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1 **Comprehensive evaluation of antioxidant and antimicrobial**
2 **properties of different mushroom species**

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13

14 **Abstract**

15 Antioxidant properties of mushroom extracts sequentially isolated by cyclohexane,
16 dichloromethane, methanol, and water from *Phaeolus schweinitzii*, *Inonotus hispidus*,
17 *Tricholoma columbetta*, *Tricholoma caligatum*, *Xerocomus chrysenteron*, *Hydnellum*
18 *ferruginum*, *Agaricus bisporus* and *Pleurotus ostreatus* were evaluated by DPPH[•], ABTS^{•+}
19 scavenging capacity, ferric reducing antioxidant power (FRAP), oxygen radical absorbance
20 capacity (ORAC), and Folin–Ciocalteu total phenolic content (TPC) methods. The integrated
21 values ('antioxidant scores') for evaluating antioxidant potential of extracts and dry mushroom
22 substances are proposed. Antimicrobial activity was screened against Gram-positive (*Bacillus*
23 *cereus*), Gram-negative (*Pseudomonas aeruginosa*) bacteria and fungi (*Candida albicans*) by
24 agar diffusion method. The highest antioxidant capacity values (in $\mu\text{M TE/g extract dw}$) were
25 found for methanol fractions of *P. schweinitzii* (9.62 ± 0.03 in DPPH[•]; 109 ± 3 in FRAP; 164 ± 1
26 in ABTS^{•+}; 340 ± 3 in ORAC assays) and *I. hispidus* (9.5 ± 0.04 in DPPH[•]; 54.27 ± 0.46 in
27 ABTS^{•+}; 88.31 ± 1.96 in FRAP; 290 ± 1 in ORAC assays). Extracts of other species possessed
28 considerably lower antioxidant activities. The extracts of *I. hispidus* were more effective against
29 tested microbial species than other mushrooms. In conclusion, our results show that some wild
30 mushrooms might be promising dietary sources of natural antioxidants and antimicrobial agents.

31

32 **Keywords:** mushrooms; extracts; antioxidant activity; antimicrobial activity; antioxidant score

33

34

35 **1. Introduction**

36

37 The role of free radicals in the development of various diseases is thoroughly discussed.
38 Deficiency in endogenous antioxidant defense may result in oxidative stress, which might be
39 associated with various health problems, including coronary heart diseases, neural disorders,
40 diabetes, arthritis and cancer (Yoshikawa, Toyokuni, Yamamoto & Naito, 2000). Therefore,
41 dietary antioxidants are believed to assist in maintaining good health, as well as in preventing
42 various diseases (Augustyniak et al., 2010). Antioxidants are present in all biological systems;
43 however, plant kingdom remains the main source of healthy compounds. Therefore, search for
44 effective and non-toxic natural antioxidants and other bioactive molecules have become a
45 regularly increasing topic. In addition, many phytochemicals possess antimicrobial activity,
46 which can also be applied for food and medical purposes.

47 Mushrooms have been widely used as a human food for centuries and have been appreciated
48 for texture and flavour as well as various medicinal and tonic properties. However, the awareness
49 of mushrooms as being an important source of biological active substances with medicinal value
50 has only recently emerged. A number of mushroom species has been reported during last decade
51 to possess significant antioxidant activity (Jones & Janardhanan, 2000; Mathew, Sudheesh, Rony,
52 Smina & Janardhanan, 2008; Nitha, Strayo, Adhikari, Devasagayam & Janardhanan, 2010;
53 Hearst et al., 2009; Kalogeropoulos, Yanni, Koutrotsios & Aloupi, 2013). Mushrooms are also
54 rich in proteins, fiber, vitamins and minerals, while the content of fat is low (Guillamón et al.,
55 2010). In addition, edible mushrooms usually contain various bioactive molecules, such as
56 phenolic compounds, polyketides, terpenes and steroids (Barros, Baptista & Ferreira, 2007).
57 Mushrooms played an important role in the treatment of various disorders, including infectious

58 diseases and therefore some naturally occurring chemical compounds identified in mushrooms
59 served as models for clinically proven drugs (Barros, Cruz, Baptista, Estevinho & Ferreira, 2008).
60 However, the interest in the use of mushrooms for the development of nutraceuticals and
61 functional food ingredients is quite recent. In fact, due to multipurpose applications and uses
62 mushrooms should be considered not only as a traditional food but also as a source of high value
63 flavourings, efficient natural dyes, as well as a raw material for functional food, food supplement
64 and pharmaceutical ingredients.

65 The aim of this study was to apply biorefinery approach in order to valorise some wild
66 mushrooms growing in Midi-Pyrénées region, which are further briefly reviewed. *Tricholoma*
67 *columbetta* is edible and can be consumed fresh, dry or pickled. A cyclopentene derivative
68 columbetdione (Vadalà, Finzi, Zanoni & Vidari, 2003) and endopeptidase (Lamaison, Pourrat &
69 Pourrat, 1980) were found in its fruiting bodies, while ethyl acetate extracts of *T. columbetta*
70 were shown to possess nematocidal activity against *Caenorhabditis elegans* and antibacterial
71 activity against *Bacillus brevis* (Stadler & Sterner, 1997).

72 *Phaeolus schweinitzii* (Fr.) is a common root and butt pathogen of conifers in North America
73 and Eurasia producing a strong, water-soluble pigments possessing five intensive colours: olive-
74 brown, olive-grey, dark-brown, brownish-grey and linoleum-brown (Cedano, Villaseñor &
75 Guzmán-Dávalos, 2001). Hispidin was isolated from acetone extract of *P. schweinitzii* (Ueno,
76 Fukushima, Saiki & Harada, 1964). *Inonotus hispidus* is a parasitic fungus preferably living on
77 deciduous trees such as *Fraxinus*, *Quercus*, *Sorbus* and *Malus*. It has been used as a traditional
78 medicine for treating dyspepsia, cancer, diabetes and stomach problems in the northeast region
79 and Xinjiang province of China (Ali, Jansen, Pilgrim, Liberra & Lindequist, 1996). *I. hispidus*
80 contains polyphenol pigments with styrylpyrone skeleton, which were reported to exhibit
81 antimicrobial, antioxidant, antiviral and anti-inflammatory activities. Two natural antioxidants,

82 named inonotusin and hispidin were isolated from the methanolic extract of the fruit bodies and
83 showed antioxidant and cytotoxic activity against human breast carcinoma cells (Zan et al.,
84 2011).

85 *Xerocomus chrysenteron* is an edible mushroom occasionally harvested in autumn. The lectin
86 was identified in *X. chrysenteron* (Birck et al., 2004), while its methanolic extract was reported to
87 possess antioxidant activity (Sarikurkcu, Tepe & Yamac, 2008; Heleno et al., 2012). *Tricholoma*
88 *caligatum* forms a small and fuscous to blackish fruit body and grows in the Mediterranean
89 region (Murata, Ota, Yamada, Yamanaka & Neda, 2013). Various aromatic derivatives were
90 identified in *T. caligatum* (Fons, Rapior, Fruchier, Saviuc & Bessièrè 2006).

91 *Agaricus bisporus* (the button mushroom) is the most widely cultivated form in the USA,
92 Europe and different parts of Australasia. It is recognized as a source of unsaturated fatty acids
93 such as linoleic, linolenic, conjugated linoleic and polyphenols (Singh, Langowski, Wanib &
94 Saengerlauba, 2010) demonstrating some medicinal properties such as anticancer activity (Zhang,
95 Huang, Xie & Holman, 2008; Shi, James, Benzie & Buswell, 2002). *Hydnellum ferrugineum* has
96 red spore deposit and is easily recognized. *Hydnellum* spp. are regarded as ‘‘nitrogen sensitive’’
97 organisms (Van der Linde, Alexander & Anderson, 2008; Ainsworth, Parfitt, Rogers & Boddy,
98 2010) and have become a concern of European conservation. *Pleurotus ostreatus* is a highly
99 nutritious edible mushroom and is considered as a source of valuable nutritional and medicinal
100 compounds; it can be easily cultivated on a large range of substrates (Gern, Wisbeck, Rampinelli,
101 Ninow & Furlan, 2008). It could be used as a cholesterol lowering additive in human diet
102 (Schneider et al., 2011).

103 Regardless above-cited articles, the reports on the antioxidant activity and antimicrobial
104 properties of the selected in this study mushrooms are rather scarce; previously published data is
105 particularly lacking a systematic approach and comprehensiveness. Therefore, the aim of the

106 present work was to evaluate antioxidant and antimicrobial properties of the selected mushroom
107 species by applying a more systematic approach. The antioxidant potential was comprehensively
108 evaluated for mushroom fractions sequentially isolated with cyclohexane, dichloromethane,
109 methanol, and water by using free radical scavenging capacity, oxygen radical absorbance
110 capacity, ferric reducing antioxidant capacity and total phenolics content assays. Antimicrobial
111 activity was screened against Gram-positive (*Bacillus cereus*) and Gram-negative (*Pseudomonas*
112 *aeruginosa*) bacteria and fungi (*Candida albicans*).

113

114 2. Materials and Methods

115

116 2.1. Mushrooms and chemicals

117 *Phaeolus schweinitzii*, *Inonotus hispidus*, *Tricholoma columbetta*, *Tricholoma caligatum*,
118 *Xerocomus chrysenteron*, *Hydnellum ferrugineum* were harvested in Midi-Pyrénées region of
119 France in autumn 2009. Taxonomic identification was carried out by Mycologist Association of
120 Faculty of Pharmacy of Toulouse University. *Agaricus bisporus* and *Pleurotus ostreatus* were
121 purchased in the local supermarket. All freeze-dried (Lyophilisateur pilote LPCCPLS15, Cryotec,
122 Saint-Gély-du-Fesc, France) mushrooms were ground in a Microfine mill (MF-10, IKA, Staufen,
123 Germany) through a 1.5-mm sieve and then stored in air-tight plastic bags in a desiccator at room
124 temperature for further analysis.

125 Stable 2,2-di-phenyl-1-picrylhydrazyl hydrate radical (DPPH[•], 95%), gallic acid, anhydrous
126 sodium carbonate, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox, 97%),
127 2,20-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), fluorescein
128 (FL) and [2,2'-azobis(2-amidino-propane) dihydrochloride (AAPH)] were from Sigma-Aldrich
129 (Steinheim, Germany); 2.0 M Folin–Ciocalteu phenol reagent, KCl, NaCl, Na₂HPO₄, Na₂CO₃ and

130 $K_2S_2O_8$ were from Merck (Darmstadt, Germany); KH_2PO_4 from Jansen Chimica (Beerse,
131 Belgium); methanol, 98% acetic acid from Lachema (Brno, Czech Republic) and agricultural
132 origin ethanol (96.6 %) from Stumbras (Kaunas, Lithuania); 2,4,6-tripyridyl-s-triazine (TPTZ)
133 was from Fluka Chemicals (Steinheim, Switzerland).

134

135 *2.2. Extraction procedure*

136 HPLC grade cyclohexane, dichloromethane, methanol and deionized water were used to
137 fractionate soluble compounds from the mushrooms in ascending polarity by sequentially
138 extracting 2-100g (depending on material availability) ground mushrooms in a Soxhlet extractor
139 for 5 h. The samples were air dried after each solvent extraction and finally the residues were
140 extracted with boiling water during 5 h constantly mixing in the Ikamag “RTC basic” magnetic
141 stirrer (IKA Labortechnik, Staufen, Germany). Organic solvents were removed in a vacuum
142 rotary evaporator RV 10 (IKA, Staufen, Germany), while water extracts were freeze-dried. All
143 extracts were kept in a refrigerator until further analysis.

144

145 *2.3. Antioxidant activity assays*

146 *2.3.1. DPPH[•]-scavenging capacity*

147 This method is based on scavenging DPPH[•] by the antioxidant (Brand-Williams, Cuvelier, &
148 Berset, 1995). The assay was performed in a 96-well microtiter plates using an UV
149 spectrophotometer EL×808 Microplate Reader (BioTex Instruments, Vermont, USA). The
150 reaction mixture in each of the 96-wells consisted of 7.5 μ L of different concentration mushroom
151 extracts (0.5%; 0.25%; 0.125%) and 300 μ L of methanolic solution of DPPH[•] (6×10^{-5} M). The
152 mixture was left to stand for 40 min in the dark and the reduction of DPPH[•] was determined by
153 measuring the absorption at 515 nm. All measurements were performed in triplicate. Radical

154 scavenging capacity (RSC) was determined from the calibration curve, which was drawn by
155 using 50, 100, 125, 250, 500, 1000 $\mu\text{M/L}$ concentration solutions of Trolox and expressed in μM
156 of Trolox equivalents (TE) per g dry extract weight ($\mu\text{M TE/g edw}$).

157

158 2.3.2. *ABTS⁺ decolourisation assay*

159 The Trolox equivalent antioxidant capacity (TEAC) assay is based on the scavenging of
160 ABTS^{++} by the antioxidant which may be measured spectrophotometrically (Re et al., 1999). A
161 stock solution of 2 mM ABTS was prepared by dissolving reagent in 50 mL of phosphate
162 buffered saline (PBS) obtained by dissolving 8.18 g NaCl, 0.27 g KH_2PO_4 , 1.42 g Na_2HPO_4 and
163 0.15 g KCl in 1 L of Milli-Q water. If pH was lower than 7.4, it was adjusted with NaOH.
164 ABTS^{++} was produced by reacting 50 mL of ABTS stock solution with 200 μL of 70 mM $\text{K}_2\text{S}_2\text{O}_8$
165 solution in purified water and allowing the mixture to stand in the dark at room temperature for
166 15–16 h before use. The radical was stable in this form for more than 2 days when stored in the
167 dark at room temperature. For the assessment of extracts, the ABTS^{++} solution was diluted with
168 PBS to obtain the absorbance of 0.800 ± 0.030 at 734 nm. One mL of ABTS^{++} solution was mixed
169 with 10 μL extract solution in 96-well microtiter plates. The absorbance was read at ambient
170 temperature every minute during 40 min. PBS solution was used as a blank; all measurements
171 were performed in triplicate. The TEAC was determined from the calibration curve, which was
172 drawn using 50, 100, 125, 250, 500, 1000 $\mu\text{M/L}$ concentration solutions of Trolox and calculated
173 in $\mu\text{M TE/g edw}$ as follows: $TEAC \left(\frac{\mu\text{M}}{\text{g}} \right) = \frac{TE_s}{1000} \times \frac{V_s}{m_s}$, TE_s – antioxidant activity of sample
174 expressed in TE (μM), V_s – sample volume (mL), m_s – sample mass (g).

175

176

177 *2.3.3. Ferric-reducing antioxidant power (FRAP) assay*

178 FRAP assay is based on the reduction of Fe^{3+} in its tripyridyltriazine complex to the blue Fe^{2+}
179 form (Benzie & Strain, 1999). The final results were expressed in $\mu\text{M TE/g edw}$. The FRAP
180 reagent was prepared from acetate buffer (pH 3.6), 10 mM TPTZ solution in 40 mM HCl and
181 20 mM iron (III) chloride solution in proportions of 10:1:1 (v/v), respectively. The FRAP reagent
182 was prepared fresh daily and was warmed to 37 °C in a water bath prior to use. Ten μL of sample
183 were added to 300 μL of the FRAP reagent and 30 μL water. The absorbance of the reaction
184 mixture was then recorded at 593 nm after 4 min. All measurements were performed in triplicate.
185 The TEAC values were determined as indicated in previous sections.

186

187 *2.3.4. Oxygen radical absorbance capacity (ORAC)*

188 ORAC method was performed as described by Prior, Wu & Schaich (2005) and Dávalos,
189 Gómez-Cordovés & Bartolomé (2004) by using fluorescein as a fluorescent probe. The reaction
190 was carried out in a 75 mM phosphate buffer (pH 7.4); stock solution of fluorescein was prepared
191 according to Prior et al (2005). Mushroom extracts were diluted 1:1000 (w/v); 25 μL of extract
192 and 150 μL of fluorescein (14 μM) solutions were placed in 96 transparent flat-bottom microplate
193 wells, the mixture was preincubated for 15 min at 37 °C and 26 μL of AAPH solution (240 mM)
194 as a peroxy radical generator added with a multichannel pipette. The microplate was
195 immediately placed in the FLUORstar Omega reader (BMG LABTECH, Ortenberg, Germany),
196 automatically shaken prior to each reading and the fluorescence was recorded every cycle (66 s),
197 totally 150 cycles. The 485-P excitation and 520-P emission filters were used. At least 4
198 independent measurements were performed for each sample. Raw data were exported from the
199 Mars software to an Excel 2003 (Microsoft, Roselle, IL) sheet for further calculations.
200 Antioxidant curves (fluorescence versus time) were first normalized and from the normalized

201 curves the area under the fluorescence decay curve (AUC) was calculated as $AUC = 1 + \sum_{i=1}^{i=80} \frac{f_i}{f_0}$,

202 where f_0 is the initial fluorescence reading at 0 min and f_i is the fluorescence reading at time i .

203 The final ORAC values were calculated by using a regression equation between the Trolox

204 concentration and the net area under the curve (AUC). The TEAC values were determined as

205 described in previous sections.

206

207 *2.3.5. Determination of total phenolic content (TPC)*

208 The TPC was measured with Folin–Ciocalteu reagent as originally described by Singleton,

209 Orthofer & Lamuela-Raventos (1999). Briefly, 30 μL (0.1%) of sample were mixed with 150 μL

210 of 10-fold diluted (v/v) Folin–Ciocalteu reagent, and 120 μL of 7.5% Na_2CO_3 . After mixing of all

211 reagents, the microplate was placed in the reader and shaken for 30 s. After incubation for 30 min

212 at room temperature the absorbance of the mixtures was measured at 765 nm. All measurements

213 were performed in triplicate. A series of gallic acid solutions in the concentration range of 0.025-

214 0.35 mg/mL was used for the calibration curve. The results were expressed in mg of gallic acid

215 equivalents per g of dry extract weight (mg GAE/g edw).

216

217 *2.4. Antibacterial assay*

218 The antimicrobial activity was assessed by the disk-diffusion method (Bauer, Kirby, Sheriss

219 & Turck, 1966). The bacterial cell suspension was prepared from 24 h culture and adjusted to an

220 inoculation of 1×10^6 colony forming units per mL (cfu/mL). Sterile nutrient agar (Bit Phar. acc

221 EN 12780:2002, 28 g/L distilled water, Scharlau, Barcelona, Spain) was inoculated with bacterial

222 cells (200 μL of bacterial cell suspension in 20 mL medium) and poured into dishes to obtain a

223 solid plate. Twenty mg of test material dissolved in the same solvent of the extraction were

224 applied on sterile 5 mm diameter paper discs, which were deposited on the surface of inoculated
225 agar plates. The plates with bacteria were incubated for 24 h at 37°C. Inhibition zone diameters
226 around each of the disc (diameter of inhibition zone plus diameter of the disc) were measured and
227 recorded at the end of the incubation time. An average zone of inhibition was calculated from 3
228 replicates. Paper discs with solvents were used as controls.

229 Minimal inhibitory concentrations (MIC) showing the lowest concentration of extract able to
230 inhibit any visible microbial growth was determined by the agar diffusion technique (Rajbhandari
231 & Schöpke, 1999). The highest concentration of extract tested during the experiment was 20
232 mg/mL. The extracts were prepared at the series of concentrations (0.01; 0.1; 1; 10; 20 mg/mL).
233 Ten µl of each concentration solution was transferred in the disk. Then the disks were transferred
234 in the Petri dishes containing microorganism culture. The plates were incubated for 24 h at 37°C
235 for bacteria. After incubation, the number of colonies in each plate was counted. Each assay
236 replicated three times.

237

238 *2.5. Statistical analysis and data assessment*

239 The values are expressed as a means of 3 replicate measurements in antimicrobial assay and 4
240 replicates in antioxidant assays with standard deviations (SD). Correlation coefficients (R) to
241 determine the relationship between two variables, RSC, FRAP, ORAC and TEAC tests were
242 calculated using MS Excel 2010 software (CORREL statistical function). The antioxidant
243 characteristics were summarized by using integrated values for extracts - 'antioxidant score' of
244 extract (ASE), which is the sum of values for the fraction obtained with the same solvent in all
245 assays, expressed in the so-called 'comparative integrated units' in g of dry extract weight (ciu/g
246 edw) and for the whole plant dry material, expressed in g of dry mushroom weight (ciu/g mdw).
247 The latter values, which may be called 'antioxidant scores of mushrooms' (ASM) take into the

248 account ASE and extract yields (EY) and were calculated as follows: $ASE_c \times EY_c / 100 +$
249 $ASE_d \times EY_d / 100 + ASE_m \times EY_m / 100 + ASE_w \times EY_w / 100$. These integrated values to some extent
250 reflect the overall total antioxidant potential of different mushroom species, which also consider
251 extract yields, as well as the effectiveness of different polarity solvents used for the extraction. It
252 is expected that the concept of antioxidant scores may help in assessing a large number of
253 antioxidant activity data, which were obtained in this study.

254

255 **3. Results and discussion**

256 *3.1. Extraction yield*

257 Efficient extraction of antioxidants and other biologically active molecules requires the use of
258 solvents with different polarities: certain antioxidants are better soluble in polar solvents such as
259 methanol, water, while cyclohexane or dichloromethane are preferable for isolating lipophilic
260 compounds. Two main approaches may be applied for exhausting isolation of various
261 components from biological material, namely parallel extraction of initial material with different
262 solvents or sequential fractionation with increasing polarity and dielectric constant solvents. The
263 latter approach was applied in our study: non-polar cyclohexane was followed by polar aprotic
264 solvent dichloromethane, the residues were further extracted with polar protic solvent methanol
265 and the process was finalized with boiling water possessing the highest dielectric constant. It is
266 obvious (Table 1) that selected mushrooms are composed of very different classes of substances
267 from the point of view of their solubility in the applied solvents. Thus, the highest total yield of
268 all fractions was obtained from *T. caligatum* (63.15 %), while the lowest one from *I. hispidus*
269 (16.48 %). Protic solvents possessing high dielectric constant gave remarkably higher extract
270 yields comparing with non-polar and aprotic solvents. It proves that all tested mushroom species

271 contain low amounts of lipophilic constituents. The yields obtained by different solvents are very
272 important characteristics in applying biorefinery concept to biomaterials for their effective,
273 preferably no-waste conversion into the fractions for different applications. For comparison,
274 previously reported yields of methanol (Yang, Lin, & Mau, 2002) and ethanol (Arbaayah &
275 Kalsom, 2013) extracts of *P. ostreatus* were 16.9 % and 12.01 %, respectively; however, in these
276 studies the initial material was extracted. Sequential extraction of *A. bisporus* with hexane, ethyl
277 acetate and aqueous methanol was also applied previously and the yields were 0.68, 0.65 and 5.84
278 %, respectively (Öztürk et al., 2011). In our study methanol and water yields were remarkably
279 higher. The yield of phenolic and polysaccharide fractions of *X. chrysenteron* from Portugal were
280 reported 12.28 and 27.40 % (Heleno et al., 2012); thus, the sum of extracts (39.68 %) is similar to
281 the sum of yields (39.93 %) obtained in our study for this species.

282

283 3.2. Antioxidant potential of different mushroom species

284 Growing interest in natural antioxidants has led to the development of a large number of
285 assays for evaluating antioxidant capacities of botanical extracts. Since the antioxidant capacity
286 of complex biological extracts is usually determined by a mixture of various antioxidatively
287 active constituents, which may act by different mechanisms and sometimes possess synergistic
288 effects, the reliability of the evaluation of overall antioxidant potential of any plant material
289 increases by applying several assays (Frankel & Meyer, 2000; Laguerre, Lecomte & Villeneuve,
290 2007).

291 ABTS^{•+} and DPPH[•] scavenging, FRAP, ORAC and TPC assays are the most common
292 methods for determining *in vitro* antioxidant capacity of plant origin substances. Huang, Ou, &
293 Prior (2005) concluded that ORAC, TPC measured with Folin-Ciocalteu reagent and one of the
294 single electron/hydrogen atom transfer assays (SET or HAT) should be recommended for the

295 representative evaluation of antioxidant properties. DPPH[•] scavenging method is mainly
296 attributed to the SET assays; however, quenching of DPPH[•] to form DPPH-H is also possible.
297 Other SET based methods include the TPC assessment using Folin–Ciocalteu reagent, ABTS^{•+}
298 decolourisation assay and ferric ion reducing antioxidant power (FRAP) assay. ORAC assay
299 evaluates radical chain breaking antioxidant activity via HAT and measures antioxidant inhibition
300 induced by peroxy radical oxidation. Following the above mentioned recommendation all these
301 methods were applied for the comprehensive assessment of antioxidant potential of the isolated
302 with different solvents mushroom fractions. To the best of our knowledge such approach is
303 applied for the selected mushroom species for the first time. Moreover, the reports on antioxidant
304 properties of *T. caligatum*, *T. columbetta* and *H. ferrugineum* have not been found in any
305 available literature sources.

306 To obtain comparable values the results of ABTS^{•+}, DPPH[•], FRAP and ORAC assays were
307 expressed in Trolox (a hydrosoluble analogue of vitamin E) equivalents, i.e. in the amount of
308 Trolox μ M possessing similar antioxidant capacity as 1 g edw, while TPC was expressed in mg
309 of gallic acid equivalents per g of edw (Table 2). Remarkable diversity in antioxidant capacity,
310 depending on mushroom species, extraction solvent and assay method, may be clearly observed.
311 Therefore, it is convenient to consistently discuss the effect of these factors in separate sections.
312 For easier assessment of antioxidant potential of different mushroom species, as well as the
313 effects of different solvents and applied antioxidant activity assays (Table 2). The antioxidant
314 characteristics were also summarized by using ‘antioxidant scores’ of extracts (ASE) and dry
315 mushroom material (ASM) and expressed in the so-called ‘comparative integrated units’ (ciu),
316 which are explained in 2.5 section.

317

318

319 3.2.1. Antioxidant capacity differences between mushroom species

320 The highest antioxidant potential demonstrated *I. hispidus* and *P. schweinitzii* extracts,
321 particularly in SET assays, while the extracts isolated from such species as *P. ostreatus* and *A.*
322 *bisporus* were the weakest antioxidant sources in these assays. For instance, the sum of TEAC
323 of *I. hispidus* extracts in ABTS^{•+} scavenging assay (225.2 $\mu\text{M TE/g}$) was more than 100 times
324 higher comparing to *P. ostreatus* (2.1 $\mu\text{M TE/g}$). These differences were less remarkable in other
325 SET assays, while in ORAC assay the values varied from 104.0 (*X. chrysenteron*) to 461.9 μM
326 TE/g (*P. schweinitzii*). TPC was from 14.52 (*T. caligatum*) to 84.57 mg GAE/g (*I. hispidus*).
327 However, extract yields were dependent both on mushroom species and extraction solvent,
328 therefore TPC values obtained for extracts were recalculated for 1 g of mushroom dry weight
329 (mdw), taking into account how much of TPC is extracted with each solvent. The TPC values
330 expressed in this way are presented in Fig. 1: they were from 0.90 mg GAE/g mdw (*P. ostreatus*)
331 to 5.96 mg GAE/g mdw (*I. hispidus*). In general the TPC values in most cases were in agreement
332 with antioxidant capacity values obtained in other assays.

333 Taking into account all measured characteristics, the ASMs of wild mushrooms expressed in
334 ciu/g mdw may be located in the following decreasing order (Fig. 2): *P. schweinitzii* (97.06) > *I.*
335 *hispidus* (78.99) > *T. caligatum* (69.95) >> *T. columbeta* (23.54) > *H. ferrugineum* (15.28) > *X.*
336 *chrysenteron* (14.35). Commercial species *A. bisporus* and *P. ostreatus* were of inferior
337 antioxidant potential comparing with the majority of studied wild mushrooms species; their
338 ASMs were 18.02 and 12.37 ciu/g mdw , respectively. It was previously reported that methanol
339 extract isolated from *P. ostreatus* was stronger DPPH[•] and OH[•] scavenger and possessed better
340 reducing properties comparing with other 5 tested commercial mushroom species (Yang et al.,
341 2002); however, in the mentioned study antioxidant indicators were expressed in percentage of

342 scavenged radicals and therefore are difficult to compare with our results. More effective
343 mushrooms species were reported to contain higher amounts of secondary metabolites such as
344 phenolics exerting multiple biological effects including antioxidant activity (Kim et al., 2008).

345

346

347 3.2.2. Effect of extraction solvent

348 Generally polar solvents are most frequently used for the extraction of antioxidants from
349 botanicals containing polyphenolics as the main antioxidatively active compounds; however,
350 some plant origin materials may also contain lipophilic compounds such as tocopherols,
351 carotenoids, terpenoids and the use of different polarity solvents may provide more
352 comprehensive information on their antioxidant potential, particularly in case of less studied
353 mushroom species. Our results clearly demonstrate (Table 1) that distribution of antioxidatively
354 active constituents in the fractions isolated with different solvents is highly dependent on
355 mushroom species. The extracts isolated from *I. hispidus* and *P. schweinitzii* (the species
356 possessing the highest antioxidant potential) with protic solvents methanol and water were
357 remarkably stronger antioxidants than cyclohexane and dichloromethane extracts of the same
358 species in all assays, while for other species the results are more complicated. For instance,
359 cyclohexane extract of the well-known commercial *A. bisporus* mushroom was stronger
360 antioxidant in all assays except for ORAC, while the TPC values were quite similar for all
361 extracts. However, it should be mentioned that this species was characterized as possessing weak
362 antioxidant potential. Dichloromethane extract of *X. chrysenteron* was stronger antioxidant in
363 SET assays, except for FRAP and TPC values; however, in ORAC assay methanol fraction was 3
364 times stronger than dichloromethane extract.

365 It is interesting noting that *P. ostreatus* cyclohexane extract was strongest antioxidant in
366 FRAP and ORAC assays, while its reducing power reflected by the TPC values was quite equally
367 distributed in all fractions isolated from this species. Previously reported TPC value of methanol
368 extract isolated from *P. ostreatus* was 15.7 ± 0.1 mg/g (Yang et al., 2002), i.e. 3 times higher than
369 in our study measured methanol extract; however, the extract in previous study was obtained
370 from the whole material, while in our study methanol was used for reextracting the residue after
371 cyclohexane and dichloromethane extraction. In another study (Yim, Chye, Tan, Ng & Ho, 2010)
372 approx. 8 mg of phenolics (in tannic acid equivalents) in 1 g dw water extract were determined.
373 The sum of TPC in all organic extracts obtained in our study was 19.98 mg GAE/g, i.e. similar as
374 in the previously assayed methanol extract (Yang et al., 2002).

375 Water is a preferable solvent in terms of toxicity and availability; however, it is not always
376 sufficiently efficient for the isolation for plant bioactive compounds. Our study shows that water
377 may be useful solvent for the extraction of remaining antioxidants from some mushroom species
378 after applying different polarity organic solvents. For instance, water extract of *I. hispidus* was
379 strongest antioxidant in ABTS⁺, FRAP and TPC assays; however, it was almost 6 times weaker
380 in ORAC assay compared to methanol fraction. It is also important noting that the yield of water
381 extract from *I. hispidus* was remarkably higher than the yields obtained with other solvents; water
382 fraction constituted 67 % of the total extractives. Water extracts of other species were also
383 remarkably less effective in ORAC assay, except for *H. ferrugineum*, when the difference
384 between methanol and water fractions constituted only 14 %. It should be noted that water
385 extracts were obtained by boiling the residues of extractions with organic solvents and in this case
386 some hydrolysis and other processes involving chemical changes may occur in extraction
387 material. Cyclohexane and dichloromethane fractions were several times weaker radical
388 scavengers than polar methanol and water extracts. It is in agreement with many previously

389 published results showing that polar solvents extract more antioxidants from botanicals than
 390 lower polarity solvents (Brahmi, Mechri, Dabbou, Dhibi & Hammami, 2012). ASEs were
 391 calculated for the extracts isolated with different solvents for comparative assessment of the
 392 effectiveness of each solvent for the tested mushroom species. They may be located in the
 393 following decreasing order (ASE in $\mu\text{g}/\text{g}$ edw is indicated in the brackets):

394 *I. hispidus*: W (569) > M (471) > D (61) > C (24);

395 *P. schweinitzii*: M (654) > W (168) > D (69) > C (50);

396 *T. columbetta*: C (177) > D (150) > M (74) > W (29);

397 *H. ferrugineum*: D (120) > W (106) > M (96) > C (64);

398 *P. ostreatus*: C (135) > D (107) > M (84) > W (13);

399 *T. caligatum*: M (171) > D (90) > W (35);

400 *A. bisporus*: M (74) > D (52) > W (38) > C (36);

401 *X. chrysenteron*: M (60) > C (42) > D (36) > W (30).

402 However, it should be noted that in this case the scores were calculated by summing the values
 403 measured for 1 g of extracts dw and are not similar to ASM which were calculated in $\mu\text{g}/\text{g}$ mdw
 404 and presented in Fig. 2; the latter reflect the antioxidant potential of all extracted fractions plus
 405 their yields in all assays.

406

407 3.2.3. Effects of assay method

408 Antioxidant activity values obtained by using different evaluation assays are in a very wide
 409 range, they depend both on mushroom species and extracted fraction. Generally the highest
 410 values were obtained in ORAC assay; their sum from all fractions were from 104 (*X.*
 411 *chrysenteron*) to 462 $\mu\text{M TE}/\text{g}$ (*P. schweinitzii*). FRAP values were from 25 (*P. ostreatus*) to 425

412 $\mu\text{M TE/g}$ (*I. hispidus*); TEAC values in ABTS^{•+} scavenging assay were from 2.1 (*P. ostreatus*) to
413 225 $\mu\text{M TE/g}$ (*I. hispidus*), while the lowest values were measured in DPPH[•] scavenging assay,
414 from 2.1 (*A. bisporus*) to 23.5 $\mu\text{M TE/g}$ (*P. schweinitzii*). Strong correlation was observed
415 between total phenolics and DPPH[•] ($R^2 = 0.8969$) and ABTS^{•+} scavenging capacity ($R^2 =$
416 0.9255), confirming that phenolic compounds are important contributors to the antioxidant
417 properties of these extracts. However, the correlations between TPC and ORAC ($R^2 = 0.7712$), as
418 well as between TPC and FRAP ($R^2 = 0.7573$) were weaker.

419 Several reasons may be considered to explain the obtained differences between the applied
420 assays. Although the principle of the applied radical scavenging or reduction assays are based on
421 SET and/or HAT, the peculiarities of reaction mechanisms in each assay are different; they may
422 largely depend on reaction media, pH, the structure of antioxidative compounds present in the
423 extracts, their interactions and other factors. For instance, Zan et al. (2011), reported that in
424 ABTS^{•+} scavenging assay, 5 from *I. hispidus* methanol extract isolated compounds exhibited
425 significant activity, from 12.71 ± 3.57 to $59 \pm 9.70 \mu\text{M TE}/\mu\text{M compound}$. These findings
426 support our results indicating high ABTS^{•+} scavenging capacity of *I. hispidus* water and methanol
427 fractions. Thus, the TEAC values in ABTS^{•+} assay of the all studied mushrooms measured in the
428 all extracts were of the following decreasing order: *I. hispidus* > *P. schweinitzii* > *T. caligatum* >
429 *H. ferruginemum* > *T. columbetta* > *X. chrysenteron* > *A. bisporus* > *P. ostreatus*. However, the
430 TEAC values recalculated for 1 g mdw, which also consider extract yields would be in different
431 order (TEAC in $\mu\text{M TE/g mdw}$ in brackets): *P. schweinitzii* (23.45) > *I. hispidus* (20.81) >> *T.*
432 *caligatum* (3.54) > *T. columbetta* > (0.60) > *X. chrysenteron* (0.59) > *H. ferruginemum* (0.52) > *A.*
433 *bisporus* (0.41) > *P. ostreatus* (0.11). Antioxidant properties of *A. bisporus* extracts sequentially
434 isolated by different polarity solvents were evaluated previously by measuring their effective

435 concentrations EC_{50} ; aqueous methanol fraction was stronger antioxidant comparing to hexane
436 and ethyl acetate extracts in β -carotene linoleic acid co-oxidation system, DPPH \cdot , ABTS $^{*+}$ and
437 CUPRAC assays (Öztürk et al., 2011). Although we used different extraction procedure and
438 antioxidant activity assays, some agreement in the obtained results may be observed, particularly
439 in case of ABTS $^{*+}$ scavenging assay, when in both studies methanol and water extracts were
440 remarkably stronger antioxidants and ABTS $^{*+}$ values were higher than DPPH \cdot values. RSC of
441 water fraction of *P. ostreatus* was also measured by Yim et al. (2010); however, it was expressed
442 in percentage of scavenged radicals, i.e. the units which are not applicable for comparison
443 purposes.

444 The sum of values measured in DPPH \cdot scavenging assay of all extracts were of the following
445 decreasing order: *P. schweinitzii* > *I. hispidus* > *T. caligatum* > *H. ferruginemum* > *X.*
446 *chryseneteron* > *T.columbetta* > *P. ostreatus* > *A. bisporus*. Again integrated DPPH \cdot scavenging
447 values recalculated for 1 g mdw would be in slightly different order (ciu/g mdw in brackets): *P.*
448 *schweinitzii* (1.93) > *I. hispidus* (1.41) > *T. caligatum* (0.55) > *T. columbetta* (0.40) > *X.*
449 *chryseneteron* (0.27) > *H. ferruginemum* (0.16) > *P. ostreatus* (0.10) > *A. bisporus* (0.07).
450 Previous reported values for *A. bisporus*, $IC_{50}=0.38$ mg/mL for ethanol extract (Liu, Jia, Kan &
451 Jin, 2013) and $IC_{50}=0.988 \pm 0.3$ mg/mL for methanol extract (Öztürk et al., 2011) are difficult to
452 compare with our results obtained for *A. bisporus* methanolic and water fractions (0.13-0.14 μ M
453 TE/g). Our study shows that DPPH \cdot scavengers from this species are more effectively extracted
454 with cyclohexane (1.53 μ M TE/g), whereas in case of other studied species cyclohexane fractions
455 were weaker DPPH \cdot scavengers, except for dichloromethane fraction isolated from *P. scweinitzii*,
456 which was almost 3 times weaker in this assay than cyclohexane fraction. DPPH \cdot scavenging
457 capacity was also recently reported for *P. ostreatus* and it was shown that it is dose-dependent
458 (Mishra et al., 2013). Effective DPPH \cdot scavenging concentration EC_{50} of *X. chryseneteron*

459 methanol/water extract was 2.06 ± 0.46 mg/mL (Heleno et al., 2012), while methanol fraction,
460 depending on extract concentration, inhibited from 27.42 ± 1.23 to 89.61 ± 0.10 % DPPH[•]
461 (Sarikurkcu et al., 2008).

462 The third method used to evaluate antioxidant potential of mushroom species was ferric ion
463 reducing ability (FRAP). In case of this assay extract's efficiency was in the following decreasing
464 order: *I. hispidus* (37.58) > *P. schweinitzii* (20.08) > *H. ferruginemum* (2.35) > *P. ostreatus*
465 (0.82) > *T. columbetta* (3.17) > *X. chrysenteron* (2.98) > *T. caligatum* (3.80) > *A. bisporus*
466 (0.73). However, the integrated FRAP values calculated for 1 g mdw as it is indicated in the
467 brackets were in different order. FRAP was also used in some other studies of mushrooms;
468 however, their data is difficult to compare due to different units used to express the data. Metal
469 chelating ability of *P. ostreatus* (Mishra et al., 2013) and *X. chrysenteron* (Sarikurkcu et al.,
470 2008) methanol extracts was reported previously as well, however, it was also expressed in
471 relative units. *A. bisporus* demonstrated strong Fe²⁺ ion chelating capacity: EC₅₀ = 310.00 ± 0.87
472 µg/mL (Öztürk et al., 2011). Chelating agents may act as secondary antioxidants by reducing
473 redox potential and stabilizing the oxidised forms of metal ions (Mishra et al., 2013).

474 Finally, the antioxidant activity of mushrooms was evaluated using ORAC assay; the values
475 for investigated species are located in the following decreasing order considering the sum of
476 values in all extracts (ORAC values calculated for 1 g mdw are presented in the brackets): *P.*
477 *schweinitzii* (51.60) > *I. hispidus* (19.19) > *T. columbetta* (19.37) > *P. ostreatus* (11.34) > *H.*
478 *ferruginemum* (12.26) > *T. caligatum* (61.06) > *A. bisporus* (16.81) > *X. chrysenteron* (10.51).
479 Methanol fractions were strongest antioxidants in ORAC assay, except for *T. columbetta*
480 (cyclohexane and dichlorometane fractions were superior), *H. ferrugineum* and *P. ostreatus*,
481 when dichloromethane extracts were stronger peroxy radical inhibitors. The lipophilic and
482 hydrophilic ORAC values of *P. ostreatus* and *A. bisporus* (white button) acetone/water extracts

483 were reported 5.67 and 49.67 (total 55.34); 6.33 and 80.00 (total 86.33) $\mu\text{M TE/g dw}$ of
484 mushroom, respectively (Dubost, Ou & Beelman, 2007). By recalculating our results from 1 g of
485 edw to 1 g of mdw we obtained 11.34 $\mu\text{M TE/g}$ for *P. ostreatus* and 16.81 $\mu\text{M TE/g}$ for *A.*
486 *bisporus*, i.e. remarkably lower, comparing with the results reported in the above cited reference.
487 ORAC value of ethanol fraction of *A. bisporus* reported in the same article was 86.33 $\mu\text{M TE/g}$
488 dw, i.e. higher than the ORAC of methanol ($65.84 \pm 1.59 \mu\text{M TE/g dw}$) and water (30.92 ± 1.61
489 $\mu\text{M TE/g dw}$) fractions determined in our study; however, the sum of ORAC values in all extracts
490 measured in our study was 148.64 $\mu\text{M TE/g dw}$ indicating that powerful scavengers of ROO^\bullet
491 radicals are also present in lower polarity fractions.

492 The values measured with Folin–Ciocalteu reagent and expressed in gallic acid or other
493 phenolic compound are generally accepted as representing the total phenolic content (TPC)
494 although it is not fully correct: Folin–Ciocalteu reagent reacts not only with phenolics but with
495 other reducing ability possessing compounds in the reaction system (Huang et al., 2005).
496 Consequently, the term TPC may be used rather conditionally; however, for the convenience we
497 are using this term in our study. Thus, the integrated TPC values (their sum in the all extracts)
498 were distributed in the following decreasing order: *I. hispidus* > *P. schweinitzii* > *H.*
499 *ferruginemum* > *X. chrysenteron* > *T. columbetta* > *P. ostreatus* > *A. bisporus* > *T. caligatum*. It
500 is interesting noting that only methanol and water fractions of *I. hispidus*, methanol fraction of *P.*
501 *schweinitzii* and water fraction of *H. ferruginemum*, contained remarkably higher concentrations of
502 TPC than the fractions isolated from the same species with other solvents. For instance, the TPC
503 values in different extracts of commercial mushrooms *A. bisporus* and *P. ostreatus* were in the
504 range of 4.21–4.64 and 4.26–5.67 mg GAE/g, respectively. TPC in acetone/water fraction of *A.*
505 *bisporus* and *P. ostreatus* was previously reported 8.00 ± 0.48 and 4.27 ± 0.69 mg GAE/g mdw
506 (Dubost et al., 2007); we obtained lower values of TPC from these species (Fig. 1). Previously

507 reported TPC in *X. chrysenteron* (36.28 ± 0.5 mg GAE/g extract) methanol/water fraction
508 (Heleno et al., 2012) is higher than the sum of TPC in all extracts obtained from this species in
509 our study. In general, we obtained the values of the same order comparing to the previously
510 published data, while the differences are reasonable, so far as the content of polyphenolics in the
511 same species may vary depending on cultivar, harvesting time, climatic conditions and other
512 factors.

513

514 3.4. Antibacterial properties of plant extracts

515 Evaluation of antioxidant activity of extracts isolated from the selected mushroom species
516 by different solvents revealed remarkable variability in the obtained values. It is known that many
517 antioxidatively active compounds may also possess different effects against microorganisms.
518 Therefore, the study was continued for the preliminary screening of antimicrobial properties of
519 the extracts against two bacteria and 1 yeast species. The results obtained for 32 extracts isolated
520 from 8 mushroom species are summarized in Table 3 listing the inhibition zones in the agar
521 diffusion assay at different applied concentrations. In general, Gram-negative *P. aeruginosa* were
522 more sensitive to the applied extracts than Gram-positive *B. cereus*. For instance, the latter
523 bacterium was not inhibited by any of the extracts concentrations of 0.1 and 1 mg/mL.
524 Comparing the solvents, methanol extracts in most cases were the strongest antimicrobial agents,
525 whereas water fractions possessed the weakest inhibitory activity in case of all mushrooms
526 (cyclohexane and dichloromethane extracts of *P. ostreatus* also did not inhibit tested
527 microorganisms at any applied concentration).

528 Comparing mushrooms species, it may be observed that the fractions of *I. hispidus* isolated with
529 different solvents possessed antibacterial activity against 2 bacteria and 1 yeast species in the agar
530 diffusion assay, however water extract was effective only against *P. aeruginosa*. All extracts of

531 *P. shweinitzii* isolated with cyclohexane, dichloromethane and methanol inhibited tested
532 microorganism. For instance, the largest inhibition zones was observed for methanol fraction of
533 *P. shweinitzii* against *P. aeruginosa* (17 ± 0.5 mm), *B. cereus* (16 ± 1.9 mm), and *C. albicans* (15
534 ± 0.0 mm). Strong effect demonstrated methanol extract of *I. hispidus* against *P. aeruginosa* (17
535 ± 1.5 mm) and *C. albicans* (15 ± 0.5 mm). It is interesting noting that these two above mentioned
536 species also possessed the highest antioxidant potential, comparing with other studied species,
537 which were remarkably less active in antimicrobial tests. *T. columbetta* methanol fraction showed
538 strong effect against *C. albicans* (17 ± 2.0 mm), *T. caligatum*, *X. chrysenteron*, *A. bisporus*, *P.*
539 *ostreatus* methanol fractions showed strong effect against *C. albicans* (15-14 mm); *H.*
540 *ferrugineum*, *A. bisporus* also showed strong effect against *P. aeruginosa* (15 mm).

541 MIC values which are evaluated by diluting the extracts and measuring the lowest inhibitory
542 concentrations are important indicators of antimicrobial activity (Table 3). None of the tested
543 extracts was active at the concentration of 0.01 mg/mL. The lowest MIC values (0.1 mg/mL)
544 against *C. albicans* were determined for cyclohexane, dichloromethane and methanol fractions of
545 *I. hispidus* and *P. schweinitzii*; cyclohexane and methanol fractions of *H. ferrugineum*, and
546 dichloromethane fraction of *X. chrysenteron*. *B. cereus* was more resistant and MIC for different
547 fractions was not lower than 10 mg/mL. Dichloromethane fraction of *X. chrysenteron* was most
548 efficient against *P. aeruginosa* (MIC=0.1 mg/mL), 7 fractions from various mushrooms had MIC
549 of 1 mg/mL, while the majority of other fractions demonstrated MIC of 10 or 20 mg/mL. Only
550 few reports on antimicrobial activity of some species tested in our studies are available.
551 Previously reported MICs for *P. ostreatus* ethanol extract were 1.25 mg/mL against *C. albicans*,
552 2.5-20 mg/mL against *P. aeruginosa*, 2.5-12.5 mg/mL against *B. cereus*; antimicrobial activity
553 was shown to be dependent on nitrogen source (Vamanu, 2012). In reported studies methanol

554 extract isolated from *A. bisporus* showed similar antimicrobial activity against *C. albicans* 16 ± 0
555 s.d. and *B. cereus* 21 ± 0 (Öztürk et al., 2011).

556

557 **Conclusions**

558 Antioxidant potential of studied mushrooms was found to be in a rather wide range: the
559 differences were observed between the tested species as well as between the fractions isolated by
560 different solvents. Assay method was also important factor in determining antioxidant properties
561 of mushroom extracts, therefore ORAC, TPC measured with Folin-Ciocalteu reagent and one of
562 the single electron transfer assays, as recommended by Huang, Ou, & Prior (2005), should be
563 performed for the representative evaluation of antioxidant properties.

564 The fractions isolated with methanol and water from *P. schweinitzii* and *I. hispidus* were
565 most powerful antioxidants almost in all tested assays. Antimicrobial activity of mushroom
566 extracts expressed in minimal inhibitory concentration was also found to be in a wide range. The
567 results suggest that different classes of antioxidatively active constituents may be present in the
568 studied mushroom species. Remarkable differences in antioxidant and antimicrobial properties of
569 extracts isolated with different polarity solvents also indicate about the presence of high variety
570 'mycochemicals' in various mushroom species. Considering that comparatively low number of
571 compounds have been identified in the selected mushroom species until now, the results may
572 foster further studies of mushroom species aimed at searching of new bioactive compounds,
573 which might be of interest for various applications, such as ingredients of functional foods,
574 nutraceuticals, pharmaceuticals, and cosmetics.

575

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577

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584

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734
735 Figure legends

- 736
- 737 Figure 1. Total content of phenolic compounds (TPC) in mushrooms
- 738
- 739 Figure 2. Antioxidant scores of mushroom species (ASM) integrating antioxidant activity values
740 in ABTS^{•+}, DPPH[•], FRAP and ORAC assays and extract yields

741 Table 1. The yields of mushroom extracts isolated by different solvents, % (w/w).

| Mushroom species | Cyclohexane | Dichloromethane | Methanol | Water | Total |
|-------------------------------|-------------|-----------------|--------------|--------------|-------|
| <i>Phaeolus schweinitzii</i> | 3.75 ± 0.30 | 2.07 ± 0.16 | 13.91 ± 0.20 | 4.82 ± 0.20 | 24.55 |
| <i>Inonotus hispidus</i> | 0.40 ± 0.15 | 0.43 ± 0.25 | 4.55 ± 0.10 | 11.10 ± 0.15 | 16.48 |
| <i>Tricholoma columbetta</i> | 1.55 ± 0.05 | 1.57 ± 0.30 | 18.74 ± 0.30 | 25.29 ± 0.25 | 47.15 |
| <i>Tricholoma caligatum</i> | 4.00 ± 0.13 | 2.79 ± 0.10 | 36.69 ± 0.25 | 19.67 ± 0.30 | 63.15 |
| <i>Xerocomus chrysenteron</i> | 2.64 ± 0.25 | 1.90 ± 0.20 | 14.16 ± 0.12 | 21.23 ± 0.17 | 39.93 |
| <i>Hydnellum ferruginenum</i> | 1.42 ± 0.12 | 1.33 ± 0.12 | 11.67 ± 0.16 | 2.94 ± 0.23 | 17.36 |
| <i>Agaricus bisporus</i> | 1.18 ± 0.19 | 0.89 ± 0.24 | 16.95 ± 0.25 | 17.52 ± 0.18 | 36.54 |
| <i>Pleurotus ostreatus</i> | 1.90 ± 0.23 | 1.38 ± 0.10 | 10.29 ± 0.10 | 4.32 ± 0.20 | 17.89 |

742 Results are expressed as a mean ± standard deviation (n=3)

743

744 Table 2. Antioxidant activity of extracts isolated from 8 mushroom species by different solvents.

| Botanical name | Extract | $\mu\text{M TE/g dw}$ | | | | TPC, mg GAE/g dw |
|-------------------------------|---------|-----------------------|-------------------|---------------|---------------|------------------|
| | | ABTS ⁺ | DPPH [•] | FRAP | ORAC | |
| <i>Inonotus hispidus</i> | C | 0.10 ± 0.02 | 0.95 ± 0.13 | 10.89 ± 0.49 | 7.50 ± 0.08 | 4.79 ± 0.36 |
| | D | 5.87 ± 1.58 | 1.72 ± 0.13 | 24.59 ± 0.64 | 18.63 ± 3.19 | 9.70 ± 0.64 |
| | M | 54.27 ± 0.46 | 9.50 ± 0.04 | 88.31 ± 1.96 | 290.00 ± 1.00 | 28.91 ± 1.49 |
| | W | 165.00 ± 1.70 | 8.71 ± 0.04 | 301.00 ± 2.00 | 53.06 ± 1.09 | 41.27 ± 0.86 |
| <i>Tricholoma caligatum</i> | D | 4.02 ± 0.40 | 3.72 ± 0.30 | 8.75 ± 0.42 | 69.74 ± 1.98 | 3.87 ± 0.01 |
| | M | 7.44 ± 1.58 | 0.12 ± 0.02 | 0.43 ± 0.12 | 158.00 ± 0.51 | 4.76 ± 0.60 |
| | W | 3.53 ± 1.77 | 2.04 ± 0.46 | 17.29 ± 0.38 | 5.83 ± 1.03 | 5.89 ± 0.24 |
| <i>Tricholoma columbetta</i> | C | 3.50 ± 0.12 | 0.44 ± 0.11 | 12.02 ± 0.35 | 155.00 ± 1.00 | 6.25 ± 0.20 |
| | D | 3.31 ± 0.18 | 0.67 ± 0.64 | 6.52 ± 0.62 | 135.00 ± 3.00 | 4.74 ± 0.06 |
| | M | 0.48 ± 0.27 | 1.24 ± 0.48 | 0.91 ± 0.61 | 64.63 ± 3.57 | 6.60 ± 3.50 |
| | W | 1.60 ± 0.13 | 0.59 ± 0.26 | 10.72 ± 0.17 | 10.83 ± 0.65 | 5.18 ± 0.28 |
| <i>Phaeolus schweinitzii</i> | C | 4.82 ± 0.70 | 3.65 ± 0.92 | 0.45 ± 0.07 | 33.55 ± 1.89 | 7.07 ± 0.49 |
| | D | 4.17 ± 0.21 | 1.33 ± 0.04 | 10.95 ± 0.25 | 42.74 ± 2.55 | 9.38 ± 0.41 |
| | M | 164.00 ± 1.00 | 9.62 ± 0.03 | 109.00 ± 3.00 | 340.00 ± 3.00 | 31.88 ± 1.67 |
| | W | 7.93 ± 1.35 | 8.89 ± 2.84 | 97.19 ± 0.85 | 45.63 ± 2.70 | 8.20 ± 0.33 |
| <i>Xerocomus chrysenteron</i> | C | 0.89 ± 0.46 | 0.03 ± 0.01 | 8.12 ± 0.14 | 26.47 ± 1.44 | 6.44 ± 0.31 |
| | D | 3.54 ± 0.28 | 2.44 ± 0.04 | 7.52 ± 0.49 | 16.35 ± 0.04 | 5.74 ± 0.14 |
| | M | 1.37 ± 0.08 | 0.51 ± 0.16 | 3.64 ± 0.35 | 49.33 ± 3.29 | 4.96 ± 0.18 |
| | W | 1.45 ± 0.36 | 0.69 ± 0.07 | 9.92 ± 1.84 | 11.85 ± 0.43 | 6.56 ± 0.12 |
| <i>Hydnellum ferrugineum</i> | C | 1.77 ± 1.47 | 0.05 ± 0.02 | 16.95 ± 0.49 | 38.41 ± 1.97 | 7.22 ± 0.53 |
| | D | 1.08 ± 0.53 | 3.02 ± 0.34 | 18.02 ± 0.42 | 90.65 ± 1.16 | 6.92 ± 0.41 |
| | M | 1.38 ± 0.21 | 0.60 ± 0.06 | 11.71 ± 0.99 | 74.02 ± 3.06 | 8.05 ± 0.23 |
| | W | 10.71 ± 0.69 | 1.65 ± 0.15 | 16.99 ± 0.07 | 63.54 ± 3.34 | 13.31 ± 0.07 |
| <i>Agaricus bisporus</i> | C | 2.06 ± 0.24 | 1.53 ± 0.16 | 10.65 ± 0.64 | 17.33 ± 0.58 | 4.21 ± 0.05 |
| | D | 0.94 ± 0.16 | 0.32 ± 0.11 | 11.62 ± 0.17 | 34.55 ± 3.16 | 4.27 ± 0.06 |
| | M | 1.41 ± 0.38 | 0.13 ± 0.03 | 2.09 ± 0.12 | 65.84 ± 3.00 | 4.23 ± 0.12 |
| | W | 0.82 ± 1.82 | 0.15 ± 0.04 | 1.35 ± 1.34 | 30.92 ± 1.61 | 4.64 ± 0.03 |
| <i>Pleurotus ostreatus</i> | C | 0.10 ± 0.04 | 0.08 ± 0.07 | 15.49 ± 2.62 | 115 ± 4.00 | 4.73 ± 0.10 |
| | D | 0.55 ± 0.19 | 1.52 ± 0.37 | 8.42 ± 1.34 | 90.73 ± 2.32 | 5.67 ± 1.30 |
| | M | 0.65 ± 0.04 | 0.74 ± 0.21 | 1.49 ± 0.83 | 76.11 ± 1.59 | 5.32 ± 0.98 |
| | W | 0.80 ± 0.18 | 0.14 ± 0.05 | 6.02 ± 2.05 | 1.73 ± 0.54 | 4.26 ± 0.01 |

745 C, cyclohexane; D, dichloromethane; M, methanol; W, water; results are expressed as a mean ± standard deviation
746 (n=4); cyclohexane extracts of *T. caligatum* was not assayed.

747 Table 3. Antimicrobial activity of mushroom extracts, in mm of inhibition zones

| Botanical name | fraction | <i>Candida albicans</i> | | | | <i>Bacillus cereus</i> | | <i>Pseudomonas aeruginosa</i> | | |
|------------------------|----------|-------------------------|------------|-----------|-----------|------------------------|-----------|-------------------------------|-----------|-----------|
| | | 20 mg/mL | 10 mg/L | 1 mg/mL | 0.1 mg/mL | 20 mg/mL | 10 mg/mL | 20 mg/mL | 10 mg/mL | 1 mg/mL |
| <i>I. hispidus</i> | C | 10 ± 1.1 | 11 ± 2.0 | 8.5 ± 0.7 | 8.0 ± 0.7 | 8.0 ± 0.5 | 7.0 ± 1.0 | 7.5 ± 0.5 | 7 ± 0.0 | 6.5 ± 0.5 |
| | D | 9.5 ± 0.7 | 9.0 ± 0.5 | 8.5 ± 0.7 | 7.0 ± 0.4 | 7.5 ± 0.5 | 6.5 ± 1.0 | 9.5 ± 0.5 | 8.5 ± 1.0 | 7.2 ± 1.2 |
| | M | 15 ± 0.5 | 13 ± 0.0 | 7.0 ± 0.9 | 6.0 ± 0.0 | 13 ± 0.1 | 10 ± 2.1 | 17 ± 1.5 | 13 ± 1.0 | 6.5 ± 0.5 |
| | W | n.a | n.a | n.a | n.a | n.a | n.a | 12 ± 1.3 | 9 ± 0.3 | n.a. |
| <i>T. caligatum</i> | C | 10 ± 0.0 | 6.5 ± 0.0 | n.a | n.a | 11 ± 1.5 | n.a | 8.5 ± 0.5 | 6.5 ± 0.5 | n.a |
| | D | 6.0 ± 0.0 | n.a | n.a | n.a | 6.0 ± 0.0 | n.a | 9.0 ± 0.0 | n.a | n.a |
| | M | 15 ± 1.0 | 13 ± 0.0 | 7.0 ± 0.0 | n.a | 11 ± 2.1 | 10 ± 1.0 | 13 ± 0.0 | 9.5 ± 1.0 | n.a |
| <i>T. columbetta</i> | C | n.a | n.a | n.a | n.a | 8.0 ± 2.5 | n.a | 9.0 ± 1.0 | 7.0 ± 0.0 | n.a |
| | D | n.a | n.a | n.a | n.a | 11 ± 0.0 | n.a | 9.0 ± 1.0 | 9.0 ± 1.5 | n.a |
| | M | 17 ± 2.0 | 13 ± 1.5 | n.a | n.a | 13 ± 1.0 | 12 ± 0.0 | 14 ± 1.0 | 10 ± 1.5 | n.a |
| <i>P. schweinitzii</i> | C | 10 ± 1.0 | 8.5 ± 0.5 | 6.0 ± 1.0 | 6.0 ± 1.5 | 11 ± 0.0 | 9.0 ± 2.2 | 7 ± 1.2 | 6.0 ± 0.5 | n.a |
| | D | 8.0 ± 1.5 | 7.0 ± 0.0 | 7.0 ± 1.0 | 6.5 ± 1.5 | 16 ± 2.3 | 10 ± 0.9 | 9 ± 0.5 | n.a | n.a |
| | M | 15 ± 0.0 | 14 ± 0.5 | 9.5 ± 0.5 | 7.0 ± 0.0 | 16 ± 1.9 | 15 ± 1.5 | 17 ± 0.5 | 16 ± 1.0 | 13 ± 1.4 |
| <i>X. chrysenteron</i> | C | n.a | n.a | n.a | n.a | n.a | n.a | 7.0 ± 0.5 | 6.0 ± 1.0 | 6.0 ± 1.0 |
| | D | 9.0 ± 0.5 | 7.0 ± 1.0 | 7.0 ± 5.0 | 6.5 ± 2.5 | n.a | n.a | 9.5 ± 0.9 | 9.0 ± 0.5 | 8.0 ± 1.5 |
| | M | 15 ± 0.9 | 14 ± 1.1 | n.a | n.a | 13 ± 0.5 | n.a | 10 ± 1.0 | 9.0 ± 0.5 | n.a |
| <i>H. ferrugineum</i> | C | 9.0 ± 1.0 | 8.0 ± 1.3 | 7.0 ± 0.5 | 7.0 ± 0.0 | n.a | n.a | n.a | n.a | n.a |
| | D | n.a | n.a | n.a | n.a | 6.5 ± 1.3 | 6.0 ± 1.0 | n.a | n.a | n.a |
| | M | 14 ± 1.0 | 14.3 ± 1.1 | 9.0 ± 0.5 | 7.0 ± 1.0 | n.a | n.a | 15 ± 1.3 | 14 ± 1.1 | n.a |
| <i>A. bisporus</i> | C | n.a | n.a | n.a | n.a | 14 ± 0.0 | 10 ± 2.3 | n.a | n.a | n.a |
| | D | n.a | n.a | n.a | n.a | 13 ± 0.5 | 12 ± 0.4 | 15 ± 0.0 | 14 ± 0.7 | n.a |
| | M | 15 ± 1.2 | 13 ± 0.0 | n.a | n.a | n.a | n.a | 12 ± 2.1 | 10 ± 1.5 | 6.5 ± 1.4 |
| <i>P. ostreatus</i> | M | 15 ± 1.4 | 13 ± 1.0 | 6 ± 1.3 | n.a | 10 ± 1.2 | 9.0 ± 1.0 | 10 ± 1.1 | 8.0 ± 1.4 | n.a |

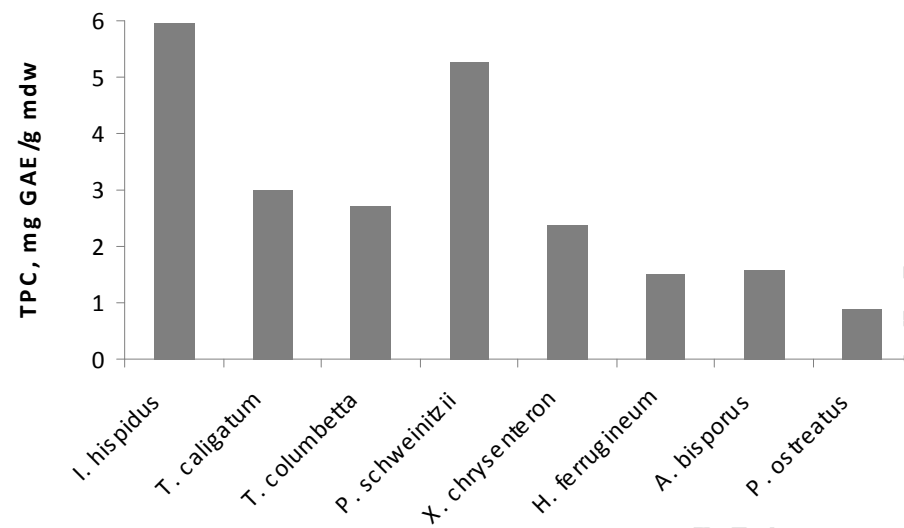
748 C, cyclohexane; D, dichloromethane; M, methanol; W, water; n.a., not active; results are expressed as a mean ± standard deviation (n=3); none of the extracts was
749 effective against *Bacillus cereus* at 0.1 and 1 mg/mL; only dichloromethane extract of *X. chrysenteron* formed inhibition zone (6.0 ± 1.5 mm) against *P.*
750 *aeruginosa* at 0.1 mg/mL; the fractions which did not form any inhibition zone at all applied concentrations are excluded from the table

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753 *Smolskaitė et al., Figure 1*

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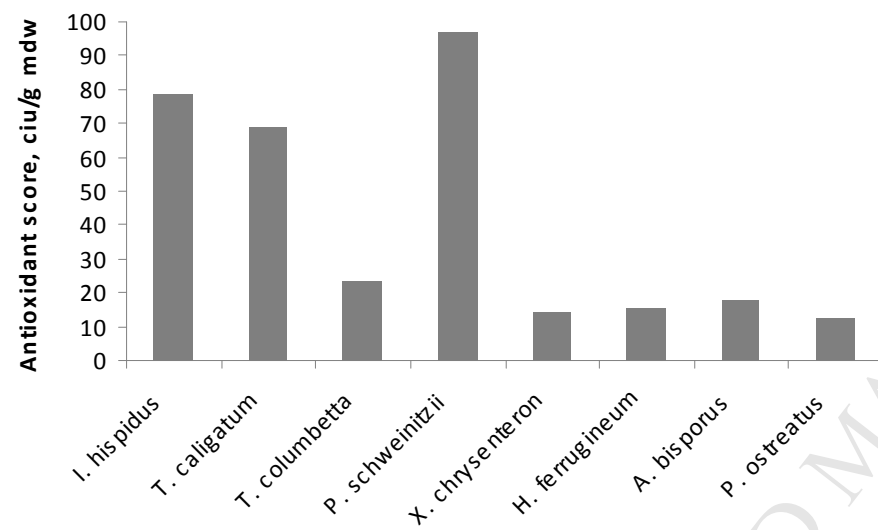


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757 *Smolskaitė et al., Figure 2*

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Highlights

- 8 mushroom species were sequentially extracted with increasing polarity solvents
- Antioxidant potential evaluated by ABTS, DPPH, FRAP, ORAC and total phenols content
- *Phaeolus schweinitzii* and *Inonotus hispidus* had strongest antioxidant potential
- The extracts of the same mushroom species were strongest antimicrobial agents
- The results foster search for new bioactive compounds in mushrooms