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Comprehensive evaluation of antioxidant and antimicrobial properties of different mushroom species

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Abstract

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

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

1. Introduction

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

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

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

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

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

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

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

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

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

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

2. Materials and Methods

2.1. Mushrooms and chemicals

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

Stable 2,2-*di*-phenyl-1-picrylhydrazyl hydrate radical (DPPH$^{\bullet}$, 95%), gallic acid, anhydrous sodium carbonate, 6-hydroxy-2-5-7-8-tetramethylchroman-2-carboxylic acid (Trolox, 97%), 2,20-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), fluorescein (FL) and [2,2'-azobis(2-amidino-propane) dihydrochloride (AAPH)] were from Sigma-Aldrich (Steinheim, Germany); 2.0 M Folin–Ciocalteu phenol reagent, KCl, NaCl, Na_2HPO_4, Na_2CO_3 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$^{\bullet}$-scavenging capacity*
147 This method is based on scavenging DPPH$^{\bullet}$ 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 µL of different concentration mushroom
151 extracts (0.5%; 0.25%; 0.125%) and 300 µL of methanolic solution of DPPH$^{\bullet}$ (6×10^{-5} M). The
152 mixture was left to stand for 40 min in the dark and the reduction of DPPH$^{\bullet}$ was determined by
153 measuring the absorption at 515 nm. All measurements were performed in triplicate. Radical

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

2.3.2. $ABTS^{\bullet+}$ decolourisation assay

The Trolox equivalent antioxidant capacity (TEAC) assay is based on the scavenging of $ABTS^{\bullet+}$ by the antioxidant which may be measured spectrophotometrically (Re et al., 1999). A stock solution of 2 mM ABTS was prepared by dissolving reagent in 50 mL of phosphate buffered saline (PBS) obtained by dissolving 8.18 g NaCl, 0.27 g KH_2PO_4, 1.42 g Na_2HPO_4 and 0.15 g KCl in 1 L of Milli-Q water. If pH was lower than 7.4, it was adjusted with NaOH. $ABTS^{\bullet+}$ was produced by reacting 50 mL of ABTS stock solution with 200 µL of 70 mM $K_2S_2O_8$ solution in purified water and allowing the mixture to stand in the dark at room temperature for 15–16 h before use. The radical was stable in this form for more than 2 days when stored in the dark at room temperature. For the assessment of extracts, the $ABTS^{\bullet+}$ solution was diluted with PBS to obtain the absorbance of 0.800 ± 0.030 at 734 nm. One mL of $ABTS^{\bullet+}$ solution was mixed with 10 µL extract solution in 96-well microtiter plates. The absorbance was read at ambient temperature every minute during 40 min. PBS solution was used as a blank; all measurements were performed in triplicate. The TEAC was determined from the calibration curve, which was drawn using 50, 100, 125, 250, 500, 1000 µM/L concentration solutions of Trolox and calculated in µM TE/g edw as follows: $TEAC\left(\frac{\mu M}{g}\right) = \frac{TE_s}{1000} \times \frac{V_s}{m_s}$, TE_s – antioxidant activity of sample expressed in TE (µM), V_s – sample volume (mL), m_s - sample mass (g).

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 µM 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 ºC and 26 µL of AAPH solution (240 mM)
194 as a peroxyl 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

curves the area under the fluorescence decay curve (AUC) was calculated as $AUC = 1 + \sum_{i=1}^{i=80} \frac{f_i}{f_0}$, where f_0 is the initial fluorescence reading at 0 min and f_i is the fluorescence reading at time i. The final ORAC values were calculated by using a regression equation between the Trolox concentration and the net area under the curve (AUC). The TEAC values were determined as described in previous sections.

2.3.5. Determination of total phenolic content (TPC)

The TPC was measured with Folin–Ciocalteu reagent as originally described by Singleton, Orthofer & Lamuela-Raventos (1999). Briefly, 30 μL (0.1%) of sample were mixed with 150 μL of 10-fold diluted (v/v) Folin−Ciocalteu reagent, and 120 μL of 7.5% Na_2CO_3. After mixing of all reagents, the microplate was placed in the reader and shaken for 30 s. After incubation for 30 min at room temperature the absorbance of the mixtures was measured at 765 nm. All measurements were performed in triplicate. A series of gallic acid solutions in the concentration range of 0.025-0.35 mg/mL was used for the calibration curve. The results were expressed in mg of gallic acid equivalents per g of dry extract weight (mg GAE/g edw).

2.4. Antibacterial assay

The antimicrobial activity was assessed by the disk-diffusion method (Bauer, Kirby, Sheriss & Turck, 1966). The bacterial cell suspension was prepared from 24 h culture and adjusted to an inoculation of $1x10^6$ colony forming units per mL (cfu/mL). Sterile nutrient agar (Bit Phar. acc EN 12780:2002, 28 g/L distilled water, Scharlau, Barcelona, Spain) was inoculated with bacterial cells (200 μL of bacterial cell suspension in 20 mL medium) and poured into dishes to obtain a 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

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

3. Results and discussion

3.1. Extraction yield

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

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

3.2. Antioxidant potential of different mushroom species

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

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

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

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

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 *bisphorus* were the weakest antioxidant sources in these assays. For instance, the sum of TEAC
323 of *I. hispidus* extracts in $ABTS^{\bullet+}$ scavenging assay (225.2 μM TE/g) was more than 100 times
324 higher comparing to *P. ostreatus* (2.1 μM 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 $DPPH^{\bullet}$ and $OH^{\bullet}$ 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.

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

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

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

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

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

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

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

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

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

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

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

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

3.2.3. Effects of assay method

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

μM TE/g (*I. hispidus*); TEAC values in $ABTS^{\bullet+}$ scavenging assay were from 2.1 (*P. ostreatus*) to 225 μM TE/g (*I. hispidus*), while the lowest values were measured in $DPPH^{\bullet}$ scavenging assay, from 2.1 (*A. bisporus*) to 23.5 μM TE/g (*P. schweinitzii*). Strong correlation was observed between total phenolics and $DPPH^{\bullet}$ (R^2 = 0.8969) and $ABTS^{\bullet+}$ scavenging capacity (R^2 = 0.9255), confirming that phenolic compounds are important contributors to the antioxidant properties of these extracts. However, the correlations between TPC and ORAC (R^2 = 0.7712), as well as between TPC and FRAP (R^2 =7573) were weaker.

Several reasons may be considered to explain the obtained differences between the applied assays. Although the principle of the applied radical scavenging or reduction assays are based on SET and/or HAT, the peculiarities of reaction mechanisms in each assay are different; they may largely depend on reaction media, pH, the structure of antioxidative compounds present in the extracts, their interactions and other factors. For instance, Zan et al. (2011), reported that in $ABTS^{\bullet+}$ scavenging assay, 5 from *I. hispidus* methanol extract isolated compounds exhibited significant activity, from 12.71 ± 3.57 to 59 ± 9.70 μM TE/μM compound. These findings support our results indicating high $ABTS^{\bullet+}$ scavenging capacity of *I. hispidus* water and methanol fractions. Thus, the TEAC values in $ABTS^{\bullet+}$ assay of the all studied mushrooms measured in the all extracts were of the following decreasing order: *I. hispidus* > *P. schweinitzii* > *T. caligatum* > *H. ferruginemum* > *T. columbetta* > *X. chrysenteron* > *A. bisporus* > *P. ostreatus*. However, the TEAC values recalculated for 1 g mdw, which also consider extract yields would be in different order (TEAC in ciu/g mdw in brackets): *P. schweinitzii* (23.45) > *I. hispidus* (20.81) >>*T. caligatum* (3.54) *T. columbetta* > (0.60) > *X. chrysenteron* (0.59) > *H. ferruginemum* (0.52) > *A. bisporus* (0.41) > *P. ostreatus* (0.11). Antioxidant properties of *A. bisporus* extracts sequentially 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, $DPPH^{\bullet}$, $ABTS^{\bullet+}$ 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 $ABTS^{\bullet+}$ scavenging assay, when in both studies methanol and water extracts were
440 remarkably stronger antioxidants and $ABTS^{\bullet+}$ values were higher than $DPPH^{\bullet}$ 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 $DPPH^{\bullet}$ scavenging assay of all extracts were of the following
445 decreasing order: *P. schweinitzii* > *I. hispidus* > *T. caligatum* > *H. ferruginemum* > *X.*
446 *chrysenteron* > *T.columbetta* > *P. ostreatus* > *A. bisporus.* Again integrated $DPPH^{\bullet}$ 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 *chrysenteron* (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 ± 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 μM
453 TE/g). Our study shows that $DPPH^{\bullet}$ scavengers from this species are more effectively extracted
454 with cyclohexane (1.53 μM TE/g), whereas in case of other studied species cyclohexane fractions
455 were weaker $DPPH^{\bullet}$ scavengers, except for dichloromethane fraction isolated from *P. scweinitzii*,
456 which was almost 3 times weaker in this assay than cyclohexane fraction. $DPPH^{\bullet}$ 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 $DPPH^{\bullet}$ scavenging concentration EC_{50} of *X. chrysenteron*

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 % $DPPH^{\bullet}$
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 Fe^{2+} ion chelating capacity: EC_{50} = 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 peroxyl radical inhibitors. The lipophilic and
482 hydrophilic ORAC values of *P. ostreatus* and *A. bisporus* (white button) acetone/water extracts

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

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

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

3.4. Antibacterial properties of plant extracts

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

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

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

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

Ainsworth, A. M., Parfitt, D., Rogers, H. J., & Boddy, L. (2010). Cryptic taxa within European species of *Hydnellum* and *Phellodon* revealed by combined molecular and morphological analysis. *Fungal Ecology, 3*, 65-80.

Ali, N. A. A., Jansen, R., Pilgrim, H., Liberra, K., & Lindequist, U. (1996). Hispolon, a yellow pigment from *Inonotus hispidus*. *Phytochemistry, 41*, 927–929.

Arbaayah, H. H., & Kalsom Y. U., (2013). Antioxidant properties in the oyster mushrooms (*Pleurotus* spp.) and split gill mushroom (*Schizophyllum commune*) ethanolic extracts. *Mycosphere, 4*, 661–673.

Augustyniak A., Bartosz, G., Čipak, A., Duburs, G., Horáková, L., Łuczaj, W., et al. (2010). Natural and synthetic antioxidants: An updated overview. *Free Radical Research, 44*, 1216-1262.

Barros, L., Baptista, P., & Ferreira, I. C.F.R. (2007). Effect of *Lactarius piperatus* fruiting body maturity stage on antioxidant activity measured by several biochemical assays. *Food and Chemical Toxicology, 45*, 1731–1737.

601 Barros, L., Cruz, T., Baptista, P., Estevinho, L. M., & Ferreira, I. C. F. R. (2008). Wild and
602 commercial mushrooms as source of nutrients and nutraceuticals. *Food and Chemical*
603 *Toxicology, 46*, 2742–2747.
604 Bauer, A. W., Kirby, W. M. M., Sheriss, J. C., & Turck, M. (1996). Antibiotic susceptibility
605 testing by standardized single disk method. *American Journal of Clinical Pathology, 45*, 493-
606 496.
607 Benzie, I. F. F., & Strain, J. J. (1999). Ferric reducing antioxidant power assay: direct measure of
608 total antioxidant activity of biological fluids and modified version for simultaneous
609 measurement of total antioxidant power and ascorbic acid concentration. *Methods in*
610 *Enzymology, 299*, 15–27.
611 Birck, C., Damian, L., Marty-Detraves, C., Lougarre, A., Schulze-Briese, C., Koehl, P., et al.
612 (2004). A new lectin family with structure similarity to actinoporins revealed by the crystal
613 structure of *Xerocomus chrysenteron* lectin XCL. *Journal of Molecular Biology, 344*, 1409–
614 1420.
615 Brahmi, F., Mechri, E., Dabbou, S., Dhibi, M., & Hammami, M. (2012). The efficacy of
616 phenolics compounds with different polarities as antioxidants from olive leaves depending on
617 seasonal variations. *Industrial Corps and Products, 38*, 146-152.
618 Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to
619 evaluate antioxidant activity. *LWT-Food Science and Technology, 28,* 25-30.
620 Cedano, M., Villaseñor, L., & Guzmán-Dávalos, L. (2001). Some aphyllophorales tested for
621 organic dyes. *Mycologist, 15,* 81-85.
622 Dávalos, A. D., Gómez-Cordovés, C., & Bartolomé, B. (2004). Extending applicability of the
623 oxygen radical absorbance capacity (Orac-fluorescein) assay. *Journal of Agricultural and*
624 *Food Chemistry, 52*, 48-54.

625 Dubost, N. J., Ou, B., & Beelman, R. B. (2007). Quantification of polyphenols and ergothioneine
626 in cultivated mushrooms and correlation to total antioxidant capacity. *Food Chemistry, 105*,
627 727–735.
628 Fons, F., Rapior, S., Fruchier, A., Saviuc, P., & Bessière, J. M. (2006). Volatile composition of
629 *Clitocybe amoenolens, Tricholoma caligatum* and *Hebeloma radicosum. Cryptogamie*
630 *Mycologie, 27*, 45-55.
631 Frankel, E. N., & Meyer, A. S. (2000). The problems of using one-dimensional methods to
632 evaluate multifunctional food and biological antioxidants. *Journal of the Science of Food and*
633 *Agriculture, 80,* 1925–1941.
634 Gern, R. M. M., Wisbeck, E., Rampinelli, J. R., Ninow, J. L., & Furlan, S. A. (2008). Alternative
635 medium for production of *Pleurotus ostreatus* biomass and potential antitumor
636 polysaccharides. *Bioresource Technology, 99*, 76–82.
637 Guillamón, E., García-Lafuente, A., Lozano, M., D´Arrigo, M., Rostagno, M. A., Villares, A., et
638 al. (2010). Edible mushrooms: Role in the prevention of cardiovascular diseases. *Fitoterapia,*
639 *81*, 715–723.
640 Hearst, R., Nelson, D., McCollum, G., Millar, B. C., Maeda, Y., Goldsmith, C. E., et al. (2009).
641 An examination of antibacterial and antifungal properties of constituents of Shiitake
642 (*Lentinula edodes*) and Oyster (*Pleurotus ostreatus*) mushrooms. *Complementary Therapies in*
643 *Clinical Practice, 15,* 5–7.
644 Heleno, A. S., Barros, L., Martins, A., Queiroz, M. J. R. P., Santos-Buelga, C., & Ferreira, C. F.
645 R. I. (2012). Phenolic, polysaccharidic, and lipidic fractions of mushrooms from northeastern
646 Portugal: chemical compounds with antioxidant properties. *Journal of Agricultural Food*
647 *Chemistry, 60,* 4634−4640.

Huang, D., Ou, B., & Prior, R. L. (2005). The chemistry behind antioxidant capacity assays. *Journal of Agricultural and Food Chemistry, 53,* 1841–1856.

Jones, S., & Janardhanan, K. K. (2000). Antioxidant and antitumor activity of *Ganoderma lucidum* (Curt: Fr) P. Karst-Reishi (Aphyllophoromycetideae) from South India. *International Journal of Medical Mushrooms, 86,* 234-241.

Kalogeropoulos, N., Yanni A. E., Koutrotsios, G., & Aloupi, M. (2013). Bioactive microconstituents and antioxidant properties of wild edible mushrooms from the island of Lesvos, Greece. *Food and Chemical Toxicology, 55*, 378–385.

Kim, M. Y., Seguin, P., Ahn, J. K, Kim, J. J., Chun, S. C., Kim, E. H., et al. (2008). Phenolic compound concentration and antioxidant activities of edible and medicinal mushrooms from Korea. *Journal of Agricultural and food chemistry, 56,* 7265-7270.

Laguerre, M., Lecomte, J., & Villeneuve, P. (2007). Evaluation of the ability of antioxidants to counteract lipid oxidation: Existing methods, new trends and challenges. *Progress in Lipid Research, 46,* 244–282.

Lamaison, J. L., Pourrat, H., & Pourratt, A. (1980). Purification et propriétés d'une protéase neutre de *Tricholoma columbetta. Phytochemistry, 19,* 1021–1023.

Liu, J., Jia, L., Kan, J., & Jin, J. (2013). In vitro and in vivo antioxidant activity of ethanolic extract of white button mushroom (*Agaricus bisporus*). *Food and Chemical Toxicology, 51*, 310–316.

Mathew, J., Sudheesh, N. P., Rony, K. A., Smina, T. P., Janardhanan, K. K. (2008). Antioxidant and antitumor activities of cultured mycelium of culinary – medicinal paddy straw mushroom *Volvariella volvacea* (Bull.: Fr.) Singer (Agaricomycetideae). *Internationl Journam of Medical Mushrooms, 10,* 139–147.

671 Mishra, K. K., Pal, R. S., ArunKumar, R., Chandrashekara, Jain, C. S. K., Bhatt, J. C.(2013).
672 Antioxidant properties of different edible mushroom species and increased bioconversion
673 efficiency of *Pleurotus eryngii* using locally available casing materials. *Food Chemistry, 138*,
674 1557–1563.
675 Murata, H., Ota, Y., Yamada, A., Ohta, A., Yamanaka T., & Neda, H. (2013). Phylogenetic
676 position of the ectomycorrhizal basidiomycete *Tricholoma dulciolens* in relation to species of
677 Tricholoma that produce "matsutake" mushrooms. *Mycoscience, 54*, 438-443.
678 Nitha, B., Strayo, D., Adhikari, S. K., Devasagayam, T. P. A., Janardhanan, K. K. (2010).
679 Evaluation of free radical scavenging activity of morel mushroom, *Morchella esculenta*
680 mycelia: a potential source of therapeutically useful antioxidants. *Pharmaceutical Biology, 48*,
681 453–460.
682 Öztürk, M., Duru, M. E., Kivrak, S., Mercan-Doğan, N., Türkoglu, A., Özler, M. A. (2011). In
683 vitro antioxidant, anticholinesterase and antimicrobial activity studies on three *Agaricus*
684 species with fatty acid compositions and iron contents: A comparative study on the three most
685 edible mushrooms. *Food and Chemical Toxicology, 49*, 1353–1360.
686 Prior, R. L., Wu, X., & Schaich, K. (2005). Standardized methods for the determination of
687 antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural*
688 *and Food Chemistry, 53*, 4290–4302.
689 Rajbhandari, M., & Schöpke, T. (1999). Antimicrobial activity of some Nepalese medicinal
690 plants. *Pharmazie, 54*, 232-233.
691 Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999).
692 Antioxidant activity applying an improved $ABTS^{\bullet+}$ radical cation decolorization assay, *Free*
693 *Radical Biology & Medicine, 26*, 1231–1237.

694 Sarikurkcu, C., Tepe, B., & Yamac. M. (2008). Evaluation of the antioxidant activity of four
695 edible mushrooms from the Central Anatolia, Eskisehir – Turkey: *Lactarius deterrimus*,
696 *Suillus collitinus, Boletus edulis, Xerocomus chrysenteron*. *Bioresource Technology, 99,*
697 6651–6655.
698 Schneider, I., Kressel, G., Meyer, A., Kings, U., Berger, R. G., & Hahn, A. (2011). Lipid
699 lowering effects of oyster mushroom (*Pleurotus ostreatus*) in humans. *Journal of Functional*
700 *Foods*, *3,* 17-24.
701 Shi, Y., James, A. E., Benzie, I. F. F., & Buswell, J. A. (2002). Mushroom-derived preparations
702 in the prevention of H_2O_2-induced oxidative damage to cellular DNA. *Teratogenesis,*
703 *Carcinogenesis, and Mutagenesis*, *22,* 103–111.
704 Singh, P., Langowski, H. C., Wani A. A., & Saengerlauba, S. (2010). Recent advances in
705 extending the shelf life of fresh *Agaricus* mushrooms: a review. *Journal of the Science of Food*
706 *and Agriculture*, *90,* 1393–1402.
707 Singleton V. L., Orthofer, R., & Lamuela-Raventos, R. M. (1999). Analysis of total phenols and
708 other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in*
709 *Enzymology*, *299*, 152–178.
710 Stadler, M., & Sterner, O. (1998). Production of bioactive secondary metabolites in the fruit
711 bodies of macrofungi as a response to injury. *Phytochemistry, 38,* 1013-1019.
712 Ueno, A., Fukushima, S., Saiki, Y., & Harada, T. (1964). Studies on the components of *Phaeolus*
713 *schweintizii* (Fr.) Pat. *Chemical and Pharmaceutical Bulletin, 12,* 376-378.
714 Vadalà, A., Finzi, P. V., Zanoni, G., & Vidari G. (2003). Columbetdione, a new cyclopentene
715 derivative from the fruiting bodies of *Tricholoma columbetta* (Basidiomycetes) - structure and
716 synthesis. *European Journal of Organic Chemistry, 4,* 642–648.

717 Vamanu, E. (2012). *In vitro* antimicrobial and antioxidant activities of ethanolic extract of
718 lyophilized mycelium of *Pleurotus ostreatus* PQMZ91109. *Molecules, 17*, 3653-3671.
719 Van der Linde, S., Alexander, I., & Anderson, I. C. (2008). A PCR-based method for detecting
720 the mycelia of stipitate hydnoid fungi in soil. *Journal of Microbiological Methods*, 75, 40–46.
721 Yang, J. H., Lin H.C., & Mau, J. L. (2002). Antioxidant properties of several commercial
722 mushrooms. *Food Chemistry*, 77, 229–235.
723 Yim, H. S., Chye, F. Y., Tan, C. T., Ng, Y. C., & Ho, C. W. (2010). Antioxidant activities and
724 total phenolic content of aqueous extract of *Pleurotus ostreatus* (cultivated oyster mushroom).
725 *Malaysian Journal of Nutrition, 16*, 281-291.
726 Yoshikawa, T., Toyokuni, S., Yamamoto, & Y., Naito, Y. (2000). *Free Radicals in Chemistry.*
727 *Biology and Medicine*. London: OICA International, (Chapter 2).
728 Zan, L., Qin, J., Zhang, Y., Yao, Y., Bao, H., & Li, X. (2011). Antioxidant hispidin derivatives
729 from medicinal mushroom *Inonotus hispidus*. *Chemical and Pharmaceutical Bulletin, 59*, 770-
730 772.
731 Zhang, M., Huang, J., Xie, X., & Holman, C. D. J. (2009). Dietary intakes of mushrooms and
732 green tea combine to reduce the risk of breast cancer in Chinese women. *International Journal*
733 *of Cancer, 124*, 1404–1408.
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735 Figure legends
736
737 Figure 1. Total content of phenolic compounds (TPC) in mushrooms
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739 Figure 2. Antioxidant scores of mushroom species (ASM) integrating antioxidant activity values
740 in $ABTS^{\bullet+}$, $DPPH^{\bullet}$, 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
Phaeolus schweinitzii	3.75 ± 0.30	2.07 ± 0.16	13.91 ± 0.20	4.82 ± 0.20	24.55
Inonotus hispidus	0.40 ± 0.15	0.43 ± 0.25	4.55 ± 0.10	11.10 ± 0.15	16.48
Tricholoma columbetta	1.55 ± 0.05	1.57 ± 0.30	18.74 ± 0.30	25.29 ± 0.25	47.15
Tricholoma caligatum	4.00 ± 0.13	2.79 ± 0.10	36.69 ± 0.25	19.67 ± 0.30	63.15
Xerocomus chrysenteron	2.64 ± 0.25	1.90 ± 0.20	14.16 ± 0.12	21.23 ± 0.17	39.93
Hydnellum ferruginemum	1.42 ± 0.12	1.33 ± 0.12	11.67 ± 0.16	2.94 ± 0.23	17.36
Agaricus bisporus	1.18 ± 0.19	0.89 ± 0.24	16.95 ± 0.25	17.52 ± 0.18	36.54
Pleurotus ostreatus	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)

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

Botanical name	Extract	µM TE/g dw				TPC, mg GAE/g dw
		$ABTS^{\bullet+}$	$DPPH^{\cdot}$	FRAP	ORAC	
Inonotus hispidus	**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
Tricholoma caligatum	**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
Tricholoma columbetta	**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
Phaeolus schweinitzii	**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
Xerocomus chrysenteron	**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
Hydnellum ferrugineum	**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
Agaricus bisporus	**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
Pleurotus ostreatus	**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	*Candida albicans*				*Bacillus cereus*			*Pseudomonas aeruginosa*	
		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. hispidus	**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.
T. caligatum	**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
T. columbetta	**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
P. schweinitzii	**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
X. chrysenteron	**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
H. ferrugineum	**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	143±1.1	9.0 ±0.5	7.0 ± 1.0	n.a	n.a	15 ± 1.3	14 ± 1.1	n.a
A. bisporus	**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
P. ostreatus	**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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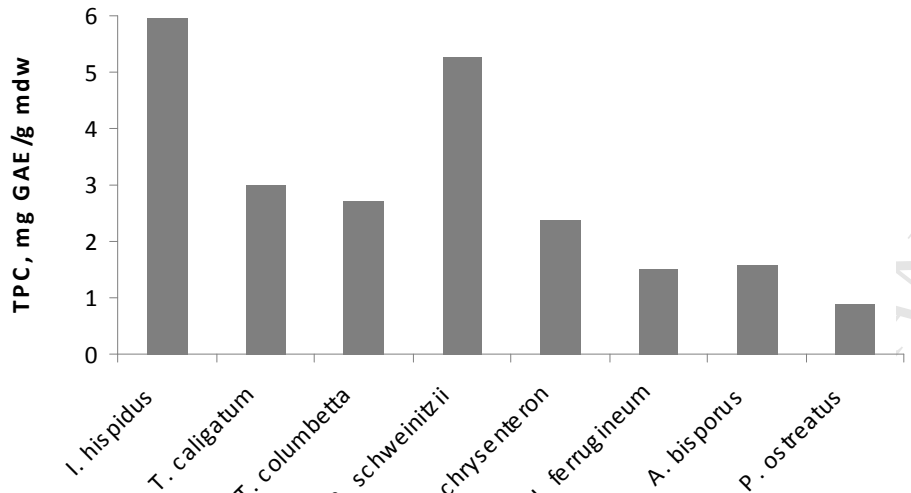

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757 *Smolskaitė et al., Figure 2*

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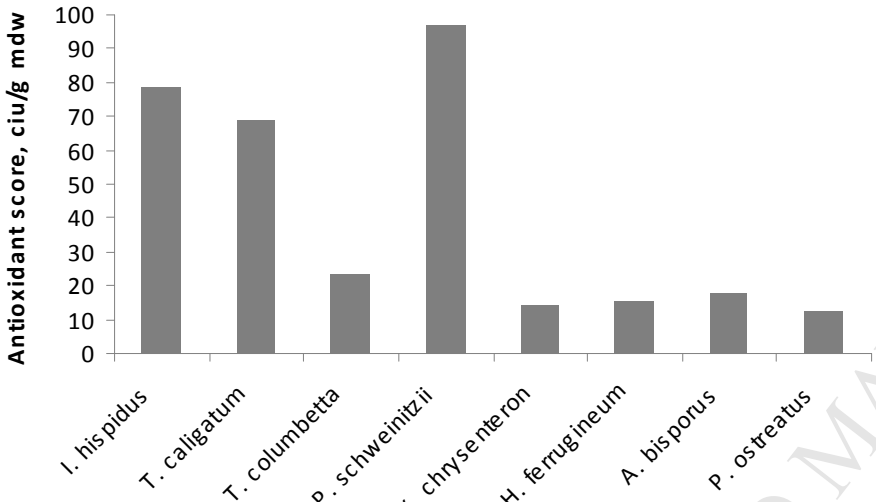

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Highlights

- 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