Food & Function

PAPER

View Article Online
View Journal | View Issue

Cite this: *Food Funct.*, 2019, **10**, 7678

Comparative investigation on edible mushrooms *Macrolepiota mastoidea*, *M. rhacodes* and *M. procera*: functional foods with diverse biological activities

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Received 19th August 2019,
Accepted 22nd October 2019

DOI: 10.1039/c9fo01900f

rsc.li/food-function

This study was oriented towards the investigation of the biological properties of three wild growing and edible *Macrolepiota* species (*M. mastoidea*, *M. rhacodes* and *Macrolepiota procera*) from Serbia. The results revealed that the mushrooms have a low caloric value; free sugars such as mannitol and trehalose were identified; oxalic and malic acids were predominant organic acids, while *p*-hydroxybenzoic and *p*-coumaric acids were identified as the main phenolic compounds. Also, they were a rich source of polyunsaturated fatty acids, which dominated over monounsaturated and saturated fatty acids. Three isoforms of tocopherols were identified and quantified: α-, β-, and δ-tocopherol. Regarding biological properties, all three species exhibited antioxidant potential, antimicrobial potential and cytotoxic activity within the different tumour cell lines tested. This study indicates that these species are indeed functional foods, due to the fact that they are edible, consumable and hold different pharmacological activities.

1. Introduction

Since time immemorial, mushrooms have been consumed all around the world for their health-beneficial effects and for being tasty. There are over 2000 mushrooms reported to be edible, including about 700 with some pharmaceutical activities.[1] Edible mushrooms were traditionally harvested in the wild; nevertheless, collection from wild areas is still important in some parts of the world.[2] It has been well demonstrated that wild mushrooms are one of the most important functional foods and good sources of nutraceuticals.[3,4]

The fungi of the genus *Macrolepiota* belong to the family Agaricaceae (Basidiomycota). Besides the pleasant taste, *Macrolepiota* species are also known for their nutritional value.[5,6]

Numerous pharmacological properties have been attributed to mushrooms, including antimicrobial, anticancer, antioxidant, antiviral, immunomodulatory, immunosuppressive, anti-allergic, anti-inflammatory, and anti-cholesterol activities.[7–9] Since mushrooms have been used as a food since ancient times and their biological functions are continuously explored nowadays, edible species are gaining extreme importance as functional foods or functional food ingredients. Microbial resistance to antibiotics, oxidative stress and cancer prevention is gaining more and more importance when linking natural product ingredients with the prevention of these medical conditions and illnesses. Antibiotic resistance is a natural phenomenon that predates the modern selective pressure of clinical antibiotic use. This fact solely implicates the search for new alternatives to the existing antibiotics.[8] The search for novel sources of antioxidants became of prime importance in contemporary science since oxidative stress may lead to various medical conditions targeting primarily nervous and cardiovascular systems.[10] Prevention of cancer, one of the most devastating diseases of the modern world, became important on a daily basis, since numerous risk factors could lead to the development of such a hardly curable state. Thus, discovering food with functions in cancer prevention is the main purpose of numerous scientific studies.[11]

The objectives of the present study were to investigate the nutritional composition of three *Macrolepiota* species: *M. mastoidea*, *M. rhacodes* and *M. procera* growing wild in

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Serbia, as well as to analyze hydrophilic and lypophilic compounds. Furthermore, we aimed to elucidate the biological properties of *Macrolepiota* species in order to classify these species as functional foods. For such purposes, antioxidant, antimicrobial and anticancer activities of methanolic extracts were tested.

2. Materials and methods

2.1. Mushroom material

Fresh fruiting bodies of *Macrolepiota mastoidea* (Fr.) Singer (Mm-151-2015) and *M. rhacodes* (Vittad.) Singer (Mr-171-2015) were collected in autumn 2015, in the village of Babušnica, in the area of Pirot city, south Serbia. Fruiting bodies of *M. procera* (Scop. ex Fr.) Sing. (Mp-171-2012) were collected from Divčibare mountain, in central Serbia, in October 2012 and authenticated by Dr Jasmina Glamočlija (Institute for Biological Research, the University of Belgrade, Serbia). Samples were prepared as previously described.[12]

2.2. Nutritional value and chemical composition of wild *Macrolepiota* species

2.2.1. Macronutrients. The samples were analyzed for chemical composition (moisture, proteins, fat, carbohydrates and ash) using the AOAC procedures.[13]

2.2.2. Hydrophilic compounds

2.2.2.1. Free sugars. Sugars were analyzed by high performance liquid chromatography (HPLC) using a refraction index (RI) detector. This assay was conducted according to a previously described methodology.[14] Quantification was carried out by the internal standard method, and the results are expressed in mg per g of lyophilized decoctions.

2.2.2.2. Organic acids. Organic acids were determined by ultra-fast liquid chromatography (UFLC, Shimadzu 20A series) coupled with a photo diode array (PDA) detector, after dissolving both water and ethanol extracted powders in metaphosphoric acid (4%), at a known concentration.[15] The organic acids were quantified by the comparison of the area of their peaks recorded at 215 nm with calibration curves obtained from commercial standards of each compound. The results were expressed in mg per g of the extract.

2.2.2.3. Phenolic compounds. The analysis was performed by the same UFLC equipment described for organic acids, according to a previously described procedure.[16] Detection was carried out with a photodiode array (PDA) detector, using 280 nm as the preferred wavelength. The phenolic acids and related compounds were quantified by comparison of the area of their peaks with calibration curves obtained from commercial standards of each compound. The results were expressed in mg per g of the extract.

2.2.3. Lipophilic compounds

2.3.3.1. Fatty acids. Fatty acids were determined by gas–liquid chromatography with a flame ionization detection (GC-FID)/capillary column as described previously.[17] Split injection (1 : 40) was carried out at 250 °C. Fatty acid identification was made by comparing the relative retention times of FAME peaks from samples with standards. The results were recorded and processed using the Clarity Data Apex 4.0 Software and expressed in the relative percentage of each fatty acid.

2.2.3.2. Tocopherols. The analysis was carried out using the HPLC equipment described above coupled to a fluorescence detector (FP-2020; Jasco) programmed for excitation at 290 nm and emission at 330 nm according to a previously described methodology.[18] The compounds were identified by chromatographic comparisons with authentic standards. Quantification was based on the fluorescence signal response of each standard, using the IS (tocol) method and by using calibration curves obtained from commercial standards of each compound. The results were expressed in mg per 100 g of dry weight.

2.3. Extract preparation of the wild *Macrolepiota* species

The extracts were prepared as previously described.[12]

2.4. Evaluation of the antioxidant activity of wild *Macrolepiota* species

The methanolic extracts were re-dissolved in methanol at 20 mg mL^{-1}. Successive dilutions from the stock solution were made and subjected to *in vitro* assays.[18] Four different assays were performed: reducing power, DPPH radical-scavenging activity, inhibition of β-carotene bleaching or β-carotene/linoleate assay and thiobarbituric acid reactive substance (TBARS) assay. The sample concentrations (mg mL^{-1}) providing 50% of antioxidant activity or 0.5 of absorbance (EC_{50}) were calculated from the graphs of antioxidant activity percentages (DPPH, β-carotene/linoleate and TBARS assays) or absorbance at 690 nm (ferricyanide/Prussian blue assay) against sample concentrations. Trolox was used as a positive control.

2.5. Evaluation of the antimicrobial activity of the wild *Macrolepiota* species

The following bacteria strains were used for antimicrobial testing: *Bacillus cereus* (clinical isolate), *Enterobacter cloacae* (ATCC 35030), *Staphylococcus aureus* (ATCC 6538), methicillin-resistant *S. aureus* (MRSA), *Escherichia coli* (ATCC 35210), antibiotic resistant *E. coli* (H2b), *Pseudomonas aeruginosa* (ATCC 27853) and antibiotic resistant *P. aeruginosa* (IBRS P001). The following micromycetes were used: *Aspergillus fumigatus* (ATCC 9197), *Aspergillus niger* (ATCC6275), *Aspergillus ochraceus* (ATCC 12066), *Aspergillus versicolor* (ATCC 11730), *Penicillium funiculosum* (ATCC 10509), *Penicillium ochrochloron* (ATCC 9112), *Penicillium verrucosum* var. *cyclopium* (food isolate) and *Trichoderma viride* (IAM 5061).

For the determination of the antimicrobial activity of methanol extracts of *Macrolepiota* species, a modified microdilution method was used.[12,19–21] The results were presented as minimum inhibitory concentrations (MIC), minimum bactericidal concentrations (MBC) and minimum fungicidal concentrations (MFC).

2.6. Evaluation of antibiotic activity – synthesis inhibition of pyocyanin in *P. aeruginosa*

The modified method for the synthesis inhibition of pyocyanin was used as previously described by authors Sandy & Foong-Yee[22] and Glamočlija *et al.*[12]

2.7. Cytotoxicity and hepatotoxicity of wild *Macrolepiota* species

The cytotoxic properties were evaluated in human tumour cell lines and in a non-tumour liver cell primary culture. The extracts of three different *Macrolepiota* species were dissolved in water/DMSO at 8 mg mL^{-1}. The positive control was elipticine.

A hepatic cell line, designated as PLP2, was used to evaluate the cytotoxicity.[23] All results were expressed in GI_{50} values (sample concentration that inhibited 50% of the net cell growth; concentrations used in the assay were in the range of 6.25 μg mL^{-1}–400 μg mL^{-1}).

2.8. Statistical analysis

All analyses regarding chemical composition and biological activities were performed in triplicate; each replicate was quantified also three times. Data were expressed as mean and standard deviation. The statistical analysis was performed using SPSS v. 23.0 (IBM Corp., Armonk, NY, USA), through one-way ANOVA and the Tukey HSD test ($\alpha = 0.05$); thus, if the presence of less than three samples was recorded, the results were treated by the Student *t*-test ($p = 0.05$).

Table 1 Macronutrients and hydrophilic compounds in the studied *Macrolepiota* spp. (mean ± SD)[a]

	Macrolepiota mastoidea	*Macrolepiota rhacodes*	*Macrolepiota procera*
Nutritional value (g per 100 g dw)			
Fat	3.62 ± 0.03a	3.8 ± 0.1a	1.9 ± 0.2b
Protein	32.36 ± 0.05b	41.42 ± 0.05a	29.2 ± 0.5c
Ash	12.44 ± 0.03b	13.9 ± 0.2a	8.5 ± 0.1c
Total carbohydrates	51.58 ± 0.01b	40.9 ± 0.2c	60.3 ± 0.2a
Energetic value (kcal per 100 g dw)	368.37 ± 0.01b	363.6 ± 0.2c	375.7 ± 0.4a
Sugars (g per 100 g dw)			
Mannitol	4.91 ± 0.08b	1.77 ± 0.01c	12.7 ± 0.4a
Trehalose	2.93 ± 0.06b	2.45 ± 0.01c	7.01 ± 0.04a
Total sugars	7.8 ± 0.2b	4.22 ± 0.02c	19.7 ± 0.4a
Organic acid (g per 100 g dw)			
Oxalic acid	0.045 ± 0.005c	0.696 ± 0.001b	0.90 ± 0.01a
Malic acid	0.32 ± 0.01c	3.07 ± 0.04b	11.4 ± 0.1a
Citric acid	nd	nd	5.5 ± 0.7
Fumaric acid	tr	tr	0.49 ± 0.01
Total organic acids	0.37 ± 0.01c	3.77 ± 0.04b	18.3 ± 0.9a
Phenolic acid (mg per 100 g dw)			
p-Hydroxybenzoic acid	0.109 ± 0.002c	0.251 ± 0.007b	1.045 ± 0.002a
p-Cumaric acid	0.040 ± 0.002b	0.016 ± 0.001c	0.225 ± 0.001a
Total phenolic compounds	0.150 ± 0.001c	0.266 ± 0.006b	1.271 ± 0.002a
Cinnanic acid	0.036 ± 0.001c	0.058 ± 0.001b	0.952 ± 0.001a

[a] nd, not detected; dw, dry weight. In each column, different letters mean significant differences between species ($p < 0.05$).

3. Results and discussion

3.1. Macronutrients and hydrophilic compounds in wild *Macrolepiota* species

Macronutrient composition and chemistry of hydrophilic compounds (sugars, organic acids and phenolic acids) for the studied wild *Macrolepiota* mushroom species are shown in Table 1. *Macrolepiota* species are used in the diet after thermal processing, mostly after cooking. A previous study has demonstrated that cooked samples have lower nutrient concentrations and lower antioxidant activities.[24]

Carbohydrates were the most abundant constituents in all of the tested mushrooms. Comparative investigation showed that *M. procera* had the highest amount of carbohydrates (60.3 ± 0.2 g per 100 g dw), followed by *M. mastoidea* (51.58 ± 0.01 g per 100 g dw) and *M. rhacodes* (40.9 ± 0.2 g per 100 g dw). Proteins were present in larger amounts in *M. rachodes* and *M. mastoidea*, while *M. procera* had the lowest amount. The studied mushrooms were poor in ash and fat contents, concurring with the literature[3,8,10,25] that has recently been reported. Energetic contributions of the samples were in the range of 363.6–375.7 kcal per 100 g dw, (Table 1), indicating that the studied species are good choices for low-caloric diet. These results are in agreement with previous studies pointing to high protein and carbohydrate contents and low fat characteristics of the different *Macrolepiota* species.[24–26] Nutritional value of *M. rhacodes* has been studied previously by Manjunathan *et al.*[25] and the study revealed that the species is a good source of carbohydrates and proteins, with a low amount of fats which is in accordance with the results obtained in this study. Our results are in agreement with another previous report[26] on nutritional analysis carried out on three mushrooms namely, *M. dolichaula*, *M. procera*, and *M. rhacodes*. These *Macrolepiota* mushrooms were rich in nutrients, nutraceutical components and minerals and low in fat,[26] as well as *Macrolepiota* species presented in our current work.

Free sugars found in the studied *Macrolepiota* species were mannitol and trehalose. *M. procera* contained higher amounts of mannitol and total sugars (12.5 ± 0.8 g per 100 g dw and 19 ± 1 g per 100 g dw, respectively) when compared to the other two studied species (Table 1). The amounts of trehalose and mannitol quantified for our samples are in agreement with previously published results referring to *M. procera*.[24,27] Other free sugars, melezitose and fructose, were found in the samples *M. procera* from Portugal, while they were not detected in our tested samples.[24,27] Mannitol and trehalose are important sugars with established biological functions. Regarding mannitol, it has four Food and Drug Administration (FDA)-approved uses: for the reduction of intracranial pressure and brain mass, for the reduction of intraocular pressure, for promoting diuresis for acute renal failure and for promoting the excretion of toxic substances, materials, and metabolites.[28–31] It has been shown that trehalose can protect mammalian tissues from desiccation as well as from oxidative stress.[32]

The organic acids detected in higher amounts were malic and oxalic acids. Fumaric acid was detected in lower amounts; there were traces in species *M. mastoidea* and *M. rhacodes*, while a higher value was recorded for *M. procera*. Citric acid was found only in *M. procera*. *M. procera* contained the highest amount of all organic acids, in comparison with *M. mastoidea* and *M. rhacodes* (Table 1), with similar values to those reported by Barros *et al.*[15] for the same species. A previous study by Tang *et al.*[33] found that both citric acid and L-malic acid have protective effects on myocardial ischemia/reperfusion injury linking the activity of these organic acids to their anti-inflammatory, antiplatelet aggregation and direct cardiomyocyte protective effects.

M. procera was rich in phenolic acids when compared to *M. rhacodes* and *M. mastoidea*, containing a much higher concentration of *p*-hydroxybenzoic and *p*-coumaric acids, as also the related compound cinnamic acid (Table 1). Jose & Radhamany[34] qualitatively detected phenolic acids in the methanolic extract of *M. mastoidea*, such as *p*-hydroxybenzoic acid, vanillic acid, gentisic acid, coumarin and *p*-coumaric acid. The differences in composition regarding phenolic compounds described by Jose and Radhamany[34] and the results presented in this study could be attributed to different extraction techniques used. On the other hand, Nowacka *et al.*[35] identified only protocatechuic acid in the ethanolic extracts of *M. procera* from Poland. Nonetheless, the ethanolic extract of *M. procera* from Portugal revealed only the presence of cinnamic acid.[36] Our results indicate a slightly different composition in comparison with the studies by Nowacka *et al.*[35] and Taofiq *et al.*,[36] which is attributed to different solvents used for the extraction (ethanol and methanol). Phenolic acids are well known for their antioxidant properties,[37] but they also revealed antimicrobial activity[24,38,39] with the potential against microbial multi-resistances.[40]

3.2. Lypophilic compounds in wild *Macrolepiota* species

The results of the lipophilic compounds obtained for three *Macrolepiota* species are shown in Table 2.

Regarding the fatty acid composition of the studied species, linoleic acid was found in higher amounts (C18:2n6, PUFA), followed by palmitic (C16:0, SFA) and oleic (C18:1n9, MUFA) acids. All tested mushrooms were rich in polyunsaturated fatty acids (PUFA), while saturated (SFA) and monounsaturated (MUFA) fatty acids were detected in lower amounts. PUFA have their important role in the prevention of cardiovascular disease, diabetes, depression, Alzheimer's disease, cancer, and dementia, although there are some controversies about their effects.[41] Comparing between species, *M. rhacodes* had the significant highest content of PUFA; *M. procera* possessed the significantly largest amount of SFA, while *M. mastoidea* gave the highest percentage of MUFA (Table 2). In a previous study by Fernandes *et al.*,[42] the fatty acids of *M. procera* included 24 compounds, linoleic, palmitic and oleic acids being the major compounds. Despite some differences regarding individual fatty acids, the general percentages obtained for SFA, MUFA and PUFA are similar to those presented in other studies.[24,27,43]

Table 2 Lipophilic compounds in the studied *Macrolepiota* spp. (mean ± SD)[a]

	Macrolepiota mastoidea	*Macrolepiota rhacodes*	*Macrolepiota procera*
C6:0	0.044 ± 0.004	0.027 ± 0.002	0.46 ± 0.08
C8:0	0.027 ± 0.002	0.027 ± 0.001	0.09 ± 0.02
C10:0	0.028 ± 0.001	0.035 ± 0.002	0.03 ± 0.01
C12:0	0.074 ± 0.001	0.084 ± 0.004	0.07 ± 0.02
C14:0	0.29 ± 0.01	0.369 ± 0.004	0.04 ± 0.07
C14:1	0.008 ± 0.001	nd	0.030 ± 0.001
C15:0	0.28 ± 0.01	0.53 ± 0.01	0.41 ± 0.05
C16:0	15.84 ± 0.05	10.00 ± 0.07	21 ± 1
C16:1	1.55 ± 0.01	0.71 ± 0.01	1.4 ± 0.1
C17:0	0.150 ± 0.002	0.157 ± 0.009	0.19 ± 0.01
C18:0	0.88 ± 0.01	1.16 ± 0.01	1.96 ± 0.05
C18:1n9	11.10 ± 0.01	6.06 ± 0.07	8.3 ± 0.2
C18:2n6	66.59 ± 0.03	78.62 ± 0.07	64 ± 1
C18:3n3	0.120 ± 0.001	0.081 ± 0.005	0.17 ± 0.01
C20:0	0.170 ± 0.001	0.112 ± 0.008	0.09 ± 0.01
C20:1	0.080 ± 0.004	0.025 ± 0.001	0.07 ± 0.01
C20:2	0.094 ± 0.001	0.12 ± 0.01	0.08 ± 0.01
C20:3n3 + C21:0	0.80 ± 0.04	0.32 ± 0.01	0.26 ± 0.02
C20:5n3	0.32 ± 0.01	0.095 ± 0.006	0.54 ± 0.05
C22:0	0.67 ± 0.01	0.75 ± 0.04	0.18 ± 0.02
C23:0	0.055 ± 0.001	0.062 ± 0.001	0.05 ± 0.01
C24:0	0.65 ± 0.01	0.66 ± 0.01	0.58 ± 0.08
C24:1	0.17 ± 0.01	nd	0.09 ± 0.01
Total SFA (% of total FA)	19.16 ± 0.06b	13.97 ± 0.03c	25 ± 1a
Total MUFA (% of total FA)	12.91 ± 0.01a	6.79 ± 0.07c	9.92 ± 0.09b
Total PUFA (% of total FA)	67.92 ± 0.06b	79.2 ± 0.1a	65 ± 1c
Tocopherols (μg per 100 g dw)			
α-Tocopherol	0.42 ± 0.03b	0.21 ± 0.01c	1.1 ± 0.1a
β-Tocopherol	82 ± 2[b]	61 ± 1[b]	nd
δ-Tocopherol	Nd	nd	26.1 ± 2
Total tocopherols	82 ± 2a	61 ± 1b	27 ± 2c

[a] nd, not detected; dw, dry weight. [b] Means statistical differences between two samples obtained by the Student *t*-test. In each line, different letters mean significant differences between species ($p < 0.05$).

For the studied mushrooms, three tocopherol isoforms were detected, with *M. mastoidea* and *M. rachodes* being the ones with higher total tocopherol content, respectively. Although Fernandes *et al.*[27] found that β-tocopherol predominated in the dried samples of *M. procera*, β-tocopherol was the most abundant isoform found in *M. mastoidea* and *M. rhacodes* tested in this study, while δ-tocopherol was only detected in *M. procera*. α-Tocopherol was found in lower amounts in all of the tested *Macrolepiota* species (Table 2). It was previously established that tocopherols are good antioxidants indicating that α-tocopherol mainly inhibits the production of new free radicals, while γ-tocopherol traps and neutralizes the existing free radicals.[44]

3.3. Antioxidant activities of wild *Macrolepiota* species

The results of the antioxidant activities of the tested methanolic extracts of *Macrolepiota* species, carried out by four different assays measuring free radical scavenging activity, reducing power and lipid peroxidation inhibition, are pre-

Table 3 Antioxidant activity in the studied *Macrolepiota* spp

		Macrolepiota mastoidea	*Macrolepiota rhacodes*	*Macrolepiota procera*
Reducing power	Folin–Ciocalteu (mg GAE per g extract)	17.35 ± 0.05b	39.5 ± 0.8a	13.9 ± 0.6c
	Ferricyanide/Prussian blue (EC_{50}; mg mL^{-1})	2.10 ± 0.01a	1.62 ± 0.01b	1.61 ± 0.01b
Radical scavenging activity	DPPH scavenging activity (EC_{50}; mg mL^{-1})	5.4 ± 0.2a	3.4 ± 0.2b	3.7 ± 0.2b
	β-Carotene/linoleate (EC_{50}; mg mL^{-1})	1.85 ± 0.09a	1.08 ± 0.08b	0.48 ± 0.04c
Lipid peroxidation inhibition	TBARS (EC_{50}; mg mL^{-1})	0.47 ± 0.02a	0.41 ± 0.03a	0.27 ± 0.02b

Concerning the Folin–Ciocalteu assay, higher values mean higher reducing power; for the other assays, the results are presented in EC_{50} values, what means that higher values correspond to lower reducing power or antioxidant potential. EC_{50}: Extract concentration corresponding to 50% of antioxidant activity or 0.5 of absorbance for the ferricyanide/Prussian blue assay.

sented in Table 3. All studied mushroom extracts possessed antioxidant potential, but *M. procera* was more potent in most of the cases. The highest total phenolic content was shown for the methanolic extract of *M. rhacodes* (39.5 ± 0.7 mg GAE per g extract). *M. rhacodes* and *M. procera* exhibited the same reducing power when evaluated through the ferricyanide/Prussian blue assay (EC_{50} = 1.62 ± 0.01 and 1.61 ± 0.01 mg mL^{-1}, respectively) and possessed similar results for DPPH radical scavenging activity (EC_{50} = 3.4 ± 0.2 and 3.7 ± 0.2 mg mL^{-1}, respectively). The methanolic extract of *M. procera* exhibited the highest lipid peroxidation inhibition, since it presented the lowest EC_{50} values for β-carotene/linoleate and TBARS assays (Table 3). *M. mastoidea* showed the lowest antioxidant activity in comparison with the other two studied mushrooms.

M. procera (EC_{50} 3.7 mg mL^{-1};[27] EC_{50} 17.76 mg dry extract per mg DPPH;[35] EC_{50} 0.331 mg mL^{-1} (ref. 45)) and *M. mastoidea* (EC_{50} 3.7 mg mL^{-1})[34] have been previously studied and have demonstrated a good DPPH radical scavenging activity, which is comparable with our results for *M. procera* (EC_{50} 3.7 mg mL^{-1}) and *M. mastoidea* (EC_{50} 5.4 mg mL^{-1}). Barros *et al.*[24] have shown the antioxidant potential of *M. mastoidea* and *M. procera* dried mushroom samples extracted with methanol: DPPH scavenging activity (EC_{50} 8.18 mg mL^{-1} and 5.38 mg mL^{-1}, respectively), reducing power (EC_{50} 4.35 mg mL^{-1} and 4.18 mg mL^{-1}, respectively), β-carotene/linoleate (EC_{50} 6.48 mg mL^{-1} and 5.19 mg mL^{-1}, respectively), TBARS assay (EC_{50} 24.20 mg mL^{-1} and >50 mg mL^{-1}, respectively). These results are comparable with the ones obtained in our study (Table 3). The ethanolic extract of *M. procera* was studied for its anti-DPP. The observed antioxidant activity is mostly a function of the bioactive constituents of the mushroom species, such as phenolic compounds, tocopherols and organic acids, as generalized for other mushroom species.[10]

3.4. Antimicrobial activities of wild *Macrolepiota* species

Methanolic extracts of *M. mastoidea*, *M. rhacodes* and *M. procera* showed antimicrobial activity against all tested strains, but at different levels (Table 4). MIC obtained in microdilution assay (section 2.5) was between 0.20 and 4.00 mg

Table 4 Antimicrobial activity of the methanolic extracts of the studied *Macrolepiota* spp. in mg mL^{-1}

Bacteria	*Macrolepiota mastoidea* MIC/MBC	*Macrolepiota rhacodes* MIC/MBC	*Macrolepiota procera* MIC/MBC	Streptomycine MIC/MBC	Ampicillin MIC/MBC
Bacillus cereus	0.20/0.25	0.35/0.50	1.50/2.00	0.0015/0.0030	0.006/0.025
Staphylococcus aureus	0.75/1.00	0.75/1.00	0.75/1.00	0.006/0.012	0.012/0.025
MRSA	2.00/4.00	2.00/4.00	2.00/4.00	0.100/—	—/—
Escherichia coli	1.00/2.00	2.00/4.00	1.50/2.00	0.050/0.100	0.100/0.200
Rez. E. coli	0.75/1.00	2.00/4.00	3.00/4.00	0.100/0.200	0.200/—
Pseudomonas aeruginosa	2.00/4.00	2.00/4.00	2.00/4.00	0.025/0.050	0.050/0.100
Rez. P. aeruginosa	1.00/2.00	2.00/4.00	2.00/4.00	0.050/0.100	0.200/—
Enterobacter cloacae	1.00/2.00	1.00/2.00	3.00/4.00	0.003/0.006	0.006/0.012

Fungi	*Macrolepiota mastoidea* MIC/MFC	*Macrolepiota rhacodes* MIC/MFC	*Macrolepiota procera* MIC/MFC	Bifonazole MIC/MFC	Ketoconazole MIC/MFC
Aspergillus fumigatus	1.00/2.00	4.00/8.00	2.00/4.00	0.150/0.200	0.200/0.500
Aspergillus versicolor	2.00/4.00	1.00/2.00	1.00/2.00	0.100/0.200	0.200/0.500
Aspergillus ochraceus	0.50/1.00	1.00/2.00	0.50/1.00	0.150/0.200	0.150/0.200
Aspergillus niger	1.00/2.00	1.00/2.00	1.50/2.00	0.150/0.200	0.200/0.500
Trichoderma viride	0.25/0.50	0.50/1.00	0.50/1.00	0.150/0.200	1.000/1.500
Penicillium funiculosum	1.00/2.00	0.75/1.00	1.00/2.00	0.200/0.250	0.200/0.500
Penicillium ochrochloron	1.00/2.00	1.00/2.00	1.00/2.00	0.200/0.250	1.000/1.500
Penicillium verrucosum var. *cyclopium*	2.00/4.00	2.00/6.00	2.00/4.00	0.100/0.200	0.200/0.300

— no activity; Rez – resistant.

mL^{-1}, while MBC was 0.25–4.00 mg mL^{-1} and MFC was 0.50–8.00 mg mL^{-1} (Table 4). The most sensitive species tested were *B. cereus* and *T. viride* for all tested extracts, while the most resistant strains were resistant *S. aureus* (MRSA), *P. aeruginosa* (IBRS P001), *A. fumigatus* and *P. verrucosum* var. *cyclopium*. The best antibacterial activity was obtained for the *M. mastoidea* methanolic extract, while the lowest activity was recorded for the methanolic extract of *M. procera*, against all the tested bacteria. The best antifungal effects were achieved with *M. mastoidea* and *M. procera* extracts, while *M. rhacodes* showed the lowest values. The tested methanol extracts exhibited a similar or lower activity compared to commercial antibiotics and fungicides, while against some resistant bacteria (*S. aureus*, *E. coli* and *P. aeruginosa*) all extracts possessed higher activity when compared to ampicillin (Table 4).

The methanol extracts of *M. procera* from Turkey were found to be able to inhibit the growth of Gram (+) and Gram (−) bacteria, as well as yeasts in the disc diffusion method.[39] The antimicrobial potential of the same extract from specimens collected in Serbia possessed slightly weaker activity. It inhibited three species of bacteria and seven tested fungi.[45] The ethanolic extracts of *M. procera* from Poland revealed moderate antimicrobial activity against several tested bacteria.[35] On the other hand, the previous study[46] indicated that *M. procera* did not possess activity against methicillin resistant strain *Staphylococcus aureus* (MRSA),[46] while we obtained activity against MRSA. In the current study, all the methanolic extracts of *Macrolepiota* species inhibited resistant bacteria *S. aureus*, *E. coli* and *P. aeruginosa* (Table 4). Our study is in agreement with the results previously reported by Alves *et al.*[47] Mushroom extracts could be an alternative to synthetic antimicrobials against pathogenic micro-organisms resistant to conventional treatments. The results on the activity of mushroom extracts against resistant bacteria investigated herein are of extreme importance since antimicrobial resistance has become an emerging medical problem during the recent decades. Among others, World Health Organization includes infectious diseases caused by pathogenic microorganisms as one of the top five major global causes of fatal outcome.[48]

Pyocyanin is a redox-active toxin produced by *P. aeruginosa*. These pigments are involved in quorum sensing, virulence, and iron acquisition.[49] Recently, a study found that the inhibited biosynthesis of pyocyanin leads to a decrease in *P. aeruginosa* pathogenicity *in vitro*. This suggests that pyocyanin is most responsible for the initial colonization of *P. aeruginosa in vivo*.[50] Our results on pyocyanin inhibition demonstrated that the extract of *M. mastoidea* had an influence on the reducing production of pyocyanin similar to antibiotics (Fig. 1).

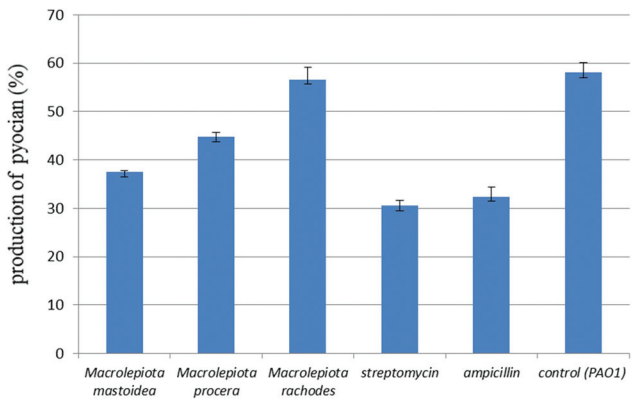

Fig. 1 Reduction of *P. aeruginosa* pyocyanin production by the methanolic extracts of *Macrolepiota* species, tested at sub-MICs (mg mL^{-1}).

3.5. Cytotoxicity of wild *Macrolepiota* species

The effect of methanolic extracts on the growth of four human tumor cell lines (MCF-7, NCI-H460, HeLa and HepG2) was determined, and the values of GI_{50} are detailed in Table 5. The extract of *M. mastoidea* revealed activity against HepG2 with GI_{50} = 181 μg mL^{-1}, being less effective against MCF-7 and HeLa, and the least effective against NCI-H460.

The tested extracts of *M. rhacodes* and *M. procera* did not show an effect against the tested cell lines at the maximum dose 400 μg mL^{-1}. Only the extract of *M. rhacodes* possessed activity against the tumor cell line MCF-7. The methanolic extracts of *Macrolepiota* species did not have cytotoxic effects towards non-tumor liver primary cells at the tested concentrations (PLP2; GI_{50} > 400 μg mL^{-1}; Table 5). It seems that *M. mastoidea* has the greatest potential to inhibit the proliferation of the hepatocellular carcinoma cell line. The results on *M. mastoidea* activity against the HepG2 cell line are of particular importance, since hepatocellular carcinoma is the most common primary liver malignancy and is a leading cause of cancer-related death worldwide.[51] The results are gaining more

Table 5 Antitumor activity and hepatotoxicity of the methanolic extracts of the studied *Macrolepiota* spp. (mean ± SD)

	Macrolepiota mastoidea	*Macrolepiota rhacodes*	*Macrolepiota procera*	Elipticine
Toxicity for human tumor cell lines				
MCF-7 (breast carcinoma) (GI_{50}, μg mL^{-1})	237 ± 14[a]	305.49 ± 19[a]	>400	0.91 ± 0.04
NCI-H460 (non-small cell lung cancer) (GI_{50}, μg mL^{-1})	287 ± 10	>400	>400	1.03 ± 0.09
HeLa (cervical carcinoma) (GI_{50}, μg mL^{-1})	250 ± 9	>400	>400	1.91 ± 0.06
HepG2 (hepatocellular carcinoma) (GI_{50}, μg mL^{-1})	181 ± 16	>400	>400	1.1 ± 0.2
Hepatotoxicity				
PLP2 (GI_{50}, μg mL^{-1})	>400	>400	>400	3.2 ± 0.7

[a] Means statistical differences between two samples obtained by the Student *t*-test.

importance, since none of the tested extracts exhibited hepatotoxicity on liver primary cells, indicating that the extracts are non-toxic to healthy cells and that they selectively act on tumour cell lines.

In the literature, there are several studies about the anticancer and cytotoxic activity for *M. procera*.[45] The study by Kosanić *et al.*[45] showed that the methanolic extract of *M. procera* exhibited the cytotoxic activity on human epithelial carcinoma HeLa cell lines, human lung carcinoma A549 cell lines, and human colon carcinoma LS174 cell lines. The *M. procera* methanolic extract showed cytotoxic activity with IC_{50} values ranging from 25.55 to 68.49 μg mL^{-1}.[45] Moreover, Arora *et al.*[52] demonstrated that the ethanolic extract of *M. procera* revealed the cytotoxic properties upon COLO-205 cancer cells.

Nowadays, medicinal mushrooms are used in many different ways as dietary foods, dietary supplement products, a new class of drugs (mushroom pharmaceuticals), natural biocontrol agents, cosmeceuticals and others.[53] Thus, the studied mushroom species could meet most of the above properties.

4. Conclusions

It was shown that the species are rich sources of proteins with a low caloric value. Mannitol and trehalose were the most abundant free sugars in the investigated species. Regarding phenolic compounds, *p*-hydroxybenzoic and *p*-coumaric acids were detected, while malic and citric acids were representatives of organic acids found in the specimens. PUFA were the most dominant fatty acids in all of the investigated samples. Tocopherols were detected in the mushrooms, with α-tocopherol present in all of the species but with the lowest concentration. The tested mushrooms were revealed to be bioactive, against possessing antimicrobial activity against resistant bacteria and therefore can be useful as potential natural antimicrobial agents. All of the tested mushroom species possessed notable antioxidant potential, tested by different assays. As for the antimicrobial activity, the most important findings are related to the antibacterial potential of *Macrolepiota* extracts against resistant strains of *Escherichia*, *Pseudomonas* and *Staphylococcus*. Regarding the studies on cytotoxicity, the most promising effect was noted for *M. mastoidea* on hepatocellular carcinoma cell lines. These wild growing mushrooms might be used directly in the diet to promote health, since it was demonstrated that the investigated edible *Macrolepiota* species are indeed functional foods.

Funding sources

The authors are grateful to the Ministry of Education, Science and Technological Development of Serbia for financial support (grant no. 173032). The authors are also grateful to the Foundation for Science and Technology (FCT, Portugal) and the FEDER under Programme PT2020 for financial support to the CIMO (UID/AGR/00690/2019); to the national funding by FCT, P.I., through the institutional scientific employment program-contract for L. Barros, A. Fernandes and R. C. Calhelha's contracts.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

The authors kindly thank Dejan Stojanović Zmaj from Babušnica, for the harvest of the *Macrolepiota mastoidea* samples.

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