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Original Article

Evaluation of metal concentration and antioxidant, antimicrobial, and anticancer potentials of two edible mushrooms *Lactarius deliciosus* and *Macrolepiota procera*

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ABSTRACT

This study is designed for the determination of metal concentrations, antioxidant, antimicrobial, and anticancer potential of two edible mushrooms *Lactarius deliciosus* and *Macrolepiota procera*. Concentrations of nine metals are determined and all metals are present in the allowable concentrations in the tested mushrooms except Cd in *M. procera*. Antioxidant activity was evaluated by free radical scavenging and reducing power. *M. procera* extract had more potent free radical scavenging activity ($IC_{50} = 311.40$ μg/mL) than *L. deliciosus* extract. Moreover, the tested extracts had effective reducing power. The total content of phenol in the extracts was examined using Folin–Ciocalteu reagent and obtained values expressed as pyrocatechol equivalents. Further, the antimicrobial potential was determined with a microdilution method on 15 microorganisms. Among the tested species, extract of *L. deliciosus* showed a better antimicrobial activity with minimum inhibitory concentration values ranging from 2.5 mg/mL to 20 mg/mL. Finally, the cytotoxic activity was tested using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide method on human epithelial carcinoma HeLa cells, human lung carcinoma A549 cells, and human colon carcinoma LS174 cells. Extract of both mushrooms expressed similar cytotoxic activity with IC_{50} values ranging from 19.01 μg/mL to 80.27 μg/mL.

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1. Introduction

A mushroom is defined as a macro fungus with a distinctive fruiting body which can be either hypogeous or epigeous, large enough to be seen with the naked eye and to be picked by hand [1]. The first growing mushrooms have received worldwide popularity in recent decades with realization to the fact that they are a good source of delicious food with high nutritional values.

Wild edible mushrooms are a popular food stuff and have high levels of proteins, vitamins, iron, zinc, sodium, and minerals [2,3]. Due to the fact that mushrooms are agents that aid in the breakdown of organic matter and play an important role in natural changes, they have a very effective mechanism to accumulate heavy metal from the environment. The accumulation of heavy metals in macrofungi has been found to be affected by environmental and fungal factors [4]. Environmental factors such as organic matter amount, pH, metal concentrations in soil, and fungal factors such as species of mushroom, morphological part of fruiting body, development stages, age of mycelium, biochemical composition, and interval between the fructifications affect metal accumulation in macrofungi [4,5].

Mushrooms can also be used for therapeutic purposes since they can produce a large variety of secondary metabolites, such as organic acids, alkaloids, terpenoids, steroids, and phenolic compounds [6]. Health promoting properties, e.g., antioxidant, antimicrobial, anticancer, cholesterol-lowering effects, and immunostimulatory effects, have been reported for some species of mushrooms [7–10]. Preliminary research has shown some medicinal mushroom isolates to have cardiovascular, anticancer, antiviral, antibacterial, antiparasitic, anti-inflammatory, and antidiabetic properties [11–13]. Currently, several extracts have widespread use in Japan, Korea, and China, as potential adjuvants to radiation treatments and chemotherapy [14,15]. Major medicinal properties attributed to mushrooms include anticancer, antiviral activities, immune response stimulating effects, and blood lipid lowering effects [16,17]. Both the fruiting body and mycelium of different mushrooms contain different compounds such as terpenoids, steroids, polyphenol, polyketides, polyglucan, flavonoids, alkaloids, polysaccharides, and dietary fibers which exert several pharmacological activities [18,19]. They are also a rich source of antioxidants, antibiotics, and antineoplastic activity [20].

Only a few studies describing the bioactive properties of mushrooms enriched with minerals could be found in literature. Thus, the aim of present work is to evaluate the metal contents and antioxidant, antimicrobial, and anticancer potentials of the methanol extract of *Lactarius deliciosus* and *Macrolepiota procera* mushrooms.

2. Methods

2.1. Fungal materials

Fungal samples of *L. deliciosus* (L. ex Fr.) and *M. procera* (Scop. ex Fr.) Sing., were collected from Kopaonik, Serbia, in June 2013. The demonstration samples are preserved in facilities of the Department of Biology and Ecology, Faculty of Science, University of Kragujevac, Kragujevac, Serbia. Determination of mushrooms was done using standard keys [21,22].

L. deliciosus contains fatty acids and various compounds such as chroman-4-one, anofinic acid, 3-hydroxyacetylindole, ergosterol, and cyclic dipeptides [23]. *M. procera* contains free amino acids, fatty acids, and sterols. Proline, glutamic acid, serine, and alanine were the most abundant free amino acids. Among the fatty acids, linoleic, oleic, and palmitic acids constituted almost all the fatty acid content. The mushroom has been shown to contain ergosterol as the principal sterol [24].

2.2. Extraction

Finely dry ground thalli of the examined mushrooms (100 g) were extracted using methanol (500 mL) in a Soxhlet extractor, Quickfit, England. The extracts were filtered and then concentrated under reduced pressure in a rotary evaporator. The dry extracts were stored at −18°C until they were used in the tests. The extracts were dissolved in 5% dimethyl sulfoxide (DMSO) for the experiments. DMSO was dissolved in sterile distilled water to the desired concentration.

2.3. Metal quantitative analysis

The collected samples of mushroom were cleaned with a plastic knife, air dried for 1 week, and further dried in an oven at 50°C until the samples reached a constant weight. Dried samples were ground to a powder using an agate mortar and stored in polyethylene bottles until analysis. All solutions were prepared from analytical reagent grade and deionized water which was generated by a Milli-Q academic water purification system (Millipore, Milford, MA, USA). Mineral acid (HNO_3) and oxidant (H_2O_2) of suprapure quality (Sigma-Aldrich, Steinheim, Germany and J.T. Baker, Center Valley, PA, USA, respectively) were used for sample digestion. For calibration, a series of standard solutions were used, prepared by diluting stock solutions of 1000 mg/L of each element supplied by J.T. Baker.

Samples (0.5 g) of powdered mushrooms were transferred in TFM vessels and digested with 7 mL of HNO_3 (65%) and 1 mL of H_2O_2 (30% in microwave digestion system; Milestone ETHOS One, Shelton, CT, USA) for 25 minutes and finally diluted to 25 mL with deionized water. A blank digest was carried out in the same way. Digestion conditions for mushroom samples in the microwave digestion system, recommended by the manufacturer, are shown in Table 1. All sample solutions were clear. The digestion procedure was done in triplicate for each sample.

Table 1 – Digestion conditions for mushroom samples in the microwave digestion system.

Step	Time (min)	Temperature (°C)	Power (W)
1	10	100	up to 500
2	7	200	up to 500
3	8	200	up to 500
Vent	10		

Metal quantitative analysis in the digested solutions was performed with inductively-coupled plasma optical emission spectrometry using a ICP-OES Spectrometer, iCAP 6000 Series, Thermo Scientific, Waltham, MA, USA. The analytical parameters of investigated metals for inductively-coupled plasma optical emission spectrometry are shown in Table 2.

2.4. Antioxidant activity

2.4.1. Scavenging 1,1-diphenyl-2-picryl-hydrazil radicals

The free radical scavenging activity of samples was measured using 1,1-diphenyl-2-picryl-hydrazil (DPPH). The method used was similar to that of Dorman et al [25] but was modified in its details. Two milliliters of methanol solution of DPPH radical in the concentration of 0.05 mg/mL and 1 mL of test samples (1000 μg/mL, 500 μg/mL, 250 μg/mL, 125 μg/mL, and 62.5 μg/mL) were placed in cuvettes. The mixture was shaken vigorously and allowed to stand at room temperature for 30 minutes. DPPH solution is initially violet in color which fades when antioxidants donate hydrogen. The change in color is monitored with a spectrophotometer (Bibby Scientific Limited, Stone, UK) at 517 nm against methanol as a blank. Ascorbic acid was used as the positive control. The DPPH radical concentration was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = [(A0 - A1)/A0] \times 100 \quad (1)$$

where A0 is the absorbance of the negative control (2 mL of methanol solution of DPPH radical + 1 mL of 5% DMSO) and A1 is the absorbance of reaction mixture or standard.

For both extract and ascorbic acid, the inhibitory concentration (IC_{50}) at 50% was determined.

2.4.2. Reducing power

The reducing power of samples was determined according to the method of Oyaizu [26]. One milliliter of test samples (1000 μg/mL, 500 μg/mL, 250 μg/mL, 125 μg/mL, and 62.5 μg/mL) were mixed with 2.5 mL of phosphate buffer (2.5 mL, 0.2M, pH 6.6) and potassium ferricyanide (2.5 mL, 1%). The mixtures were incubated at 50°C for 20 minutes. Then, 2.5 mL of 10% trichloroacetic acid was added to the mixture and centrifuged (HermLe, Wehingen, Germany). Finally, the upper layer was mixed with distilled water (2.5 mL) and ferric chloride (0.5 mL of 0.1%). The absorbance of the solution was measured at 700 nm in a spectrophotometer (Bibby Scientific Limited). The blank was prepared with all the reaction agents without extract. Higher absorbance of the reaction mixture indicated that the reducing power is increased. Ascorbic acid was used as the positive control.

2.4.3. Determination of total phenolic compounds

Total soluble phenolic compounds in the acetone extracts were determined with Folin–Ciocalteu reagent according to the method of Slinkard and Singleton [27] using pyrocatechol as a standard phenolic compound. Briefly, 1 mL of the extract (1 mg/mL) was placed in a volumetric flask and diluted with distilled water (46 mL). One milliliter of Folin–Ciocalteu reagent was added and the contents of the flask were mixed thoroughly. After 3 minutes, 3 mL of 2% sodium carbonate was added and then was allowed to stand for 2 hours with intermittent shaking. The absorbance was measured at 760 nm in a spectrophotometer (Bibby Scientific Limited). The total concentration of phenolic compounds in the extract was determined as micrograms of pyrocatechol equivalents (PE) per milligram of dry extract by using an equation that was obtained from a standard pyrocatechol graph as follows:

$$\text{Absorbance} = 0.0057 \times \text{total phenols (μg PE/mg of dry extracts)} - 0.1646 \quad (2)$$

($R^2 = 0.9203$)

2.5. Antimicrobial activity

2.5.1. Microorganisms and media

The following bacteria were used as test organisms in this study: *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* [American Type Culture Collection (ATCC) 6633], *Bacillus cereus* (ATCC 10987), *Escherichia coli* (ATCC 25922), and *Proteus mirabilis* (ATCC 12453). All of the bacteria used were obtained from the ATCC. The bacterial cultures were maintained on Müller–Hinton agar substrates (Torlak, Belgrade, Serbia). The fungi used as test organisms were: *Aspergillus flavus* (ATCC 9170), *Aspergillus niger* (ATCC 16888), *Candida albicans* (ATCC 10259), *Penicillium expansum* (ATCC 20466), *Penicillium chrysogenum* (ATCC 10106), *Mucor mucedo* (ATCC 20094), *Trichoderma viride* (ATCC 13233), *Cladosporium cladosporioides* (ATCC 11680), *Fusarium oxysporum* (ATCC 62506), and *Alternaria alternata* (ATCC 36376). All of the fungi were from the ATCC. The fungal cultures were maintained on potato dextrose agar, except for *C. albicans* that was maintained on Sabourad dextrose agar (Torlak). All of the cultures were stored at 4°C and subcultured every 15 days.

Bacterial inoculi were obtained from bacterial cultures incubated for 24 hours at 37°C on Müller–Hinton agar substrate and brought up by dilution according to the 0.5 McFarland standard to approximately 10^8 CFU/mL. Suspensions of fungal spores were prepared from fresh mature (3–7-day-old) cultures that grew at 30°C on a potato dextrose agar substrate. Spores were rinsed with sterile distilled water, used to determine turbidity spectrophotometrically at 530 nm, and then further diluted to approximately 10^6 CFU/mL according to the

Table 2 – Instrumental parameters of investigated metals for inductively coupled plasma optical emission spectrometry.

Parameter					
Power	1150 W				
Plasma position	Axial				
Pump rate	50 rpm				
Coolant gas flow	12 L/min				
Auxiliary gas flow	0.5 L/min				
Nebulizer gas flow	0.6 L/min				
Nebulizer pressure	2.9 bar				
Sample flow rate	2.5 mL/min				
Element	Cd	Co	Cr	Cu	Fe
wavelength (nm)	228.802	228.616	267.716	324.754	238.204
	Mn	Ni	Pb	Zn	
	257.610	231.604	216.999	213.856	

procedure recommended by the National Committee for Clinical Laboratory Standards [28].

2.5.2. *Minimal inhibitory concentration*
The minimal inhibitory concentration (MIC) was determined using 96-well microtiter plates (Spektar, Čačak, Serbia) using the broth microdilution method [29]. A series of dilutions with concentrations ranging from 40 mg/mL to 0.0195 mg/mL for extracts was used in the experiment against every microorganism tested. The starting solutions of test samples were obtained by measuring off a certain quantity of samples and dissolving it in DMSO. Two-fold dilutions of test samples were prepared in Müller–Hinton broth for bacterial cultures and Sabourad dextrose agar broth for fungal cultures. The MIC was determined with resazurin. Resazurin is an oxidation–reduction indicator used for the evaluation of microbial growth. It is a blue nonfluorescent dye that becomes pink and fluorescent when reduced to resorufin by oxidoreductases within viable cells. The boundary dilution without any changing color of resazurin was defined as the MIC for the tested microorganism at the given concentration. Streptomycin (for bacteria) and ketoconazole (for fungi) were used as a positive control. A solvent control test was performed to study an effect of 5% DMSO on the growth of a microorganism. All experiments were performed in triplicate.

2.6. *Cytotoxic activity*

2.6.1. *Cell culture*
Human epithelial carcinoma HeLa cells, human lung carcinoma A549 cells, and human colon carcinoma LS174 cells were obtained from ATCC (Manassas, VA, USA). All cancer cell lines were cultured as a monolayer in the Roswell Park Memorial Institute 1640 nutrient medium, with 10% (inactivated at 56°C) fetal bovine serum, 3mM of L-glutamine, and antibiotics, at 37°C in humidified air atmosphere with 5% CO_2.

2.6.2. In vitro *cytotoxic assay*
In vitro assay for cytotoxic activity of investigated extracts was performed when the cells reached 70–80% of confluence. Stock solution (50 mg/mL) of extracts was dissolved in corresponding medium to the required working concentrations. Neoplastic HeLa cells (5000 cells per well), A549 cells (5000 cells per well), and LS174 cells (5000 cells per well) were seeded into 96-well microtiter plates, and 24 hours later, after cell adherence, five different, double diluted concentrations of investigated extracts were added to the wells. Final concentrations of the extracts were 200 μg/mL, 100 μg/mL, 50 μg/mL, 25 μg/mL, and 12.5 μg/mL except for the control wells, where only nutrient medium was added. The cultures were incubated for the next 72 hours. The effect on cancer cell survival was determined 72 hours after the addition of extract, with the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test [30]. Briefly, 20 μL of MTT solution (5 mg/mL phosphate-buffered saline) was added to each well and incubated for a further 4 hours at 37°C in 5% CO_2 and humidified air. Subsequently, 100 μL of 10% sodium dodecyl sulfate was added to solubilize the formazan crystals formed from MTT after the conversion by mitochondrial dehydrogenases of viable cells. Absorbencies proportional to the number of viable cells were measured using a microplate reader (Multiskan EX, Thermo Scientific, Finland) at 570 nm. Each experiment was performed in triplicate and independently repeated at least four times.

2.7. *Data analyses*

Data analyses were performed with the EXCEL (Microsoft, Redmond, WA, USA) and SPSS (SPSS Inc., Chicago, IL, USA) software packages. To determine the statistical significance of antioxidant activity, Student *t* test was used. All values are expressed as mean ± standard deviation of three parallel measurements.

3. Results

Obtained data of metal quantitative analysis of the tested mushroom species are shown in Table 3. The average metal concentrations are expressed as mg/kg [dry weight (dw) of mushroom] in fruiting bodies samples of mushrooms. According to the results, Fe was the most abundant element in the samples of *L. deliciosus,* with a values of 216.83 mg/kg dw, followed by Zn with a value of 123.5 mg/kg dw. In the sample *M. procera,* the most abundant element was Cu with a value of 109.57 mg/kg dw, followed by Fe and Zn with values of 89.53 mg/kg dw and 53.85 mg/kg dw, respectively. The levels of Mn, Ni, Cd, Pb, and Co in *L. deliciosus* were 5.98 mg/kg dw, 1.58 mg/kg dw, 0.54 mg/kg dw, 0.44 mg/kg dw, and 0.12 mg/kg dw, while in *M. procera* were 9.38 mg/kg dw, 0.26 mg/kg dw, 6.23 mg/kg dw, 1.96 mg/kg dw, 0.31 mg/kg dw, respectively. Cr content in *L. deliciosus* was 1.11 mg/kg dw, while in *M. procera* Cr was not detected. Among the elements tested, Co has the lowest concentration value.

The scavenging DPPH radicals and reducing power of the studied extracts are represented in Tables 4 and 5. As a shown in tables, extract from *M. procera* showed larger antioxidant activities than *L. deliciosus*. In various antioxidant activities, there was a statistically significant difference between extracts and control ($p < 0.05$). Various antioxidant activities

Table 3 – Metal concentrations (mg/kg, dry weight) of the mushroom samples.

Mushroom	Fe	Zn	Cu	Mn	Ni	Cd	Pb	Cr	Co
Lactarius deliciosus	216.83 ± 1.38	123.57 ± 0.30	15.49 ± 0.12	5.98 ± 0.04	1.58 ± 0.01	0.54 ± 0.00	0.44 ± 0.30	1.11 ± 0.01	0.12 ± 0.00
Macrolepiota procera	89.53 ± 0.24	53.85 ± 0.17	109.57 ± 0.60	9.38 ± 0.03	0.26 ± 0.01	6.23 ± 0.02	1.69 ± 0.14	nd	0.31 ± 0.01

Data are presented as mean ± standard deviation; $n = 3$.
nd = not detected.

Table 4 – 1,1-diphenyl-2-picryl-hydrazil (DPPH) radical scavenging activity of methanol extracts of *Lactarius deliciosus* and *Macrolepiota procera*.

Mushroom species	DPPH radical scavenging IC_{50} (μg/mL)
Lactarius deliciosus	493.82 ± 2.35
Macrolepiota procera	311.40 ± 1.28
Ascorbic acid	6.42 ± 0.18

Data are presented as mean ± standard deviation of three parallel measurements.

Table 6 – Total phenolic content of methanol extracts of *Lactarius deliciosus* and *Macrolepiota procera*.

Mushroom species	Phenolic content (μg PE/mg of extract)
Lactarius deliciosus	42.68 ± 1.065
Macrolepiota procera	67.98 ± 1.013

Data are presented as mean ± standard deviation of three parallel measurements.
PE = pyrocatechol equivalents.

were compared to ascorbic acid. The results showed that standard antioxidant had stronger activity than tested samples.

The total phenolic content of the mushrooms extracts is given in Table 6. The total phenolic content in extracts of *L. deliciosus* and *M. procera* was 42.68 μg PE/mg and 67.98 μg PE/mg, respectively.

The antimicrobial activity of the mushrooms extracts against the test microorganisms is shown in Table 7. Extracts of these mushrooms acted selectively on the microorganisms tested. Extract from *L. deliciosus* inhibited all tested microorganisms except *A. flavus*. The MIC fluctuated in a range of 2.5–20 mg/mL. *M. procera* extract showed slightly weaker activity. It inhibited three species of bacteria and seven tested fungi with MIC values from 5 mg/mL to 20 mg/mL. The most sensitive, among the bacteria, was *B. cereus*, and the highest resistance was shown in *E. coli* and *S. aureus*. Among the fungi, the most sensitive appeared to be *C. albicans* and *F. oxysporum*. The most resistant fungi were *A. flavus*.

The antimicrobial activity was compared with the standard antibiotics, streptomycin (for bacteria) and ketoconazole (for fungi). The results showed that standard antibiotics had stronger activity than tested samples as shown in Table 7. In a negative control, DMSO had no inhibitory effect on the tested organisms.

The data obtained for anticancer effects of *L. deliciosus* and *M. procera* extracts are shown in Table 8. Both tested mushrooms showed similar anticancer potential. The IC_{50} value for both samples relative to the tested cells ranged from 19.01 μg/mL to 80.27 μg/mL. The IC_{50} for *L. deliciosus* against HeLa cells, A549 cells, and LS174 cells was 19.01 μg/mL, 33.05 μg/mL, and 74.01 μg/mL, respectively. The IC_{50} value for *M. procera* was 29.39 μg/mL related to HeLa cells, 25.55 μg/mL related to A549 cells, and 68.49 μg/mL related to LS174 cell line. Furthermore, both extracts showed less activity compared with cis-dichlorodiammine platinum (II) as a positive control.

4. Discussion

Concentrations of nine metals (Fe, Zn, Cu, Mn, Ni, Cd, Pb, Cr, and Co) have been determined in this study. Several factors may affect the accumulation and concentration of metals in mushrooms. Concentrations of the metals are generally assumed to be species dependent, but substrate composition or pH of the soil are also considered to be important factors [31,32]. The analysis of the metal concentration of these and related mushrooms was previously reported from other researchers [33–36]. Compared with their results, results of mineral content in our mushroom samples are in agreement with previous studies, with small differences related to Zn, Mn, and Cd.

According to the Food and Agriculture Organization and World Health Organization standards for toxic metals (Cd and Pb) acceptable daily intake of Cd and Pb for an adult (of 60 kg body weight) are 0.06 mg and 0.214 mg, respectively. Also, for an adult (60 kg body weight) the provisional tolerable daily intake for metals Fe, Zn, and Cu is 48 mg, 60 mg, and 3 mg, respectively [37,38]. For the calculations, we used the fact that a 300-g portion of fresh mushrooms per meal contains 30 g of dry matter [5]. In our experiment, metal concentrations in the analyzed samples of mushrooms were low and within the legal limit suggested by the Food and Agriculture Organization and World Health Organization standards, with the exception of *M. procera*. In the sample *M. procera*, Cd level was three times higher than allowed, and it was around 0.19 mg. Our findings are consistent with some other studies [39], which emphasized that *M. procera* can accumulate extremely high amounts of metals, and should therefore be omitted from human consumption because of possible risk to human health.

The tested mushroom extracts have moderate antioxidant activity. DPPH fast test (H-donor method) was used as a simple, fast, and principal test in antioxidant activity studies.

Table 5 – Reducing power of methanol extracts of *Lactarius deliciosus* and *Macrolepiota procera*.

Mushroom species	Absorbance (700 nm)			
	1000 μg/mL	500 μg/mL	250 μg/mL	125 μg/mL
Lactarius deliciosus	0.1189 ± 0.031	0.0947 ± 0.025	0.0445 ± 0.008	0.0372 ± 0.004
Macrolepiota procera	0.9001 ± 0.043	0.4453 ± 0.030	0.3722 ± 0.012	0.1182 ± 0.009
Ascorbic acid	2.113 ± 0.032	1.654 ± 0.021	0.0957 ± 0.008	0.0478 ± 0.008

Data are presented as mean ± standard deviation of three parallel measurements.

Table 7 – Minimum inhibitory concentration of methanol extracts of *Lactarius deliciosus* and *Macrolepiota procera*.

Mushroom species	*L. deliciosus*	*M. procera*	S	K
Bacillus cereus	2.5	5	0.016	–
Bacillus subtilis	5	5	0.016	–
Eschrichia coli	20	–	0.062	–
Proteus mirabilis	5	10	0.062	–
Staphylococcus aureus	20	–	0.031	–
Aspergillus flavus	–	–	–	0.312
Aspergillus niger	20	20	–	0.078
Candida albicans	5	5	–	0.039
Penicillium expansum	10	20	–	0.156
Penicillium chrysogenum	20	–	–	0.078
Alternaria alternata	5	10	–	0.078
Trichoderma viride	5	10	–	0.078
Cladosporium cladosporioides	5	10	–	0.039
Fusarium oxysporum	5	5	–	0.078
Mucor mucedo	20	–	–	0.156

Data are presented as the mean of three replicate (mg/mL).
K = ketoconazole; S = streptomycin.

Table 8 – Growth inhibitory effects of methanol extracts of *Lactarius deliciosus* and *Macrolepiota procera* on HeLa, A549, and LS174 cell lines.

Cell lines	HeLa	A549	LS174
Mushroom species	IC_{50} (μg/mL)		
Lactarius deliciosus	19.01 ± 0.38	33.05 ± 1.61	74.01 ± 2.04
Macrolepiota procera	29.39 ± 0.52	25.55 ± 1.42	68.49 ± 1.53
Cis-DDP	0.86 ± 0.33	4.91 ± 0.42	3.18 ± 0.29

Cis-DDP = cis-dichlorodiammine platinum.

Other methods such as superoxide anion radical and hydroxy radical scavenging activity are used to know and understand the mechanism of antioxidant activity. In this study, we used DPPH test just to verify that extracts exhibit antioxidant activity, and in future complex research we would use some other tests to explain the mechanisms of antioxidant activity. The intensity of antioxidant activity depended on the tested mushroom species and the solvent which was used for extraction. The differences in the antioxidant activity of various solvents may be a result of different capabilities to extract bioactive substances. When antioxidative capacities of the extracts are compared with their phenolic constituents, it could be concluded that the antioxidative nature of the extracts might depend on their phenolics. We found that the tested extracts exhibited the highest radical scavenging activity with the greatest amount of phenolic content. Several previous reports also demonstrated a significant relationship between total phenolic content and antioxidant activity [9,17,40,41]. Phenolic components are potential antioxidants. Phenolic compounds can donate hydrogen to free radicals to stop the chain reaction of lipid oxidation at the initial stage. This ability of phenolic compounds to scavenge radicals comes due to the presence of their phenolic hydroxyl groups [42].

In the literature there are several data for the antioxidant activity of *L. deliciosus* and *M. procera* [8,43–45]. They determined antioxidant activity for these species, but for other extraction solvents used. In this study, the antioxidant activity of selected mushrooms was confirmed by methanol extracts. Different extraction solvents, according to their polarity, may extract various compounds which can participate in great antioxidant activity. This means that synergistic effects may occur between these constituents leading to the pronounced antioxidant activity of mushroom extract (containing the antioxidant active components).

Similar to our results, numerous researchers found antimicrobial activity for *L. deliciosus* and *M. procera* [8,46–48]. In this study, methanol extract of tested mushrooms exhibited a stronger antimicrobial effect than previously reported for other extracts. The probable mechanisms of antimicrobial action of tested mushrooms are inhibition of cell wall synthesis, protein synthesis, or nucleid acid synthesis, like antibiotics, but less effective. The intensity of the antimicrobial effect depended on the species of mushroom, its concentration, and the tested organism. Differences in antimicrobial activity of tested mushroom are probably a consequence of the presence of different components with antimicrobial activity [49]. However, it is necessary to understand that extracts are mixtures of natural compounds, and their antimicrobial activity is not only a result of the different activities of individual components but may be the result of their interactions, which can have different effects on the overall activity of extracts.

Generally, slightly higher activities of mushrooms against gram-positive than gram-negative strains were observed. These results are comparable with previous results regarding the antimicrobial activity of mushrooms. For example, Nowacka et al [17] studied the antimicrobial activity of 31 wild growing fungi from Poland and they found that mushrooms were more active against gram-positive bacteria. In our experiments, the examined mushroom in the same concentrations showed a stronger antibacterial than antifungal activity. These results could be expected due to the fact that numerous tests proved that bacteria are more sensitive to antibiotics compared with fungi [50]. The reason for different sensitivity between the fungi and bacteria can be found in different transparencies of the cell wall. The cell wall of the gram-positive bacteria consists of peptidoglucans (mureins) and teichoic acids, while the cell wall of the gram-negative bacteria consists of lipopolysaccharides and lipopoliproteins, whereas, the cell wall of fungi consists of polysaccharides such as hitchin and glucan [51].

The importance of mushrooms as anticancer agents has been confirmed in recent years, which suggests that mushroom can be used as biological agents in the treatment of cancer. Some nonedible mushrooms like *Ganoderma lucidum* have a history of use for the treatment of various diseases including cancer. With similar content of putative effective anticancer compounds such as polysaccharides, proteoglycans, steroids, etc., one might predict that edible mushrooms would also demonstrate anticancer activity [52]. Because of that we wanted to examine edible mushrooms as potential anticancer agents. In this study, *M. procera* showed better anticancer effects on A549 and LS174 cell lines, while *L. deliciosus* exerted a stronger effect on HeLa cells. In literature, there are several studies about the anticancer activity of *L.*

deliciosus and *M. procera*. For instance, Kim et al [53] showed that heteropolysaccharides isolated from *L. deliciosus* exhibited significant antitumor activates *in vivo*. Also, water extract from *M. procera* inhibited tumor metastasis of colon 26-M3.1 cells compared with a tumor control [54]. The mechanism of action of the tested extracts is yet to be tested. It is known that anticancer compounds can play a crucial role as a reactive oxygen species inducer, mitotic kinase inhibitor, antimitotic, angiogenesis inhibitor, topoisomerase inhibitor, leading to apoptosis, and eventually checking cancer proliferation [55]. In this study, we have not proven whether extracts act selectively, or whether extracts affect cell viability of normal cells. Further research will be necessary in order to identify compounds responsible for the observed antitumor effects and to establish the reinforcement activities as well as to improve the selectivity.

In conclusion, it can be stated that tested mushroom extracts have certain levels of antioxidant, antimicrobial, and anticancer activities *in vitro* and also allowed metals concentrations, except Cd in *M. procera*. Therefore, people should be careful when it comes to the consumption of this mushroom because of the potential risk to human health. It is very possible that the antimicrobial and anticancer activities of *M. procera* in this study are a result of Cd toxicity. Identification of the active compounds of these mushroom species will lead to their evaluation as a commercial potential in medicine, food production, and the cosmetic industry.

Conflicts of interest

All contributing authors declare no conflicts of interest.

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