

## Antioxidant properties of edible mushrooms occurring in Slovakia

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### Summary

Edible mushrooms are appreciated as a valuable food due to their nutritional qualities. They contain biologically active compounds, e.g. polyphenols, flavonoids, proteins, saccharides and vitamins with antioxidant activity that are known to protect cells from damage and apoptosis. This study was focused on the antioxidant properties of ten selected edible mushrooms occurring in Slovakia, namely, *Agaricus arvensis*, *Boletus edulis* (Bull.), *Cantharellus cibarius* (Fr.), *Imleria badia* (Fr.), *Lactarius volemus* (Fr.), *Leccinum pseudoscabrum*, *Lentinula edodes*, *Lepista nuda* (Bull.), *Morchella esculenta* (L.) and *Pleurotus ostreatus*. Free radical-scavenging activity (RSA) using 2,2-diphenyl-1-picrylhydrazyl (DPPH), total phenolics content, total flavonoids content and ascorbic acid, the latter determined using ultra-high performance liquid chromatography, were studied in methanolic and aqueous extracts obtained from the above-mentioned mushrooms. All the extracts exhibited RSA (in the range of 14.2–88.2 % for methanolic extracts and 48.9–91.4 % for water extracts) due to the presence of phenolics. These were contained in methanolic extracts at 398.9–4 455.4 mg·kg<sup>-1</sup> dry weight (DW) and in aqueous extracts at 2 314.9–15 171.9 mg·kg<sup>-1</sup> DW (expressed as gallic acid equivalents). Flavonoid compounds were contained in methanolic extracts at 1 136.7–1 332.0 mg·kg<sup>-1</sup> DW and in aqueous extracts at 1 247.3–1 735.0 mg·kg<sup>-1</sup> DW (expressed as quercetin equivalents). Ascorbic acid was contained in the methanolic extracts at 525.42–2 752.75 mg·kg<sup>-1</sup> DW and in aqueous extracts at 26.89–2 178.96 mg·kg<sup>-1</sup> DW.

### Keywords

mushroom; phenolic; radical-scavenging activity; flavonoid; ultra-high performance liquid chromatography

There is a wide spectrum of mushroom species that are rich in proteins, minerals, vitamins and antioxidants. Mushrooms represent a very small portion of human diet but their consumption increases due to their pleasant taste, texture and aroma. Their functional benefits are due to the presence of bioactive compounds [1, 2].

Due to the content of functional proteins, mushrooms are used as unconventional meat alternatives [2]. Polyunsaturated fatty acids and vitamins that are contained at nutritionally significant levels (B1, B2, B12, C, D and E) are also significant assets [3, 4]. The low glycemic index (GI) and high mannitol content make mushrooms suitable for the people with diabetes, while the low content of sodium make them appropriate for individuals suffering from hypertension. The contents

of magnesium, calcium, zinc, sodium, potassium and phosphorus enhance their health benefits as these are considered vital ions and co-factors of enzymes [5, 6].

Mushrooms are widely used in the food industry, for example to produce fermented meal or as food dyes [7]. In addition to their rich chemical composition, some compounds in mushrooms have a high antioxidant potential and thus reduce the level of oxidative stress [8]. Oxidative stress is caused by many generally known factors, including chemical (e.g. toxic chemicals presented in cigarette smoke or in fried foods), physical (UV irradiation) or biological ones (aging) [9, 10]. It often causes damage to biologically important molecules and cells in the body, potentially leading to many diseases [11]. Several studies indicated a correla-

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tion between the oxidative stress and the diseases. Free radicals causing oxidative stress can participate in the processes leading to the development of chronic non-infectious diseases (Down's syndrome, schizophrenia, manic-depressive psychosis, cystic fibrosis of the lungs, and others) [11]. Oxidative stress is considered to be one of the initiation factors of atherosclerosis [12], cardiovascular diseases [12, 13] and stroke [14, 15]. It is also involved in the development and progression of Alzheimer's disease [16] and Parkinson's disease [17]. Due to the excessive formation of free radicals, especially reactive forms of oxygen (hydroxyl  $\cdot\text{OH}$ , superoxide ion  $\text{O}_2^{\cdot-}$  and nitrogen ( $\text{NO}^\cdot$ ,  $\text{NO}_2^\cdot$ ), oxidative stress causes premature skin aging [18]. The antioxidant defence is activated by in vivo processes by intracellular enzymes, such as superoxide dismutases, glutathione peroxidases, catalases or by extracellular compounds, for example transferrin, coenzyme Q, uric acid and by supplying the non-enzymatic substances with antioxidant effects (A, C, D and E vitamins,  $\beta$ -carotene, amino acids, elements Se, Zn, Fe, Mg and others) from the diet [19].

In the present study, ten aqueous and methanolic extracts from mushrooms (*Agaricus arvensis*, *Boletus edulis* (Bull.), *Cantharellus cibarius* (Fr.), *Imleria badia* (Fr.), *Lactarius volemus* (Fr.), *Leccinum pseudoscabrum*, *Lentinula edodes*, *Lepista nuda* (Bull.), *Morchella esculenta* (L.) and *Pleurotus ostreatus*) were screened for their antioxidant properties.

## MATERIALS AND METHODS

### Mushrooms

Edible mushrooms were purchased in local markets in Košice (Slovakia). The cultivated ones were only shiitake and oyster mushrooms, while the remaining mushrooms were wild. Amounts of 100 g of mushrooms were immediately sliced and dried at room temperature (approximately 25 °C). Then they were milled by a grinder TSM6A013B (Bosch, Munich, Germany) to obtain the powdered form. The samples were stored in dark at room temperature ( $\pm 25$  °C) and used for analyses within two weeks.

### Chemicals

2,2-Diphenyl-1-picrylhydrazyl radical (DPPH), gallic acid, Folin-Ciocalteu's reagent, quercetin, formic acid and methanol for high performance liquid chromatography (HPLC) were obtained from Sigma Aldrich (St. Louis, Missouri, USA). L-ascorbic acid was obtained from Lachner (Nera-

tovice, Czech Republic) and sodium carbonate, aluminium (III<sup>+</sup>) chloride, sodium nitrite, sodium hydroxide and methanol for extraction were obtained from Mikrochem (Pezinok, Slovakia). All chemicals were of p. a. or HPLC grade.

### Equipment

For determination of antioxidant activity, total phenolics content (TPC) and total flavonoids content (TFC), UV-Vis spectrophotometer Cary 60 (Agilent Technologies, Santa Clara, California, USA) was used. Ultra-high performance liquid chromatography (UHPLC) was performed using Dionex Ultimate 3000 chromatograph (Thermo Fisher Scientific, Waltham, Massachusetts, USA) with a diode array detector (DAD) using the octadecylsilyl (ODS) Hypersil column (250 mm  $\times$  4.6 mm, particle size 3  $\mu\text{m}$ ; Thermo Fisher Scientific).

### Sample preparation

Extracts were prepared by suspending 100 mg of mushroom powder in 2 ml of distilled water or methanol. Extractions proceeded at 8 °C with stirring on a magnetic stirrer (Heidolph MR HEI-TEC, Schwabach, Germany) for 24 h. After that, they were heated up to a room temperature (approximately 25 °C) and the extracts were filtered through Whatman 1 filter (Whatman, Maidstone, United Kingdom). The filtrates were stored at 8 °C for a maximum of 3 days until analysis.

### Radical-scavenging activity determination

A volume of 2 ml of 2 mmol $\cdot\text{l}^{-1}$  methanolic solution of DPPH radical were added to 250  $\mu\text{l}$  of an extract. The mixtures were incubated for 30 min in the dark at room temperature (approximately 25 °C). Subsequently, the decrease in absorbance at 517 nm was measured spectrophotometrically. The control sample was a solution of 2 mmol $\cdot\text{l}^{-1}$  DPPH radical with 250  $\mu\text{l}$  of methanol. L-ascorbic acid calibration solutions of 10, 20, 40, 60, 80, 100, 200 and 400  $\mu\text{g}\cdot\text{ml}^{-1}$  in methanol were used as reference standards for the evaluation of ascorbic acid equivalents (AAE). The radical-scavenging activity (RSA) was determined using the Brand-Williams method [20] as a percentage of DPPH radical inhibition with respect to a decrease in absorption of the control sample using Eq. 1,

$$\text{RSA} = \frac{(A_0 - A_x)}{A_0} \cdot 100 \quad (1)$$

where  $A_x$  is absorbance of the sample solution and  $A_0$  is absorbance of the control sample.

*RSA* values were expressed as L-ascorbic acid equivalents in milligrams per litre of extract. The results were obtained from calibration curve (Eq. 2).

$$y = -0.0189x + 2.0082; R^2 = 0.9990 \quad (2)$$

#### Total phenolics content determination

*TPC* was determined using spectrophotometry, by applying a modified Waterhouse method [21] with Folin-Ciocalteu reagent. Standard solutions of gallic acid were prepared at concentrations of 50, 100, 150, 250, 500, 750 and 1000 mg·l<sup>-1</sup> in distilled water. Distilled water was used as a blank sample. A volume of 1.6 ml of distilled water and 100 µl of Folin-Ciocalteu reagent were added to 20 µl of the standard solution or the extract sample. After mixing, 300 µl of 100 g·l<sup>-1</sup> sodium carbonate was added. The solutions were incubated in the dark at room temperature (approximately 25 °C) for 2 h. Absorbance at 765 nm was measured spectrophotometrically. The data obtained from standard solutions were used for the construction of the calibration line. Results were expressed as milligrams of gallic acid equivalents (GAE) per kilogram of dry weight (DW) of mushrooms.

#### Total flavonoids content determination

*TFC* was determined using spectrophotometry, using a modified method developed by Do et al. [22]. Standard solutions of quercetin were prepared at concentrations of 50, 100, 150, 250, 500, 750 and 1000 mg·l<sup>-1</sup> in distilled water. Distilled water was used as a blank sample. A volume of 50 µl of 50 g·l<sup>-1</sup> NaNO<sub>2</sub> were added to 1 ml of the standard solution. Subsequently, solutions were incubated for 6 min at room temperature (approximately 25 °C). In the next step, 300 µl of 10 g·l<sup>-1</sup> AlCl<sub>3</sub> were added and the reaction mixture was incubated for further 5 min at room temperature (approximately 25 °C). Finally, 2 ml of 1 mol·l<sup>-1</sup> NaOH was added. The solutions were filtered through Whatman 1 filter and absorbance was measured at 410 nm. The data obtained from standard solutions were used for the construction of the calibration line. Results were expressed as milligrams of quercetin equivalents (QE) per kilogram DW of mushrooms.

#### L-ascorbic acid determination

For determination of L-ascorbic acid, an UHPLC analysis used with gradient elution using methanol as eluent A and 0.2 % (v/v) formic acid in demineralized water as eluent B with the elution mode of 0–20 min, 0–75 % A; 20–29 min,

75 % A; 29–29.2 min, 75–0 % A; and 29.2–35 min, 0 % A. The injection volume was 10 µl. The column temperature was maintained at 25 °C. The analysis was controlled by Chromeleon 7 software (Thermo Fisher Scientific) and the detection wavelength was 240 nm. During the chromatographic separation, a flow rate of the mobile phase was 0.8 ml·min<sup>-1</sup>. The repeatability of sample measurements was ensured by using three replicates of the same sample, and it was expressed as relative standard deviation (*RSD*). The calibration line of L-ascorbic acid was constructed for the concentration range of 0.005–1.0 mg·ml<sup>-1</sup>. Limit of detection (*LOD*) and limit of quantification (*LOQ*) were defined as the signal-to-noise ratios (*S/N*) of 3.3 and 10, respectively.

*RSA* values were expressed as L-ascorbic acid equivalent in milligrams per litre of extract. The results were obtained from calibration curve (Eq. 2) of ascorbic acid as well, and are in correlation with *RSA* (Tab. 1).

#### Statistical analysis

All analyses were performed in triplicate. The results were analysed using one-way analysis of variance (ANOVA) and Tukey's post-hoc test using SPSS 26.0 software (SPSS, Chicago, Illinois, USA). The level of *p* < 0.001 was used.

## RESULTS AND DISCUSSION

#### Radical-scavenging activity

Data on the scavenging effects of the extracts on DPPH free radicals are shown in Tab. 1. As can be seen, the highest *RSA* for both extract types were observed for cep mushroom (*B. edulis*) – 91.4 % and 88.2 % (water and methanol extract, respectively). Generally, better *RSA* was observed for water extracts. To compare methanolic and aqueous extracts of mushrooms, statistically significant differences were observed for *C. cibarius*, *L. volemus*, *L. edodes*, *L. nuda*, *M. esculenta* and *P. ostreatus*. Additionally, methanolic extract of *L. volemus* showed better *RSA* than the water extract (84.1 % vs 58.7 %). Statistically not significant was *RSA* of *I. badia*, where the aqueous extract also showed lower *RSA* (84.6 %) than the methanolic one (85.6 %). As we compare cultivated shiitake and oyster mushrooms with wild ones, methanolic extracts of wild fungi showed generally better antioxidant effects. Both types of extract of oyster mushroom showed the lowest *RSA* (aqueous extract 49.0 % and methanolic extract 14.2 %). We mean that the cultivation conditions are important for the content

**Tab. 1.** Antioxidant properties of fungal extracts.

Common name	Latin name	RSA [%]	AAE [mg·l <sup>-1</sup> ]	TPC [mg·kg <sup>-1</sup> ]	TFC [mg·kg <sup>-1</sup> ]
<b>Water extracts</b>					
Horse mushroom	<i>Agaricus arvensis</i>	86.8 ± 0.9	94.27 ± 0.81	8 349.8 ± 394.0	1 387.3 ± 2.9
Cep	<i>Boletus edulis</i>	91.4 ± 0.1	98.48 ± 0.10	15 171.9 ± 810.5 *	1 735.0 ± 95.3
Chanterelle mushroom	<i>Cantharellus cibarius</i>	62.4 ± 1.5 *	72.21 ± 1.33 *	2 314.9 ± 198.4	1 261.7 ± 26.0
Bay bolete	<i>Imleria badia</i>	84.6 ± 1.5	92.30 ± 1.33	6 663.3 ± 286.5	1 247.7 ± 11.2
Weeping milk cap	<i>Lactarius volemus</i>	58.7 ± 2.6 *	68.84 ± 2.40 *	4 907.2 ± 114.5	1 320.3 ± 5.5
Hazel bolete mushroom	<i>Leccinum pseudoscabrum</i>	83.7 ± 1.3	91.50 ± 1.22	12 708.8 ± 1838.4 *	1 247.3 ± 11.7
Shiitake	<i>Lentinula edodes</i>	89.3 ± 0.3 *	96.55 ± 0.31 *	7 462.8 ± 718.9	1 289.0 ± 44.1
Wood blewit	<i>Lepista nuda</i>	77.9 ± 1.7 *	86.23 ± 1.51 *	9 224.5 ± 725.2	1 446.3 ± 23.0
Morel mushroom	<i>Morchella esculenta</i>	85.3 ± 1.8 *	92.95 ± 1.62 *	11 205.1 ± 1051.0	1 345.7 ± 40.2
Oyster mushroom	<i>Pleurotus ostreatus</i>	48.9 ± 2.2 *	60.01 ± 2.04 *	6 251.0 ± 313.6	1 281.3 ± 33.2
<b>Methanolic extracts</b>					
Horse mushroom	<i>Agaricus arvensis</i>	84.2 ± 0.2	93.20 ± 0.18	2 419.6 ± 216.2	1 167.3 ± 2.3
Cep	<i>Boletus edulis</i>	88.2 ± 0.1	96.47 ± 0.08	4 455.4 ± 677.6 *	1 260.0 ± 7.6
Chanterelle mushroom	<i>Cantharellus cibarius</i>	15.2 ± 12.3 *	36.16 ± 10.20 *	398.9 ± 228.9	1 163.3 ± 6.4
Bay bolete	<i>Imleria badia</i>	85.6 ± 1.0	94.34 ± 0.81	2 467.0 ± 432.2	1 248.0 ± 10.6
Weeping milk cap	<i>Lactarius volemus</i>	84.1 ± 5.9 *	93.11 ± 4.84 *	2 747.8 ± 291.9	1 168.0 ± 2.0
Hazel bolete mushroom	<i>Leccinum pseudoscabrum</i>	78.8 ± 3.6	88.71 ± 2.99	3 053.1 ± 88.8 *	1 184.7 ± 5.0
Shiitake	<i>Lentinula edodes</i>	15.4 ± 0.9 *	36.37 ± 0.76 *	903.7 ± 169.8	1 249.3 ± 7.6
Wood blewit	<i>Lepista nuda</i>	29.9 ± 2.8 *	48.30 ± 2.29 *	1 285.3 ± 85.6	1 332.0 ± 10.4
Morel mushroom	<i>Morchella esculenta</i>	56.9 ± 6.0 *	70.65 ± 4.92 *	2 382.3 ± 112.1	1 136.7 ± 1.2
Oyster mushroom	<i>Pleurotus ostreatus</i>	14.2 ± 0.3 *	35.32 ± 0.21 *	561.0 ± 26.5	1 281.3 ± 33.8

Comparisons between groups were performed using one-way ANOVA. All results are shown as mean ± standard deviation.

\* – significant difference between methanolic and water extracts at  $p < 0.001$  level.

RSA – radical scavenging activity, AAE – ascorbic acid equivalents, TPC – total phenolics content (expressed as milligrams of gallic acid equivalents per kilogram of dry weight), TFC – total flavonoids content (expressed as milligrams of quercetin equivalents per kilogram of dry weight).

of antioxidants and a study on that topic would be interesting. RSA values were in correlation with ascorbic acid content (Tab. 1).

#### Total phenolics content

Phenolic compounds are secondary metabolites, generally considered as antioxidants blocking free radicals activated by oxidative stress or environmental pollution. They also have protective effects on living organisms [23]. Tab. 1 presents TPC as milligrams of gallic acid equivalents per kilogram DW of mushrooms. The amounts of gallic acid equivalents were calculated using the line equation (Eq. 3)

$$y = 1.1966x + 0.0093; R^2 = 0.9994 \quad (3)$$

Higher total phenolics content values were generally observed in water extracts (2314.9–15 171.9 mg·kg<sup>-1</sup>) in comparison to methanolic samples (398.9–4 455.4 mg·kg<sup>-1</sup>). The highest TPC was identified in *B. edulis* (15 171.9 mg·kg<sup>-1</sup>),

followed by *L. pseudoscabrum* (12 708.8 mg·kg<sup>-1</sup>) and *M. esculenta* (11 205.1 mg·kg<sup>-1</sup>), whereas the lowest content was observed for *C. cibarius* (2314.9 mg·kg<sup>-1</sup>). Similarly, the highest TPC in a methanolic extract was observed for *B. edulis* (4 455.4 mg·kg<sup>-1</sup>), followed by *L. pseudoscabrum* (3 053.1 mg·kg<sup>-1</sup>) and *L. volemus* (2 747.8 mg·kg<sup>-1</sup>). The lowest content was determined for *C. cibarius* (398.9 mg·kg<sup>-1</sup>). These results confirm the fact that the content of phenolic compounds depends on the analysed mushroom species and origin of the mushrooms (whether cultivated or wild). For the cultivated ones, the cultivation substrate or medium is also important, along with the polarity of the solvent used for extraction [24]. Statistically significant results if we compare water and methanolic extracts were observed for *B. edulis* and *L. pseudoscabrum* ( $p < 0.001$ ).

According to other authors, TPC in the cep mushroom methanolic extract were of 31 640 mg·kg<sup>-1</sup> DW [25], in the weeping milk cap



it was 2331.11 mg·kg<sup>-1</sup> DW [26], in morel mushroom it was 3.5 mg·kg<sup>-1</sup> DW [27], for *Agaricus arvensis* the value was 170 mg·kg<sup>-1</sup> DW, for wood blewit it was 70 mg·kg<sup>-1</sup> [28], for oyster mushroom it was 2488 mg·kg<sup>-1</sup> DW [29], and for chanterelle it was 1750 mg·kg<sup>-1</sup> DW [28]. Values of 2390 mg·kg<sup>-1</sup> DW [30] and 36190 mg·kg<sup>-1</sup> DW [31] were identified in the water extract of the chanterelle mushroom while *B. edulis* exhibited a *TPC* of 2670 mg·kg<sup>-1</sup> [32].

#### Total flavonoids content

Flavonoids represent a broad spectrum of secondary metabolites structurally based on the flavan ring. The applied determination method was based on the formation of a stable complex of Al<sup>3+</sup> ion with the carbonyl and hydroxyl groups of flavones. Aluminium chloride reacts with quercetin to form a stable yellow-coloured complex [33]. Tab. 1 shows *TFC* values as quercetin equivalents in milligrams per kilogram DW of mushrooms. The contents of quercetin equivalents were calculated using the line equation (Eq. 4)

$$y = 175.4x - 9.67; R^2 = 0.9999 \quad (4)$$

Higher *TFC* values were determined in water extracts (1247.3–1735.0 mg·kg<sup>-1</sup>) than in methanolic extracts (1136.7–1281.3 mg·kg<sup>-1</sup>). The values were in correlation with *TPC*. No statistically significant differences between methanolic and aqueous extracts were observed. As for aqueous extracts of the investigated mushrooms, the highest *TFC* was observed for *B. edulis* (1735.0 mg·kg<sup>-1</sup>), followed by *L. nuda* (1446.3 mg·kg<sup>-1</sup>) and *A. arvensis* (1387.3 mg·kg<sup>-1</sup>). The lowest content was observed for *I. badia* and *L. pseudoscabrum*

(1247.7 mg·kg<sup>-1</sup> and 1247.3 mg·kg<sup>-1</sup>). Similarly in methanolic extracts, the highest *TFC* was observed for *L. nuda* (1332.0 mg·kg<sup>-1</sup>), followed by oyster (1281.3 mg·kg<sup>-1</sup>) and *B. edulis* (1260.0 mg·kg<sup>-1</sup>). The lowest content was observed for *M. esculenta* (1136.7 mg·kg<sup>-1</sup>). When comparing *TPC* and *TFC*, content of phenolics was higher than that of flavonoids, which is in agreement with literature data. Flavonoid contents presented by other authors were 28.4 mg·kg<sup>-1</sup> DW for oyster mushroom and 3750 mg·kg<sup>-1</sup> DW for a water extract of *Lentinus edodes* [31]. JAWORSKA et al. [32] determined total flavonoids of *B. edulis* at 260 mg·kg<sup>-1</sup> DW of raw mushrooms.

#### L-ascorbic acid content

Contents of L-ascorbic acid in aqueous and methanolic extracts are presented in Tab. 2. In general, higher amounts of ascorbic acid were detected in methanolic extracts. The calibration curve of ascorbic acid was linear in a relatively wide concentration range (0.005–1.000 mg·ml<sup>-1</sup>). Linear regression exhibited a high correlation ( $R^2 = 0.9968$ ) while *RSD* was 1.2 %. According to the calculation, *LOD* and *LOQ* values of L-ascorbic acid were 0.0171 mg·ml<sup>-1</sup> and 0.0519 mg·ml<sup>-1</sup>, respectively. The determined content of ascorbic acid was expressed in milligrams per kilogram of dry weight (dried mushrooms).

IGILE et al. [34] determined ascorbic acid contents in oyster mushrooms, which were cultivated in laboratory ( $278.8 \pm 0.05$  mg·kg<sup>-1</sup>). Fresh *B. edulis* mushrooms contained of 10.1 mg·kg<sup>-1</sup> of ascorbic acid [32]. We determined higher content of ascorbic acid in water extracts for these mentioned mushrooms, i.e.  $1682.36 \pm 90.55$  mg·kg<sup>-1</sup> DW (oyster) and  $103.72 \pm 9.62$  mg·kg<sup>-1</sup> DW. When

Tab. 2. Content of ascorbic acid in extracts.

Common name	Latin name	Water extract * [mg·kg <sup>-1</sup> ]	Methanolic extract * [mg·kg <sup>-1</sup> ]
Horse mushroom	<i>Agaricus arvensis</i>	ND	ND
Cep	<i>Boletus edulis</i>	103.72 ± 9.62	2752.75 ± 23.77
Chanterelle mushroom	<i>Cantharellus cibarius</i>	ND	ND
Bay bolete	<i>Imleria badia</i>	2178.96 ± 52.63	1052.90 ± 66.21
Weeping milk cap	<i>Lactarius volemus</i>	26.89 ± 2.83	1970.87 ± 65.08
Hazel bolete mushroom	<i>Leccinum pseudoscabrum</i>	190.33 ± 20.69	1976.49 ± 79.89
Shiitake	<i>Lentinula edodes</i>	ND	1012.45 ± 60.03
Wood blewit	<i>Lepista nuda</i>	ND	2559.11 ± 130.21
Morel mushroom	<i>Morchella esculenta</i>	563.15 ± 25.52	525.42 ± 24.54
Oyster mushroom	<i>Pleurotus ostreatus</i>	1682.36 ± 90.55	2428.26 ± 88.28

Content of ascorbic acid is expressed per kilogram of dry weight. Comparisons between groups were performed using one-way ANOVA. All data are shown as mean ± standard deviation.

\* – significant difference between methanolic and water extracts at  $p < 0.001$  level, ND – not detected.

comparing methanolic and aqueous extracts, significant differences between them could be seen. The content of ascorbic acid in methanolic extracts was generally higher, except for morel, where no significant difference between both types of extracts was determined. In bay bolete mushroom, determined higher content of ascorbic acid was determined in the aqueous extract ( $2178.96 \pm 52.63 \text{ mg}\cdot\text{kg}^{-1}$ ) than in the methanolic one ( $1052.9 \pm 66.21 \text{ mg}\cdot\text{kg}^{-1}$ ) (Tab. 2). The differences between contents in aqueous and methanolic extracts may be due to the fact that the release of ascorbic acid from the complex structures of the mushrooms was easier into the less polar solvent.

## CONCLUSIONS

This study was focused on antioxidant properties of extracts from various mushrooms, which originate in the content of complex of various chemical compounds with properties leading to inhibit free radicals, for example phenolics, flavonoids, vitamins or polysaccharides, peptides, amino acids and micronutrients. We confirmed that each of the studied mushrooms contained substances with antioxidant activity. Phenolic substances and flavonoids were found in each sample of the studied mushrooms and each sample exhibited the scavenging activity against DPPH radicals. The best RSA was observed for *Boletus edulis* aqueous extract (91.4 %). The results obtained for the individual extracts indicate that it is more appropriate to use water for the extraction when determining contents of phenolic substances and flavonoids. Out of all the investigated mushrooms, the one with the highest TPC ( $15171.4 \text{ mg}\cdot\text{kg}^{-1} \text{ DW}$ ) and TFC ( $1735.0 \text{ mg}\cdot\text{kg}^{-1} \text{ DW}$ ) was the cep mushroom (*B. edulis*). The highest amount of ascorbic acid was observed in the methanolic extract of *B. edulis* ( $2752.75 \text{ mg}\cdot\text{kg}^{-1} \text{ DW}$ ). As we look at the species of Boletaceae, for all the studied mushrooms we observed good antioxidant properties in all tested parameters including ascorbic acid content. Surprisingly, the lowest antioxidant activity was shown by *Pleurotus ostreatus* (a member of Agaricaceae) in which no ascorbic acid was detected using our method. This suggested that high RSA originated in high TPC and TFC. A wide range of potential applications of mushrooms and their extracts in the food industry, medicine, pharmacy, as well as other sectors substantiates further research into this commodity.

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