impacts on antioxidant activity.[69,78] The results obtained confirm that the chemical composition of the submerged cultivated biomass of *Sch. commune* 64 was affected by the substrate composition, which in turn affected the extract content and ultimately influenced their bioactivity.

F. Identification of Phenolic Compounds

Upon testing the extracts, it was established that the content of total phenolic compounds varied depending on the type of solvent and the growth substrate employed during mushroom cultivation. Due to the fact that mandarin juice production waste (MJPW) is a rich substrate and in turn contains phenolic compounds, the differences in composition and content of phenolic compounds in water and ethanol extracts obtained from native MJPW (N1, N2) and biomass of *Sch. commune* 64 grown on MJPW contained nutrient medium (L1, L2) were investigated. For this reason, chromatographic and mass spectrometry analysis of the extracts was carried out and results are presented in Figs. 3 and 4 and Table 7. Peak number 1 has deprotonated pseudo molecular ion at m/z 593 which produces during fragmentation major product ions at m/z 473 as well as m/z 353 corresponding to the loss of one [M-H-120] or two [M-H-240] hexose residues. Absence of agly- cone spectral line on the fragmentation spectrum is typical for flavone with C-glucosidic linkage. Thus, compound number 1 was tentatively identified as flavone-di-C-glucoside or, in accordance to literature, as apigenin-di-C-glucoside.[79] Peak number 2 with corresponding m/z at 609 was identified as rutin. This was confirmed by MS fragmentation analysis as well as comparison of retention time and fragmentation pat- tern with analytical standard. Peak number 3 was characterized with ion at m/z 579 and has very intensive spectral line on fragmentation spectrum at m/z 271 belonging to aglycone (naringenin). The loss of 308 Da is typical for rutinoside moiety and, thus, this compound could be identified as naringenin-7-O-rutinoside. Analogically, compound number 4 was suggested as hesperetin-7-O-rutinoside presenting the same mass loss and strong product ion at m/z 301 (hesperetin). Peak number 5 belongs to flavonone – eriodictyol. It was confirmed by fragmentation of precursor ion at m/z 287 which produces product ion at m/z 151.

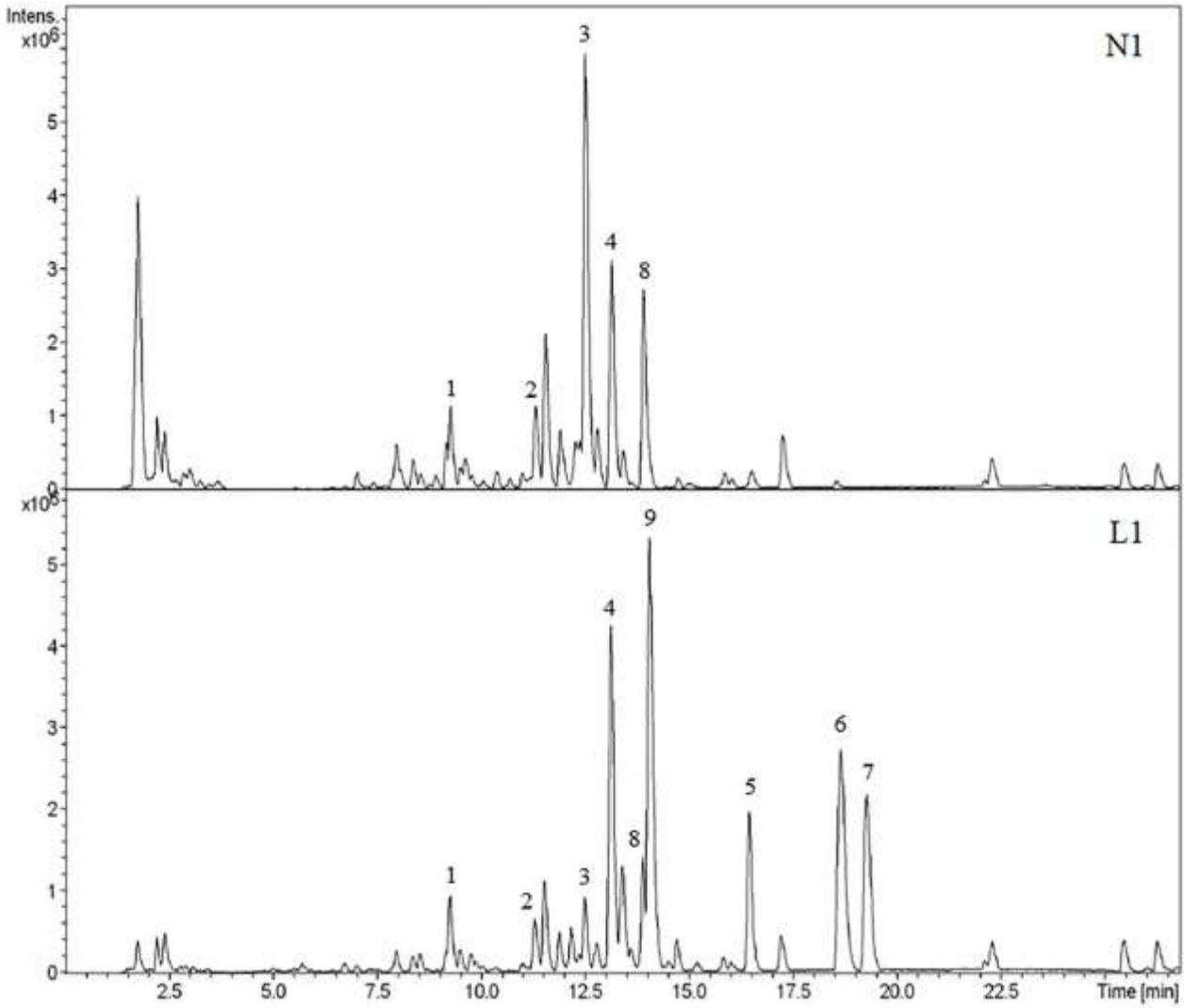

FIG 3: Chromatograms of water extracts. N1, water extract from native MJPW; L1, water extract from *Sch. commune* 64 biomass growth on MJPW contained nutrient medium

Characteristic fragmentation pattern of pseudo molecular ions at m/z 271 (peak 6) and m/z 301 (peak 7) confirms the presence of pure aglycones – naringenin and hesperetin, respectively. Additionally, to major compounds, found and identified in ethanolic samples, two more compounds in water extracts were sub- jected to characterization. Peak number 8 has deprotonated ion at m/z 711 and main product ions at m/z 651 and m/z 607. Since this compound didn't have any UV absorption in the range of 254–280 nm, typical for polyphenols, this could suppose the non-polyphenolic nature of this compound. Intensive chromatographic

peak number 9 at t_R 14,1 min belongs to monoglycosides and was characterized by the product ion at m/z 301 corresponding to aglycone with the loss of one glucose moiety (M-H-162 Da) at the 3-O position. The results show that extracts from native MJPW (N1 and N2) contain two main flavanone diglucosides (peaks 3 and 4) - narirutin and hesperidin, which are main polyphenolic compounds in MJPW.

After growing of *Sch. commune* 64 on MJPW, decrease in MS signal intensity of these compounds could be observed in the extracts L1 and L2. The appearance of three flavonons (peaks 5, 6, 7) on chromatograms

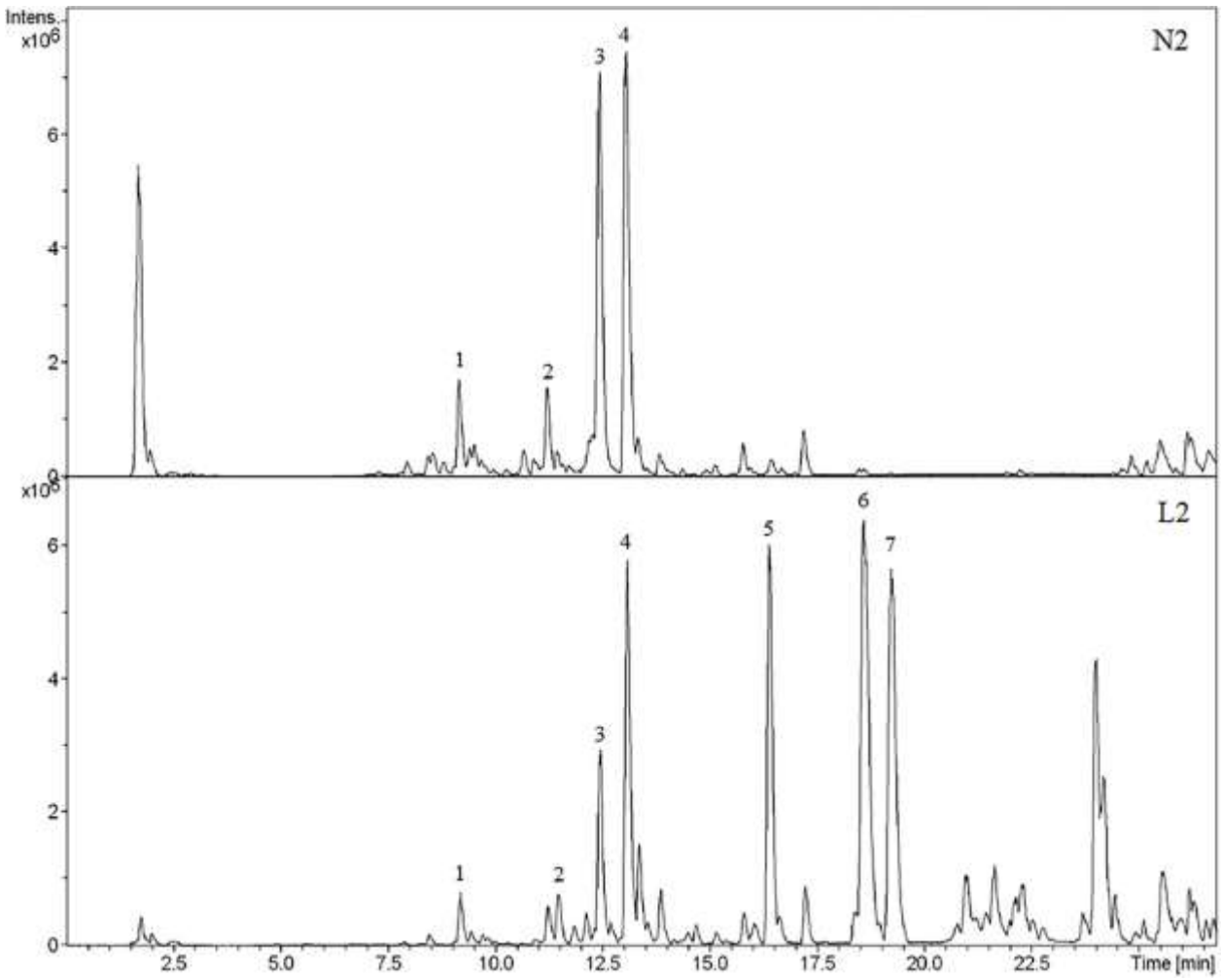

FIG 4: Chromatograms of ethanol extracts. N2, ethanol extract from native MJPW; L2, ethanol extract from *Sch. commune* 64 biomass growth on MJPW contained nutrient medium

TABLE 7: Major polyphenolic profile of extracts

Peak number	t_R, min	Precursor ion (M-H), m/z	Product ions, m/z	Compound
1	9.1	593	473, 353	Apigenin-di-C-glucoside
2	11.2	609	301	Quercetin-3-O-rutinoside (Rutin)
3	12.5	579	271	Naringenin-7-O-rutinoside (Narirutin)
4	13.1	609	301	Hesperetin-7-O-rutinoside (Hesperidin)
5	16.4	287	151	Eriodictyol
6	18.5	271	151, 119, 107	Naringenin
7	19.2	301	286, 257, 242	Hesperetin
8	13.8	711	651, 607	unknown
9	14.1	463	301	Hesperetin-3-O-glucoside

could be attributed to vital activity of tested mushroom. Proposed continuous consumption of sugar moieties by mushroom resulted in the appearance of intensive monoglycoside peak 9 (hesperetin-3-O-glucoside) and peaks 6 and 7, which correspond to aglycons of narirutin and hesperidin. The appearance of intensive peak of flavanone eriodictyol (peak 5) was also detected in extracts L1 and L2. All these compounds have different bioactive properties, which could contribute to measured overall anti-fungal and antioxidant activ- ity of the studied extracts. It can be assumed that, the antifungal and antioxidant activity could potentially be attributed to the formation of new aglucones during the degradation of the MJPW substrate by tested mushroom, *Sch. commune* 64.

IV. CONCLUSIONS

In the present study, a significant number of the tested submerged-cultivated Agaricomycetes mushrooms exhibited varying capacities of antifungal activity against *A. niger*, *B. cinerea*, *F. oxysporum*, and *G. bid- welii*. Among the tested species, *Sch. commune* demonstrated the most favorable outcomes in inhibiting pathogenic fungi. Among various growth substrates, glycerol and MJPW were found to have positive ef- fects on the antifungal activity of *Sch. commune*. It was also observed that compared with glycerol, the use of MJPW as a growth substrate improved the antifungal potential of the tested mushroom. Among the nitrogen sources tested, the presence of 6% peptone in the nutrient medium enhanced the antifungal activity of *Sch. commune*. Moreover, extracts obtained from submerged mycelial biomass and culture liquids using different solvents demonstrate considerable antifungal activity and content of bioactive substances such as, total phenolic and flavonoid contents, as well as demonstrate significant antioxidant capacity inhibiting DPPH free radicals in dose dependent manner. It is also worth noting that future studies are needed to per- form fractionation of the obtained extracts to identify the active fractions with antifungal and antioxidant properties and finally to determine the active substances which provides the biological activity of tested mushroom. From present study, it is confirmed that cultural conditions, growth substrates and content of nutrient growth medium have a significant influence on the biological activity of mushrooms. Based on the results obtained, it can be assumed that *Sch. commune* has promising antifungal and antioxidant potential, and it can be recommended as a promising producer of bioactive compounds.

REFERENCE

1. Cohen N, Cohen J, Asatiani MD, Varshney VK, Yu HT, Yang YC, Li YH, Mau JL, Wasser SP. Chemical composition and nutritional and medicinal value of fruit bodies and submerged cultured mycelia of culinary-medicinal higher Basidiomycetes mushrooms. Int J Med Mushrooms. 2014;16:273–91.
2. Mwangi RW, Macharia JM, Wagara IN, Bence RL. The antioxidant potential of different edible and medicinal mushrooms.
   Biomed Pharmacother. 2022;147:112621.
3. Panda SK, Luyten W. Medicinal mushrooms: Clinical perspective and challenges. Drug Discov Today. 2022;27:636–51.
4. Nowak R, Nowacka-Jechalke N, Pietrzak W, Gawlik-Dziki U. A new look at edible and medicinal mushrooms as a source of
   ergosterol and ergosterol peroxide - UHPLC-MS/MS analysis. Food Chem. 2022;369:130927.
5. Chugh RM, Mittal P, Mp N, Arora T, Bhattacharya T, Chopra H, Cavalu S, Gautam RK. Fungal mushrooms: A natural com- pound with therapeutic applications. Front Pharmacol. 2022;13:925387.
6. Wasser SP. Medicinal mushroom science: History, current status, future trends, and unsolved problems. Int J Med Mush- rooms. 2010;12:1–16.
7. Lo HC, Wasser SP. Medicinal mushrooms for glycemic control in diabetes mellitus: History, current status, future perspec- tives, and unsolved problems (review). Int J Med Mushrooms. 2011;13;401–26.
8. Huang C, Hung Y, Chen L, Asatiani M, Elisashvili VI, Klarsfeld G, Melamed D, Fares B, Wasser SP, Mau JL. Chemical

com- position and antioxidant properties of different combinations of submerged cultured mycelia of medicinal mushrooms. Int J Med Mushrooms. 2021;23:1–24.
9. Hyder MS, Dutta SD. Mushroom-derived polysaccharides as antitumor and anticancer agent: A concise review. Biocatalysis Agricult Biotechnol. 2021;35;102085.
10. Shan Z, L Lin, Yun Z, Fa-Yin Y, Guo-Hua Z. Roles of mushroom polysaccharides in chronic disease management. J Integr Agric. 2022;21;1839–66.
11. Pathak MP, Pathak K, Saikia R, Gogoi U, Ahmad M, Patowary P, Das A. Immunomodulatory effect of mushrooms and their
bioactive compounds in cancer: A comprehensive review. Biomed Pharmacother. 2022;149;112901.
12. Buckel I, Andernach L, Schüffler A, Piepenbring M, Opatz T, Thines E. Phytotoxic dioxolanones are potential virulence fac- tors in the infection process of Guignardia bidwellii. Sci Rep. 2017;7:8926.
13. El-Baky NA, Abdel Rahman RA, Sharaf MM, Amara AAAF. The development of a phytopathogenic fungi control trial: As- pergillus flavus and Aspergillus niger infection in jojoba tissue culture as a model. Sci World J. 2021;2021:6639850.
14. Zhao Y, Lin X. Cryptococcus neoformans: Sex, morphogenesis, and virulence. Infect Genet Evol. 2021;89:104731.
15. Rabaaoui A, Dall'Asta C, Righetti L, Susca A, Logrieco AF, Namsi A, Gdoura R, Werbrouck SPO, Moretti A, Masiello M. Phylogeny and mycotoxin profile of pathogenic Fusarium species isolated from sudden decline syndrome and leaf wilt symp- toms on date palms (Phoenix dactylifera) in Tunisia. Toxins. 2021;13:463.
16. Blandenet M, Gonçalves IR, Rascle C, Dupuy JW, Gillet FX, Poussereau N, Choquer M, Bruel C. Evidencing new roles for
the glycosyl-transferase Cps1 in the phytopathogenic fungus Botrytis cinerea. J Fungi. 2022:8:899.
17. Lopes JP, Lionakis MS. Pathogenesis and virulence of Candida albicans. Virulence. 2022;13:89–121.
18. Scharf DH, Heinekamp T, Brakhage AA. Human and plant fungal pathogens: The role of secondary metabolites. PLoS Pat- hog. 2014;10:e1003859.
19. Pais P, Costa C, Cavalheiro M, Romão D, Teixeira MC. Transcriptional control of drug resistance, virulence and immune system evasion in pathogenic fungi: A cross-species comparison. Front Cell Infect Microbiol. 2016;6:131.
20. Yoon MY, Cha B, Kim JC. Recent trends in studies on botanical fungicides in agriculture. Plant Pathol J. 2013;29:1–9.
21. Meis JF, Chowdhary A, Rhodes JL, Fisher MC, Verweij PE. Clinical implications of globally emerging azole resistance in Aspergillus fumigatus. Philos Trans R Soc Lond B Biol Sci. 2016;371:20150460.
22. Fisher MC, Hawkins NJ, Sanglard D, Gurr SJ. Worldwide emergence of resistance to antifungal drugs challenges human
health and food security. Science. 2018;360:739–42.
23. Brauer VS, Rezende CP, Pessoni AM, De Paula RG, Rangappa KS, Nayaka SC, Gupta VK, Almeida F. Antifungal agents in
agriculture: Friends and foes of public health. Biomolecules. 2019;9:521.
24. Al-Samarrai G, Singh H, Syarhabil M. Evaluating eco-friendly botanicals (natural plant extracts) as alternatives to synthetic fungicides. Ann Agricult Environment Med. 2012;19:673–76.
25. Potočnik I, Stepanovic M, Rekanovic E, Todorovic B, Milijasevic-Marcic S. Disease control by chemical and biological fungicides in cultivated mushrooms: Button mushroom, oyster mushroom and shiitake. Pesticides Phytomed. 2015;30: 201–8.
26. Gupta PK. Reproductive and developmental toxicology. In: Herbicides and fungicides. San Diego: Academic Press; 2017. p.
657–79.
27. Chen ZZ, Wei Y, Li X, Peng CC, Long ZF. Antifungal activity and mechanism of major compound isolated from hexane extract of Curcuma longa. Asian J Chem. 2013;25:6597–600.
28. Jun HI, Song GS, Yang EI, Youn Y, Kim YS. Antioxidant activities and phenolic compounds of pigmented rice bran extracts.
J Food Sci. 2012;77:C759–64.
29. Kaewnarin K, Niamsup H, Shank L, Rakariyatham N. Antioxidant and antiglycation activities of some edible and medicinal
plants. Chiang Mai J Sci. 2008;41:105–16.
30. Xu BJ, Chang SKC. A comparative study on phenolic profiles and antioxidant activities of legumes as affected by extraction
solvents. J Food Sci. 2007;72:S159–66.
31. Thajuddin N. Fungicides – beneficial and harmful aspects [Internet]. InTech; 2011. doi: 10.5772/2308.
32. Shuping DSS, Eloff JN. The use of plants to protect plants and food against fungal pathogens: A review. Afr J Trad Comple- ment Alternat Med. 2017;14:120–7.
33. Dhandapani K, Sivarajan K, Ravindhiran R, Sekar JN. Fungal infections as an uprising threat to human health: Chemosensi- tization of fungal pathogens with AFP from Aspergillus giganteus. Front Cell Infect Microbiol.

2022;12:887971.
34. Atchade E, Jean-Baptiste S, Houzé S, Chabut C, Massias L, Castier Y, Brugière O, Mal H, Montravers P. Fatal invasive as- pergillosis caused by Aspergillus niger after bilateral lung transplantation. Med Mycol Case Rep. 2017;17:4–7.
35. Mannix MK, Marlin L, Rothman I, Islam S. Refractory tinea caused by Aspergillus niger. BMJ Case Rep. 2020;13:e235269.
36. Gao Y, Qu M, Song C, Yin L, Zhang M. Cerebral vasculitis caused by Talaromyces marneffei and Aspergillus niger in a HIV positive patient: A case report and literature review. J Neurovirol. 2022;28:274–80.
37. Onesti G, González-Domínguez E, Rossi V. Production of pycnidia and conidia by Guignardia bidwellii, the causal agent of grape black rot, as affected by temperature and humidity. Phytopathology. 2017;107:173–83.
38. Jaina A, Sarsaiya S, Wua Q, Lua Y, Shi J. A review of plant leaf fungal diseases and its environment speciation. Bioengi- neered. 2019;10:409–24.
39. Syed S, Tollamadugu NVKVP, Lian B. Aspergillus and Fusarium control in the early stages of Arachis hypogaea (groundnut crop) by plant growth-promoting rhizobacteria (PGPR) consortium. Microbiol Res. 2020;240:126562.
40. Xu Y, Tong Z, Zhang X, Zhang X, Luo Z, Shao W, Li L, Ma Q, Zheng X, Fang W. Plant volatile organic compound (E)-2-hexenal facilitates Botrytis cinerea infection of fruits by inducing sulfate assimilation. New Phytol. 2021;231:432–46.
41. Gul S, Hussain A, Ali Q, Alam I, Alshegaihi RM, Meng Q, Zaman W, Manghwar H, Munis MFH. Hydropriming and osmotic priming induce resistance against Aspergillus niger in wheat (Triticum aestivum L.) by activating β-1,3-glucanase, chitinase, and thaumatin-like protein genes. Life. 2022;12:2061.
42. Gordon TR. Fusarium oxysporum and the Fusarium wilt syndrome. Ann Rev Phytopathol. 2017;55:23–39.
43. Esteban A, Abarca ML, Bragulat MR, FJ Cabañes. Effect of pH on ochratoxin A production by Aspergillus niger aggregate species. Food Addit Contam. 2006;23:616–22.
44. Rascle C, Dieryckx C, Dupuy JW, Muszkieta L, Souibgui E, Droux M, Bruel C, Girard V, Poussereau N. The pH regulator PacC: A host-dependent virulence factor in Botrytis cinerea. Environ Microbiol Rep. 2018;10:555–68.
45. Gordon TR, Stueven M, Pastrana M, Henry PM, Dennehy CM, Kirkpatrick SC, Daugovish O. The effect of pH on spore germination, growth, and infection of strawberry roots by Fusarium oxysporum f. sp. fragariae, cause of Fusarium wilt of strawberry. Plant Dis. 2019;103:697–704.
46. Tovar-Herrera OE, Martha-Paz AM, Pérez-LLano Y, Aranda E, Tacoronte-Morales JE, Pedroso-Cabrera MT, Arévalo-Niño K, Folch-Mallol JL, Batista-García RA. Schizophyllum commune: An unexploited source for lignocellulose degrading en- zymes. Microbiol Open. 2018;7:e00637.
47. Wasser SP. Review of medicinal mushrooms advances: Good news from good allies. HerbalGram. 2002;56:28–33.
48. Wongaem A, Reamtong O, Srimongkol P, Sangtanoo P, Saisavoey T, Karnchanatat A. Antioxidant properties of peptides obtained from the split gill mushroom (Schizophyllum commune). J Food Sci Technol. 2021;58:680–91.
49. Arunachalam K, Sasidharan SP, Yang X. A concise review of mushrooms antiviral and immunomodulatory properties that may combat against COVID-19. Food Chem Adv. 2022;1:100023.
50. Khardziani T, Metreveli E, Didebulidze K, Elisashvili V. Screening of Georgian medicinal mushrooms for their antibacterial activity and optimization of cultivation conditions for the split gill medicinal mushroom, Schizophyllum commune BCC64 (Agaricomycetes). Int J Med Mushrooms. 2020;22:659–69.
51. Mkhize SS, Simelane MBC, Mongalo IN, Pooe OJ. The effect of supplementing mushroom growing substrates on the bioac- tive compounds, antimicrobial activity, and antioxidant activity of Pleurotus ostreatus. Biochem Res Int. 2022;9436614.
52. Kour R, Rastogi A, Sharma RK, Kumar A, Raghuwanshi P. Chemical composition, anti-oxidative activity, and in vitro dry matter degradability of Kinnow mandarin fruit waste. Vet World. 2014;7(10):803–6.
53. Costanzo G, Iesce MR, Naviglio D, Ciaravolo M, Vitale E, Arena C. Comparative studies on different citrus cultivars: A revaluation of waste mandarin components. Antioxidants. 2020;9:517.
54. Vamanu E. In vitro antioxidant and antimicrobial activities of two edible mushroom mycelia obtained in the presence of dif- ferent nitrogen sources. J Med Food. 2013;16:155–66.
55. Pekşena A, Kibar B. Effects of various carbon and nitrogen sources on mycelial biomass production of Macrolepiota procera and Polyporus squamosus in submerged culture. Anadolu J Agricult Sci. 2016;31:6–24.
56. Dresch P, D´Aguanno MN, Rosam K, Grienke U, Rollinger JM, Peintner U. Fungal strain matters: Colony growth and bioac- tivity of the European medicinal polypores Fomes fomentarius, Fomitopsis pinicola and Piptoporus betulinus. AMB Express. 2015;5:4.
57. Nowakowski P, Naliwajko SK, Markiewicz-Żukowska R, Borawska MH, Socha K. The two faces of Coprinus

comatus-func- tional properties and potential hazards. Phytother Res. 2020;34:2932–44.
58. Appiah T, Boakye YD, Agyare C. Antimicrobial activities and time-kill kinetics of extracts of selected Ghanaian mushrooms.
Evid Based Complement Alternat Med. 2017;2017:4534350.
59. Razak DLA, Fadzil NHM, Jamaluddin A, Rashid NYA, Sani NA, Manan MA. Effects of different extracting conditions on an- ti-tyrosinase and antioxidant activities of Schizophyllum commune fruit bodies. Biocat Agricult Biotechnol. 2019;19:101116.
60. Mišković J, Karaman M, Rašeta M, Krsmanović N, Berežni S, Jakovljević D, Piattoni F, Zambonelli A, Gargano ML, Ven- turella G. Comparison of two Schizophyllum commune strains in production of acetylcholinesterase inhibitors and antioxi- dants from submerged cultivation. J Fungi. 2021;7:115.
61. Alves MJ, Ferreira IC, Dias J, Teixeira V, Martins A, Pintado M. A review on antifungal activity of mushroom (Basidiomy- cetes) extracts and isolated compounds. Curr Top Med Chem. 2013;13(21):2648–59.
62. Jacinto-Azevedo B, Valderrama N, Henríquez K, Aranda M, Aqueveque P. Nutritional value and biological properties of
Chilean wild and commercial edible mushrooms. Food Chem. 2021;356:129651.
63. Román MPG, Mantilla NB, Flórez SAC, DeMandal S, Passari AK, Ruiz-Villáfan B, Rodríguez-Sanoja R, Sánchez S. Antimi- crobial and antioxidant potential of wild edible mushrooms. An introduction to mushroom [Internet]. IntechOpen; 2020. doi: 10.5772/intechopen.90945.
64. Kour H, Kour D, Kour S, Singh S, Hashmi SAJ, Yadav AN, Kumar K, Sharma YP, Ahluwalia AS. Bioactive compounds from
mushrooms: Emerging bioresources of food and nutraceuticals. Food Biosci. 2022;50:102124.
65. Podkowa A, Kryczyk Poprawa A, Opoka W, Muszyńska B. Culinary–medicinal mushrooms: A review of organic compounds and bioelements with antioxidant activity. Eur Food Res Technol. 2021;247:513–33.
66. Unsal V, Dalkıran T, Çiçek M, Kolükçü E. The role of natural antioxidants against reactive oxygen species produced by cad- mium toxicity: A review. Adv Pharmaceut Bull. 2020;10:184.
67. Kumar A, Bharti AK, Bezie Y. Schizophyllum commune: A fungal cell-factory for production of valuable metabolites and enzymes. BioResources. 2022;17:3.
68. McCue P, Horii A, Shetty K. Solid-state bioconversion of phenolic antioxidants from defatted soybean powders by Rhizopus oligosporus: Role of carbohydrate-cleaving enzymes. J Food Biochem. 2003;27:501–14.
69. Boonthatui Y, Chongsuwat R, Kittisakulnam S. Production of antioxidant bioactive compounds during mycelium growth of
Schizophyllum commune on different cereal media. CMU J Nat Sci. 2021;20:e2021032.
70. Basso V, Schiavenin C, Mendonça S, Gonçalves de Siqueira F, Salvador M, Camassola M. Chemical features and antioxidant profile by Schizophyllum commune produced on different agroindustrial wastes and byproducts of biodiesel production. Food Chem. 2020;329:127089.
71. Nowacka N, Nowak R, Drozd M, Olech M, Los R, Malm A. Antibacterial, antiradical potential and phenolic compounds of
thirty-one Polish mushrooms. PLoS One. 2015;10:e0140355.
72. Sharma A, Chauhan P, Sharma K, Kalotra V, Kaur A, Chadha P, Kaur S, Kaur A. An endophytic Schizophyllum commune possessing antioxidant activity exhibits genoprotective and organ protective effects in freshwater fish Channa punctatus ex- posed to bisphenol A. BMC Microbiol. 2022;22:291.
73. Klaus A, Kozarski M, Niksic M, Jakovljevic D, Todorovic N, Van Griensven LJLD. Antioxidative activities and chem- ical characterization of polysaccharides extracted from the Basidiomycete Schizophyllum commune. Food Sci Technol. 2011;44:2005–11.
74. Wang Q, Wang F, Xu Z, Ding Z. Bioactive mushroom polysaccharides: A review on monosaccharide composition, biosynthe- sis and regulation. Molecules. 2017;22:955.
75. Sun Y, He H, Wang Q, Yang X, Jiang S, Wang D. A review of development and utilization for edible fungal polysaccharides: Extraction, chemical characteristics, and bioactivities. Polymers. 2022;14:4454.
76. Tu L, Fan Y, Deng Y, Hu L, Sun H, Zheng B, Lu D, Guo C, Zhou L. Production and anti-inflammatory performance of PVA hydrogels loaded with curcumin encapsulated in octenyl succinic anhydride modified schizophyllan as wound dressings. Molecules. 2023;28:1321.
77. Çayan F, Tel-Çayan G, Deveci E, Duru ME. HPLC–DAD characterization of phenolic profile and in vitro antioxidant, anticholinesterase, and antidiabetic activities of five mushroom species from Turkey. 3 Biotech. 2021;11:273.
78. Chun S, Gopal J, Muthu M. Antioxidant activity of mushroom extracts/polysaccharides - their antiviral properties and plau- sible anti-COVID-19 properties. Antioxidants. 2021;10:1899.
79. Cao J, Yin C, Qin Y, Cheng Z, Chen D. Approach to the study of flavone di-C-glycosides by high performance liquid chro- matography-tandem ion trap mass spectrometry and its application to characterization of flavonoid composition in Viola yedoensis. J Mass Spectrom. 2014;49:1010–24.