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Original article

Comparative study of antioxidant and antibacterial properties of the edible mushrooms *Pleurotus levis*, *P. ostreatus*, *P. pulmonarius* and *P. tuber-regium*

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Summary Comparative studies of functional properties among closely related mushroom species, supported by molecular identification, standard cultivation and extraction protocols, are not well documented. We compared antioxidant and antibacterial properties of standardised hydroalcoholic extracts of four *Pleurotus* species (*P. levis*, *P. ostreatus*, *P. pulmonarius* and *P. tuber-regium*). Antioxidant properties were investigated using DPPH and ABTS radical scavenging capacity, total phenolic content, β-carotene-linoleic and ORAC assays. Antibacterial effect was assessed using the microplate method. The functional properties of standardised mushroom extracts were different in species studied. β-carotene–linoleic acid and ORAC assays showed high antioxidant activity, particularly in *P. ostreatus*. *Pleurotus tuber-regium* exhibited the lowest antioxidant activity in the ORAC assay (3316.0 μmol of trolox equivalent mL^{-1}), but exerted the most potent bacteriostatic and bactericidal activity. *Bacillus subtilis* showed a high degree of susceptibility to a very low concentration (3.33 μg mL^{-1}) of *P. levis* extract. Remarkable antioxidant and antibacterial properties were found in *P. levis* and *P. tuber-regium* compared to the other species studied that are cultivated commercially.

Keywords Antibacterial properties, antioxidant properties, comparative analysis, edible mushrooms, *Pleurotus* species.

Introduction

Antioxidants are important for maintaining human health, and there is accordingly an increasing interest to determine the antioxidant capacity of foods and beverages. Edible mushrooms contain bioactive compounds with functional and medicinal properties. They are a natural source of antioxidants and help to prevent oxidative stress when integrated to the diet. Mushrooms are also a unique food because their antioxidant capacity is naturally associated with antimicrobial properties. Diverse bioactive compounds have been identified, including polyphenols, flavonoids, minerals, polysaccharides, vitamins and carotenoids (Kozarski *et al.*, 2015). Antioxidant and antimicrobial properties have been studied in widely cultivated species (Chang & Miles, 2004; Kozarski *et al.*, 2015; Petrović *et al.*, 2015; Shen *et al.*, 2017), including the common cultivated mushroom (*Agaricus bisporus*), oyster mushrooms (*Pleurotus* spp.), shiitake (*Lentinula edodes*), enokitake (*Flammulina velutipes*), reishi (*Ganoderma lucidum*), the monkey-head mushroom (*Hericium erinaceus*), the paddy straw mushroom (*Volvariella volvacea*) and the pioppino mushroom (*Cyclocybe aegerita*). These properties have actively promoted research and development of mushroom

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products as ingredients for the food industry (Lu *et al*., 2016; Wang *et al*., 2016; Bach *et al*., 2017). Although thorough characterisation and biosynthesis of mushroom antioxidants are in progress, they show clear free radical scavenging and reducing effects, the inhibition of lipid peroxidation, as well as metal-chelating activity (Cheung, 2008). Antimicrobial properties of diverse mushroom metabolites against Gram-positive and Gram-negative bacteria, as well as other fungi, have also been studied (Chang & Miles, 2004; Shen *et al*., 2017). However, comparative analysis of functional and medicinal properties of edible mushrooms is problematic, as in many studies, species are not molecularly identified, cultivation and extraction protocols are not standardised, and there is heterogeneity in the chemical composition and purification of mushroom-based products, extracts or compounds (Meneses *et al*., 2016).

Oyster mushrooms belong to the genus *Pleurotus* (Fungi: Basidiomycota). They are cosmopolitan, commercially cultivated worldwide, and show diverse biotechnological applications (Martínez-Carrera, 1998). At least, fifteen species of *Pleurotus* representing intersterile groups have been recognised (Vilgalys *et al*., 1996). However, large-scale cultivation and studies on functional properties have mainly been concentrated on three species, *P. ostreatus*, *P. pulmonarius* and *P. eryngii*. Other interesting species, such as *P. levis* and *P. tuber-regium*, have received less or no consideration. *Pleurotus levis* is an edible mushroom found in North America, whose whitish basidiocarps are centrally stipitate, show decurrent lamellae and a partial veil. This is an important feature, which may reduce the release of enormous amounts of spores into the atmosphere of growing rooms, a major health drawback in the commercial cultivation of oyster mushrooms (Martínez-Carrera, 1998). Although *P. levis* is used as a food in Mexico, their functional properties have not yet been studied. *Pleurotus tuber-regium*, known as the king tuber oyster mushroom, is an edible and medicinal species found in Asia, Australia and Africa, where its sclerotium is regarded as a delicacy. Anticancer, antiviral, anti-inflammatory, immunomodulating, hypoglycaemic and antioxidant properties have been found in *P. tuber-regium* (Roupas *et al*., 2012).

Potent antioxidant and antibacterial properties have been reported for several species, such as *P. cystidiosus*, *P. citrinopileatus*, *P. djamor*, *P. eryngii*, *P. eryngii* var. *ferulae*, *P. ostreatus*, *P. pulmonarius* and *P. tuber-regium* (Cohen *et al*., 2002; Chang & Miles, 2004; Cheung, 2008; Kozarski *et al*., 2015; Shen *et al*., 2017). However, comparative studies between *Pleurotus* species cultivated commercially on a large scale and those showing promising features are poorly documented. In this study, several *Pleurotus* species were selected, including *P. levis*, *P. ostreatus*, *P. pulmonarius* and *P. tuber-regium*. After mushroom harvesting, we compared their antioxidant and antibacterial properties of standardised hydroalcoholic extracts obtained from mature basidiocarps. Analyses were supported by molecular identification of strains studied, standard cultivation and extraction techniques.

Materials and methods

Chemicals and spectral measurements

Butylated hydroxyanisole (BHA), 2,2-diphenyl-1-picrylhydrazyl (DPPH), β-carotene, linoleic acid, 2,2-azino-bis (3-ethylbenzo-thiazoline-6-sulphonic acid) diammonium salt (ABTS), 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH), trolox, potassium persulfate, L-ascorbic acid (AA), α-tocopherol (α-T), butylated hydroxytoluene (BHT), Folin–Ciocalteu's reagent (FCR), gallic acid, tween-80, chloroform, fluorescein and sodium carbonate were obtained from Sigma-Aldrich (St. Louis, MO, USA). Coomassie brilliant blue G-250 dye and bovine serum albumin were from Bio-Rad (Hercules, CA, USA). Bioactivity measurements were carried out on an Epoch 96-well microplate reader or a Synergy HT plate reader (Bio-Tek Instruments, Winooski, VT, USA). Measurements and calculations of the bioactivity results were assessed using the software Gen5 and Microsoft Excel.

Strains, mushroom cultivation and standard extracts

The origin and code of *Pleurotus* species studied are shown in Table 1. Four species and six strains were included, as follows: (i) *P. levis* (Berk. & M. A. Curtis) Singer (CP-30); (ii) *P. ostreatus* (Jacq.) P. Kumm. (CP-50, CP-800); (iii) *P. pulmonarius* (Fr.) Quél. (CP-16, CP-799); and (iv) *P. tuber-regium* (Fr.) Singer

Table 1 *Pleurotus* species studied originating from differing geographical regions and their accession numbers at the GenBank (www.ncbi.nlm.nih.gov/genbank/)

Species	Code	Country of origin	Region	Accession number
P. levis (Berk. & M.A. Curtis) Singer	CP-30	Mexico	North America	GU722279
P. ostreatus (Jacq.) P. Kumm.	CP-50	USA and Ukraine	North America x Eastern Europe	LT627806
	CP-800	Nigeria	West Africa	JF736659
P. pulmonarius (Fr.) Quél.	CP-16	Hong Kong	South-East Asia	LT627807
	CP-799	Nigeria	West Africa	JF736658
P. tuber-regium (Fr.) Singer	CP-182	Nigeria	West Africa	AF109983

(CP-182). The identity of all strains was confirmed by sequencing their ITS1-5.8S-ITS2 rDNA region (Challen *et al*., 1995; Huerta *et al*., 2010); derived sequences were submitted to GenBank, and the pertinent accession numbers appear in Table 1. All strains are deposited at the Centre for Genetic Resources of Edible, Functional and Medicinal Mushrooms (CREGEN-HCFM), CP, *Campus* Puebla, Mexico (Martínez-Carrera *et al*., 2010). The strain CP-30 of *Pleurotus levis* was isolated by tissue culture from a wild basidiocarp growing on dead tree in the city of Puebla (2172 m altitude), Mexico. The strain CP-50 of *P. ostreatus* is a commercial hybrid (Morales *et al*., 1995) derived from a cross between strains CP-25 (USA) and CP-41 (Ukraine). The strain CP-800 of *P. ostreatus* was isolated from a wild basidiocarp growing on dead palm oil tree inside a Nigerian cocoa plantation and was obtained from the Pure and Applied Biology Department, Ladoke Akintola University of Technology (LAUTECH), Ogbomoso (347 m altitude), Nigeria (Adebayo *et al*., 2014). The strain CP-16 of *P. pulmonarius* was kindly provided by Prof. S. T. Chang from the Chinese University of Hong Kong. The strain CP-799 of *P. pulmonarius* was isolated from a wild basidiocarp growing on dead tree inside a Nigerian cocoa plantation and was also obtained from the LAUTECH (Adebayo *et al*., 2012). The strain CP-182 of *P. tuber-regium* was kindly provided by Dr. O. S. Isikhuemhen from the North Carolina A&T State University, USA, and was isolated from the Edo State, Nigeria (Isikhuemhen *et al*., 2000). Strains were maintained and subcultured on potato-dextrose-agar (PDA, BD Bioxon, Cuautitlán Izcalli, Mexico) medium, routinely autoclaved at 121 °C for 15 min and incubated at 27–28 °C. Wheat grain spawn was prepared according to standard methods.

Pleurotus species were cultivated on sterilised barley straw as previously described (Martínez-Carrera & Ramírez Juárez, 2016). Six replicate bags were done for each strain. Mature basidiocarps from each bag were harvested, cut into slices (*ca*. 0.5–1 cm), dried at 40 °C in a forced air drying oven (SMO28-2; Shel Lab, Cornelius, OR, USA) for 5 days, and stored at −80 °C inside plastic bags until use. The biological efficiency (BE, %) was determined according to the following formula: BE = (total mushroom fresh weight/total substrate dry weight) × 100.

Standardised mushroom extracts were obtained for analyses. Dried mushroom slices were chopped in a blender, and 10 g of the product was put into a filter paper (8 μm) bag for maceration (24 h). Hydroalcoholic extracts (32% by volume) were obtained according to previous patent (Martínez-Carrera *et al*., 2014). Mushroom extracts were concentrated to 10 mL in a rotary evaporator (HS-2000NS; Hahn Shin Scientific, Bucheon, South Korea) at 19 °C. They were then filter-sterilised (0.45 μm; Merck Millipore, Molsheim, France), freeze-dried overnight using a vacuum apparatus (Freezone 4.5; Labconco, Kansas City, MO, USA), and stored at 4 °C until use. The following parameters were determined in mushroom extracts: pH, conductivity and resistance (Ω) using a seven excellence multiparameter (Mettler Toledo, Columbus, OH, USA), dry weight (mg mL^{-1}) and comparative direct visualisation in a spectrophotometer (Epoch, Biotek, Winooski, VT, USA; wavelength range: 200–1000 nm) using microplates. The concentration of mushroom extracts was determined recording the dry weight (mg mL^{-1}) of 1.0 mL of each extract, processed at 40 °C for 48 h.

Determination of protein content

The concentration of protein present as equivalent of bovine serum albumin (BSA, Bio-Rad) protein standard in each sample was determined using the method of Bradford (1976). Samples (80 μL) were prepared in concentrations ranging from 100 to 800 μg mL^{-1}. The absorbance was measured at 595 nm using a 96-well microplate reader, and data were analysed.

Determination of total phenolic content

The concentration of phenolic compounds in mushroom extracts, expressed as gallic acid equivalent (GAE), was measured according to a modified method of Cheung *et al*. (2003). A sample (20 μL) of the mushroom extract at a concentration of 20.0 μg mL^{-1} was mixed with 100 μL of Folin and Ciocalteu's phenol reagent. After 3 min, 75 μL of saturated solution of sodium carbonate was added to the mixture. The reaction was incubated in the dark for 2 h. Sample was added immediately as blank after incubation, and the absorbance was measured at 740 nm using a 96-well microplate reader. A calibration curve was constructed with different concentrations of gallic acid (0.0–200.0 μg mL^{-1}) and ascorbic acid (0–200.0 μg mL^{-1}) as standard.

DPPH free radical scavenging activity

This bioassay was determined spectrophotometrically following a modified DPPH assay (Öztürk *et al*., 2011). Different concentrations (0.0–20.0 μg mL^{-1}) of standardised mushroom extracts were dissolved in the solvent (alcohol from tequila, 32% by volume) and properly mixed by vortexing. The reaction was then initiated by the addition of 40 μL of DPPH (0.4 mM) prepared in methanol to the samples (160 μL). After 30 min of incubation at 30 °C in the dark, the absorbance was measured at 517 nm using a 96-well microplate reader. The solvent was used as control. As $DPPH^{\cdot}$ radical absorbs at 517 nm, but due to a

reduction by an antioxidant or a radical species, its absorption decreases. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The capability for scavenging the DPPH$^{\cdot}$ radical was calculated by the following equation:

$$\text{DPPH}^{\cdot}\text{radical scavenging effect}(\%) = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

where A_{Control} is the initial concentration of the DPPH$^{\cdot}$ and A_{Sample} is the absorbance of the remaining concentration of DPPH$^{\cdot}$ in the presence of the extract and positive control (solvent). The extract concentration providing 50% radical scavenging activity (EC_{50}) was calculated using the method of Alexander *et al.* (1999). Different concentrations of the following antioxidant standards were used for comparison of the activity: BHA (0.0–25.0 μg mL^{-1}), BHT (0.0–25.0 μg mL^{-1}), α-tocopherol (0.0–25.0 μg mL^{-1}) and ascorbic acid (0.0–40.0 μg mL^{-1}).

Inhibition of β-carotene bleaching

The inhibition of β-carotene bleaching was determined spectrophotometrically following a modified β-carotene–linoleic assay (Prieto *et al.*, 2012). β-carotene (4 mg), linoleic acid (0.5 mL) and tween-80 (4 g) were mixed in 20 mL of chloroform in a round-bottomed flask, with vigorous shaking of the suspension. The chloroform was evaporated in a rotary evaporator at 40 °C in a short period of time (*ca.* 15 min). The oily residue was distributed in aliquots of 1 mL in 30 mL tubes, which were maintained in the dark at −28 °C. A single tube provides sufficient reagent to fill a microplate (96 wells), which is obtained adding 30 mL of 100 mM Briton buffer, pH 6.5 in preheated mili-Q water at the assay temperature (45 °C). The reaction was performed by combining 35 μL (0.0–5.0 mg mL^{-1}) of sample at different concentrations and 165 μL of reagent, measuring the initial absorbance. The microplate was incubated at 45 °C in the dark, and the absorbance was measured in different periods (10, 20, 30, 60, 90 and 120 min) at 517 nm using a 96-well microplate reader. β-carotene bleaching inhibition was calculated using the following equation: (β-carotene content after 2 h of assay/initial β-carotene content) × 100. The mushroom extract concentration providing 50% antioxidant activity (EC_{50}) was calculated by interpolation from the graph of β-carotene bleaching inhibition percentage against extract concentration (Vaz *et al.*, 2011). Different concentrations of following antioxidant standards were used for comparison of the activity: BHA (0.0–5.0 μg mL^{-1}), BHT (0.0–20.0 μg mL^{-1}), α-tocopherol (0.0–40.0 μg mL^{-1}) and ascorbic acid (0.0–200.0 μg mL^{-1}).

ABTS radical cation decolourisation assay

The ABTS scavenging activity was determined spectrophotometrically according to a modified method of Öztürk *et al.* (2011). The ABTS$^{\bullet+}$ radical cation was produced by the reaction between 7 mM ABTS in water and 2.45 mM potassium persulfate, stored in the dark at room temperature for 13 h. Before use, the ABTS$^{\bullet+}$ solution was adjusted to the absorbance of 0.710 ± 0.020 at 734 nm using 99% ethanol at a ratio of 65.67:1 (ethanol: reagent). Oxidation of ABTS$^{\bullet+}$ was carried out adding 160 μL of ABTS$^{\bullet+}$ solution to 40 μL (0.0–5.0 μg mL^{-1}) of sample solution in ethanol at different concentrations. After 10 min, the absorbance was measured at 734 nm using a 96-well microplate reader. The percentage of inhibition was calculated for each concentration relative to a control (ethanol). The scavenging capability of ABTS was calculated using the following equation:

$$\text{ABTS}^{\bullet+}\text{radical scavenging effect}(\%) = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

where A_{Control} is the absorbance of ABTS$^{\bullet+}$ and ethanol, while A_{Sample} is the absorbance of the ABTS$^{\bullet+}$ and tested samples. The mushroom extract concentration providing 50% radical scavenging activity (EC_{50}) was calculated from the graph of ABTS$^{\bullet+}$ scavenging effect percentage against extract concentration. BHT (0.01–5 μg mL^{-1}), BHA (0.0–25 μg mL^{-1}), α-tocopherol (0.0–100.0 μg mL^{-1}) and ascorbic acid (0.0–50 μg mL^{-1}) were used as antioxidant standards for comparison of the activity.

Oxygen radical absorbance capacity (ORAC) assay

Antioxidant activity was measured by the ORAC assay (Huang *et al.*, 2002). Dried basidiocarps of strains studied were ground to a fine powder. The analysis of basidiocarps [μmol of trolox equivalent (TE) g^{-1}] included the addition of 20 mL acetone per water (50:50, v/v) extraction solvent to 1.0 g of the powder. The extract was centrifuged at 21 036 ***g*** for 15 min, and the supernatant was used for analysis after appropriate dilution with buffer solution. In the case of mushroom extracts studied, they were directly diluted with the buffer solution and analysed (μmol of TE mL^{-1}). The reagent and standard preparation were made as follows: 0.414 g of AAPH was dissolved in 10 mL of 75 mM phosphate buffer (pH 7.4) to a final concentration of 153 mM kept in ice bath. A stock solution (0.02 M) of trolox standard was prepared in 50 mL of 75 mM phosphate buffer (pH 7.4). The stock solution was then diluted with the same phosphate buffer up to 50, 25, 12.5 and 6.25 μM working

solutions. Fluorescein (4 nM, 150 μL) was added to each reaction mixture. The fluorescence assay was measured in a BioTek Synergy HT plate reader (Bio-Tek Instruments).

Antibacterial assay

The following species of bacteria were used for the assay: *Bacillus subtilis* (Ehrenberg) Cohn (ATCC-6633, CPB-9, Gram positive); *Escherichia coli* (Migula) Castellani and Chalmers (ATCC-25922, CPB-8, Gram negative); *Listeria monocytogenes* (Murray *et al.*) Pirie (ATCC-19111, CPB-11, Gram positive); *Pseudomonas aeruginosa* (Schroeter) Migula (ATCC-27853, CPB-13, Gram negative); *Salmonella typhi* (Schroeter) Warren and Scott [CPB-1, Gram negative, clinical isolate from the culture collection of the Autonomous University of Puebla (BUAP), Mexico]; *Staphylococcus aureus* Rosenbach (ATCC-25923, CPB-10, Gram positive); *Stenotrophomonas* spp. (CPB-7, Gram negative, clinical isolate from the BUAP, Mexico); and *Streptococcus agalactiae* Lehmann and Neumann (CPB-4, Gram positive, clinical isolate from the BUAP, Mexico). The antimicrobial activity of mushroom extracts was assayed by the standard broth microdilution method (CLSI, 2006; Wiegand *et al.*, 2008) to determine the minimal inhibitory concentration (MIC), as well as the minimum bactericidal concentration (MBC). All experiments were performed in triplicate.

Statistical analysis

Antioxidant, antibacterial and protein concentration analyses were carried out in triplicate, whereas for ORAC analysis in quintuplicate. Results were expressed as means ± standard deviation (SD). Statistical analysis was performed using the Tukey's test, and $P < 0.05$ was considered to be significant. Correlations among data obtained were processed using statistical software (2012 SAS Institute Inc., Cary, NC, USA).

Results

Yields varied in most species from 141.8 to 236.6 g of fresh basidiocarps (*P. levis*, *P. ostreatus* and *P. pulmonarius*), reaching biological efficiencies (BEs) of 37.7–62.0%. *Pleurotus tuber-regium* showed the lowest mushroom yield (38.8 g) and BE (10.4%). The analysis of basidiocarps by ORAC assay indicated that the greatest antioxidant activity is found in the strain CP-16 of *P. pulmonarius* [24661.6 μmol of trolox equivalent (TE) g^{-1}], as shown in Table 2. This was followed by *P. ostreatus* (CP-50: 14105.6 μmol of TE g^{-1}, CP-800: 13455.2 μmol of TE g^{-1}), the other strain of *P. pulmonarius* (CP-799: 9555.2 μmol of TE g^{-1}) and *P. levis* (CP-30: 3900.8 μmol of TE g^{-1}).

Table 2 Mushroom yield of fresh basidiocarps cultivated on sterilised barley straw, antioxidant activity and the characterisation of standardised mushroom extracts from *Pleurotus* species studied

Species	Strain	Dry substrate weight (g)	Mushroom yield (g)	BE (%)	Antioxidant activity of basidiocarps (ORAC assay, μmol of trolox equivalent g^{-1})	Mushroom extract volume (mL)	Concentration of the hydroalcoholic extract (mg mL^{-1})	Conductivity (μS cm^{-1}; 20.6 °C)	Resistance (Ω)	pH
P. levis	CP-30	364.0 ± 8.8 a	196.4 ± 54.8 ab	54.2 ab	3900.8 ± 653.8 c	50	213.12 ± 5.1 d	6015.94 ± 525.5 c	108.80 ± 11.3 a	7.01 a
P. ostreatus	CP-50	377.0 ± 8.2 a	141.8 ± 23.1 b	37.7 b	14105.6 ± 2422.3 b	50	250.64 ± 3.8 c	6057.28 ± 281.5 c	108.20 ± 5.5 a	6.90 a
	CP-800	382.0 ± 11.5 a	236.6 ± 12.6 a	62.0 a	13455.2 ± 1930.4 b	50	291.04 ± 4.6 a	12101.68 ± 1485.4 a	54.00 ± 7.3 d	6.13 c
P. pulmonarius	CP-16	360.0 ± 23.3 a	194.0 ± 13.1 ab	54.1 ab	24661.6 ± 4461.4 a	50	273.28 ± 3.8 b	7280.08 ± 1123.5 c	91.40 ± 15.1 ab	6.78 ab
	CP-799	367.0 ± 8.5 a	196.6 ± 49.0 ab	53.6 ab	9555.2 ± 1953.1 b	50	252.28 ± 3.3 c	9969.72 ± 2075.8 ab	67.00 ± 13.6 cd	6.54 b
P. tuber-regium	CP-182	379.0 ± 6.0 a	38.8 ± 12.0 c	10.4 c	ND	30	192.92 ± 3.8 e	8007.10 ± 1520.5 bc	82.00 ± 14.1 bc	6.99 a

BE, biological efficiency; ND, not determined.
Means in a column followed by differing letters indicate significant difference, $P < 0.05$, according to the Tukey's test.

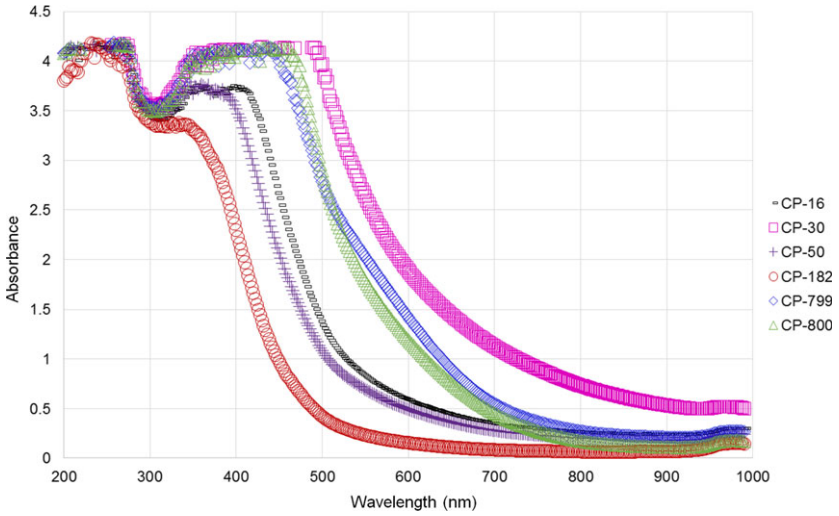

Figure 1 Comparative direct visualisation of standardised extracts from *Pleurotus* species studied in a spectrophotometer (Epoch, Biotek, USA; wavelength range: 200–1000 nm) using microplates ($n = 3$). Standardised extracts were obtained from basidiocarps cultivated on sterilised barley straw. CP-16: *P. pulmonarius*. CP-30: *P. levis*. CP-50: *P. ostreatus*. CP-182: *P. tuber-regium*. CP-799: *P. pulmonarius*. CP-800: *P. ostreatus*. [Colour figure can be viewed at wileyonlinelibrary.com]

Mushroom extracts varied in yield, conductivity, resistance and pH according to species and strains. The amount of hydroalcoholic extract (dry weight) obtained from each *Pleurotus* species is shown in Table 2. *Pleurotus levis* yielded 213.12 mg mL^{-1}, *P. ostreatus* ranged from 250.64 to 291.04 mg mL^{-1}, *P. pulmonarius* varied between 252.28 and 273.28 mg mL^{-1} and *P. tuber-regium* produced 192.92 mg mL^{-1}. The extract from *P. ostreatus* (CP-800) showed the greatest conductivity (12101.68 μS cm^{-1}) and the lowest pH 6.13. Conversely, the lowest conductivity (6015.94 μS cm^{-1}) was recorded in *P. levis*, which showed the highest pH 7.01. The composition of standardised mushroom extracts was shown to be different by comparative direct visualisation in a spectrophotometer, although absorbance patterns of the strain CP-800 of *P. ostreatus* and the strain CP-799 of *P. pulmonarius*, both from West Africa, were similar (Fig. 1).

The protein concentration of standardised mushroom extracts is shown in Table 3. Protein concentration varied from 281.16 μg mL^{-1} to 696.56 μg mL^{-1}.

Table 3 Protein concentration in standardised hydroalcoholic extracts from *Pleurotus* species studied as equivalent of bovine serum albumin

Species	Strain	Protein (μg mL^{-1})
P. levis	CP-30	689.20 ± 35.89 a
P. ostreatus	CP-50	386.93 ± 38.06 b
	CP-800	696.56 ± 52.80 a
P. pulmonarius	CP-16	605.29 ± 38.06 a
	CP-799	622.77 ± 68.31 a
P. tuber-regium	CP-182	281.16 ± 23.75 b

Means in a column followed by differing letters indicate significant difference, $P < 0.05$, according to the Tukey's test.

The greatest concentration of protein was recorded in the strain CP-800 of *P. ostreatus* (696.56 μg mL^{-1}), followed by *P. levis* (CP-30: 689.20 μg mL^{-1}), *P. pulmonarius* (CP-799: 622.77 μg mL^{-1}; CP-16: 605.29 μg mL^{-1}), *P. ostreatus* (CP-50: 386.93 μg mL^{-1}) and *P. tuber-regium* (CP-182: 281.16 μg mL^{-1}).

Total phenolic content

Phenolic compounds in mushroom extracts varied from 50.32 GAE μg mL^{-1} to 129.06 GAE μg mL^{-1} at a concentration of the extracts of 2.5 μg mL^{-1} (Table 4). The extract from strain CP-800 of *P. ostreatus* showed the highest phenolic content as gallic acid equivalents (129.06 GAE μg mL^{-1}), but the strain CP-50 of this species had only 65.76 GAE μg mL^{-1}. This was followed by strains CP-799 (98.36 GAE μg mL^{-1}) and CP-16 (80.19 GAE μg mL^{-1}) of *P. pulmonarius*, *P. levis* (CP-30: 56.80 GAE μg mL^{-1}) and *P. tuber-regium* (CP-182: 50.32 GAE μg mL^{-1}).

Radical scavenging activity and bleaching inhibition assays

Data from radical scavenging activity, radical decolourisation and bleaching inhibition of mushroom extracts are shown in Fig. 2. They were determined using DPPH, ABTS and β-carotene–linoleic assays, respectively. Extracts from mushroom species and strains showed different activities, depending on the technique used. *Pleurotus levis* (CP-30) showed the greatest DPPH free radical scavenging activity (80.9%), using the lowest amount of extract (1.27 μg mL^{-1}). However, ABTS radical cation decolourisation and β-carotene bleaching activities were lower in *P. levis* than the rest of strains (Fig. 2a–c). Similar was the case for *P. ostreatus* (CP-50,

Table 4 Antioxidant activity considering the effective concentration (EC_{50}) of standardised extracts from different *Pleurotus* species obtained from DPPH, ABTS and β-carotene–linoleic acid assays. The total phenolic content, as well as the antioxidant activity assessed by the ORAC assay, performed independently is also included

Species	Strain	DPPH assay (μg mL^{-1})	ABTS assay (μg mL^{-1})	β-carotene–linoleic acid assay (μg mL^{-1})	Total phenolic content (GAE μg mL^{-1})	ORAC assay (μmol of trolox equivalent mL^{-1})
P. levis	CP-30	0.51 ± 0.0 c	0.50 ± 0.04 ab	0.59 ± 0.17 f	56.80 ± 0.00 e	5080.8 ± 452.3 bc
P. ostreatus	CP-50	1.05 ± 0.2 b	0.42 ± 0.01 bc	0.34 ± 0.31 f	65.76 ± 0.00 d	8199.2 ± 1468.9 b
	CP-800	0.63 ± 0.02 c	0.24 ± 0.02 d	0.17 ± 0.05 f	129.06 ± 0.68 a	34646.0 ± 4124.4 a
P. pulmonarius	CP-16	0.70 ± 0.08 c	0.35 ± 0.06 c	>5.0 ± 0.00 b	80.19 ± 0.54 c	6496.8 ± 2647.1 bc
	CP-799	0.49 ± 0.01 cd	0.37 ± 0.0 c	0.22 ± 0.05 f	98.36 ± 0.31 b	4455.2 ± 1942.1 bc
P. tuber-regium	CP-182	1.68 ± 0.22 a	0.50 ± 0.01 a	0.16 ± 0.04 f	50.32 ± 0.32 f	3316.0 ± 774.8 c
Antioxidant standards						
Ascorbic acid		0.06 ± 0.002 e	0.16 ± 0.02 de	>35.22 ± 0.00 a	–	–
BHA		0.56 ± 0.05 c	0.37 ± 0.04 c	4.30 ± 0.14 c	–	–
α-Tocopherol		0.01 ± 0.006 e	0.12 ± 0.01 e	1.35 ± 0.17 e	–	–
BHT		0.19 ± 0.04 de	0.00027 ± 0.0 f	2.16 ± 0.32 d	–	–

Means in a column followed by differing letters indicate significant difference, $P < 0.05$, according to the Tukey's test.

CP-800), *P. pulmonarius* (CP-16, CP-799) and *P. tuber-regium* (CP-182).

EC_{50} values in antioxidant properties

Experimental data were normalised and expressed as EC_{50} values (μg mL^{-1}) for each mushroom extract. The effective concentration, which is required to show 50% antioxidant activity of standardised extracts from *Pleurotus* species, was determined by DPPH and ABTS assays for radical scavenging activity and the β-carotene-linoleic acid assay for bleaching inhibition (Table 4), as compared with antioxidant standards (BHA, BHT, α-tocopherol and ascorbic acid). The EC_{50} varied among the species and strains, showing better antioxidant activity at lower concentrations of EC_{50} in comparison with the standards. In general, *P. ostreatus* and *P. pulmonarius* showed better antioxidant activity considering the effective concentration obtained from DPPH, ABTS and β-carotene–linoleic acid assays. Lower values of effective concentration in DPPH assay were recorded in *P. pulmonarius* (CP-799: 0.49 μg mL^{-1}), followed by *P. levis* (CP-30: 0.51 μg mL^{-1}) and *P. ostreatus* (CP-800: 0.63 μg mL^{-1}). Greater values of effective concentration were found in *P. pulmonarius* (CP-16: 0.70 μg mL^{-1}), *P. ostreatus* (CP-50: 1.05 μg mL^{-1}) and *P. tuber-regium* (CP-182: 1.68 μg mL^{-1}). Effective concentration of the standards were 0.01 μg mL^{-1} for α-tocopherol, followed by 0.06 μg mL^{-1} for ascorbic acid, 0.19 μg mL^{-1} for BHT and 0.56 μg mL^{-1} for BHA. Only *P. pulmonarius* (CP-799) and *P. levis* (CP-30) showed antioxidant activity equivalent to the BHA standard. The other mushroom species and strains had lower antioxidant activity than the standards.

In the case of ABTS assay, the lowest EC_{50} value was obtained in *P. ostreatus* (CP-800: 0.24 μg mL^{-1}), although the other strain of this species had a higher value (CP-50: 0.42 μg mL^{-1}). This was followed by *P. pulmonarius* (CP-16: 0.35 μg mL^{-1}, CP-799: 0.37 μg mL^{-1}), *P. levis* (CP-30: 0.50 μg mL^{-1}) and *P. tuber-regium* (CP-182: 0.50 μg mL^{-1}) (Table 4). Antioxidant standards had the following EC_{50} values: 0.00027 μg mL^{-1}, 0.12 μg mL^{-1}, 0.16 μg mL^{-1} and 0.37 μg mL^{-1} for BHT, α-tocopherol, ascorbic acid and BHA, respectively. Antioxidant activity equivalent to ascorbic acid and BHA standards was only recorded in *P. ostreatus* (CP-800) and *P. pulmonarius* (CP-16, CP-799). The other mushroom species and strains had lower antioxidant activity than the standards.

In β-carotene–linoleic acid assay, low EC_{50} values were obtained from standardised extracts of *P. ostreatus* (CP-50, CP-800: 0.17–0.34 μg mL^{-1}) and *P. tuber-regium* (0.16 μg mL^{-1}). Low and higher values were recorded in *P. pulmonarius* (0.22- >5.0 μg mL^{-1}) and *P. levis* (0.59 μg mL^{-1}). The EC_{50} values of the standards varied as follows: 1.35 μg mL^{-1}, 2.16 μg mL^{-1}, 4.30 μg mL^{-1} and >35.22 μg mL^{-1}, for α-tocopherol, BHT, BHA and ascorbic acid, respectively. The extracts of all mushroom species and strains showed better antioxidant activity than standards studied (Table 4).

The ORAC assay provides an accurate measurement of antioxidant activity as inhibition time and degree of inhibition are combined into a single quantity (Huang *et al.*, 2002). The greatest antioxidant activity was recorded in the strain CP-800 of *P. ostreatus* reaching 34 646 μmol of TE mL^{-1}, although the strain CP-50 of this species only showed 8199.2 μmol of TE mL^{-1} (Table 4). This was followed by *P. pulmonarius*

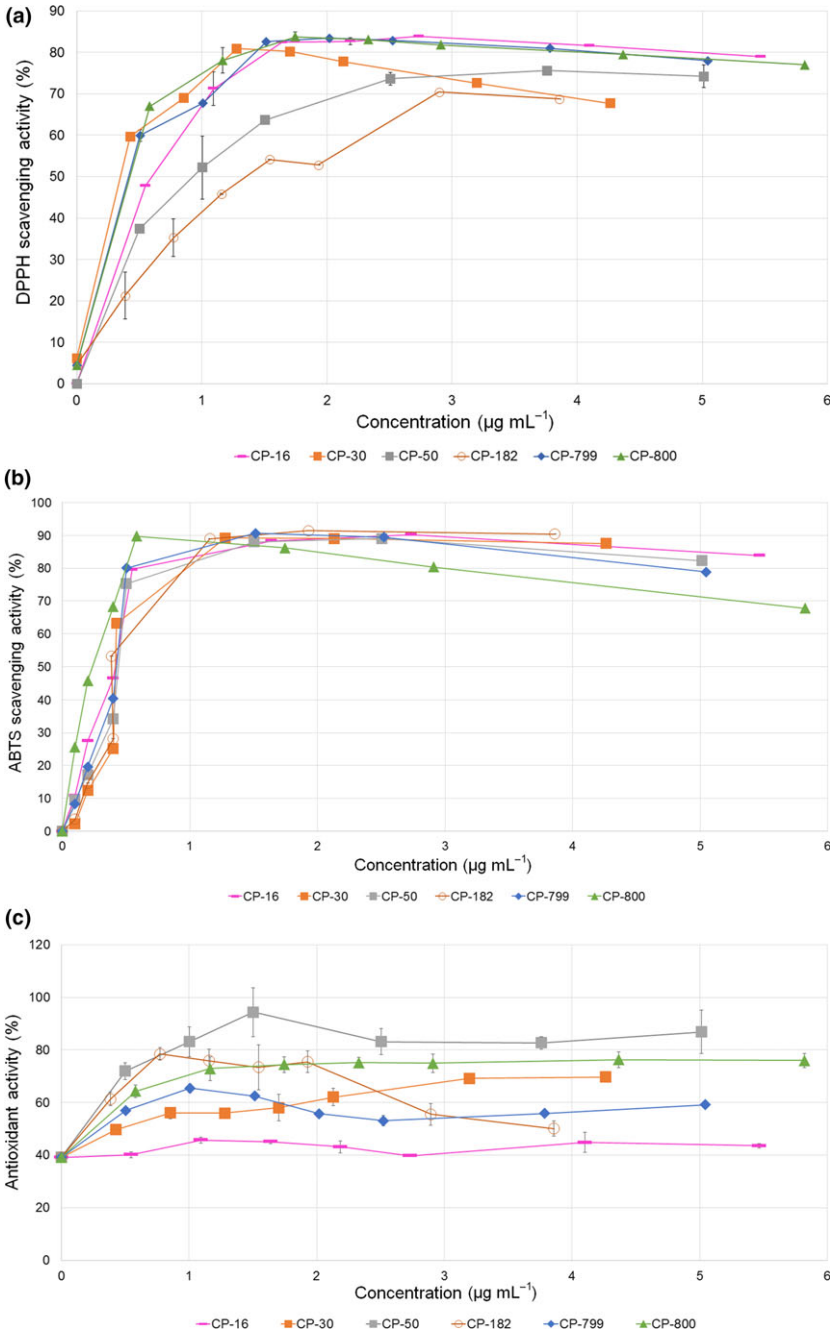

Figure 2 Free radical scavenging activity, radical decolourisation and inhibition of β-carotene bleaching at different concentrations of standardised extracts from *Pleurotus* species. (a) DPPH free radical scavenging activity. (b) ABTS free radical scavenging activity. (c) antioxidant activity by the β-carotene–linoleic assay. CP-16: *P. pulmonarius*. CP-30: *P. levis*. CP-50: *P. ostreatus*. CP-182: *P. tuber-regium*. CP-799: *P. pulmonarius*. CP-800: *P. ostreatus*. [Colour figure can be viewed at wileyonlinelibrary.com]

(CP-16: 6496.8 μmol of TE mL^{-1}; CP-799: 4455.2 μmol of TE mL^{-1}), *P. levis* (CP-30: 5080.8 μmol of TE mL^{-1}) and *P. tuber-regium* (CP-182: 3316.0 μmol of TE mL^{-1}).

Minimal inhibitory concentration (MIC) assay

Bacterial susceptibility to mushroom extracts of eight clinically relevant species is shown in Table 5. All bacterial species tested were susceptible to at least three mushroom extracts, showing a MIC ranging from 3.33 to 145.52 μg mL^{-1}. *Escherichia coli* and *Pseudomonas aeruginosa* were resistant to the evaluated concentration of extracts from *P. levis* (CP-30), *P. ostreatus* (CP-50) and *P. pulmonarius* (CP-16), while *Salmonella typhi* was only not susceptible to *P. levis* (CP-30). The MIC of extracts assessed was grouped into three categories for analysis: 1–10 μg mL^{-1} (low), 11–100 μg mL^{-1}

(regular) and >101 μg mL^{-1} (high). Several bacterial species were highly susceptible to a MIC ranging from 1 to 10 μg mL^{-1} of mushroom extracts. This was the case of *Bacillus subtilis* against *P. levis* (CP-30: 3.33 μg mL^{-1}), *Streptococcus agalactiae* against *P. tuber-regium* (CP-182: 6.03 μg mL^{-1}) and *P. ostreatus* (CP-50: 7.83 μg mL^{-1}). Most bacteria were susceptible to a MIC varying from 11 to 100 μg mL^{-1} of mushroom extracts. The inhibitory effect varied according to mushroom species and strain. *Pleurotus levis* inhibited *S. agalactiae* (CP-30: 13.32 μg mL^{-1}) and *Staphylococcus aureus* (CP-30: 26.64 μg mL^{-1}). *Pleurotus ostreatus* had inhibitory effect on *S. agalactiae* (CP-800: 72.76 μg mL^{-1}), *Stenotrophomonas* spp. (CP-50: 62.66 μg mL^{-1}, CP-800: 36.38 μg mL^{-1}), *E. coli*, *B. subtilis*, *S. aureus*, *Listeria monocytogenes* and *P. aeruginosa* (CP-800: 72.76 μg mL^{-1}). *Pleurotus pulmonarius* exerted inhibition on *S. agalactiae* (CP-799: 63.07 μg mL^{-1}), *Stenotrophomas* spp. (CP-16: 68.32 μg mL^{-1}, CP-799: 63.07 μg mL^{-1}), *B. subtilis* (CP-16: 68.32 μg mL^{-1}), *S. aureus* (CP-16: 68.32 μg mL^{-1}, CP-799: 63.07 μg mL^{-1}) and *L. monocytogenes* (CP-16: 68.32 μg mL^{-1}). The extract from strain CP-182 of *P. tuber-regium* inhibited *S. typhi* (24.12 μg mL^{-1}), *Stenotrophomonas* spp. (24.12 μg mL^{-1}), *E. coli* (48.23 μg mL^{-1}), *B. subtilis* (48.23 μg mL^{-1}), *S. aureus* (12.06 μg mL^{-1}), *L. monocytogenes* (24.12 μg mL^{-1}) and *P. aeruginosa* (12.06 μg mL^{-1}). Other bacteria were weakly or no susceptible to a MIC greater than 101 μg mL^{-1}. This was so for *S. typhi*, *Stenotrophomonas* spp., *E. coli*, *L. monocytogenes* and *P. aeruginosa* against *P. levis* (CP-30: ≥106.56 μg mL^{-1}). *Salmonella typhi* against *P. ostreatus* (CP-50: 125.32 μg mL^{-1}, CP-800: 145.52 μg mL^{-1}). *Escherichia coli*, *B. subtilis*, *S. aureus*, *L. monocytogenes* and *P. aeruginosa* against *P. ostreatus* (CP-50: ≥125.32 μg mL^{-1}). *Salmonella typhi* against *P. pulmonarius* (CP-16: 136.64 μg mL^{-1}, CP-799: 126.14 μg mL^{-1}). *Streptococcus agalactiae* against *P. pulmonarius* (CP-16: 136.64 μg mL^{-1}). *Escherichia coli*, *B. subtilis*, *L. monocytogenes* and *P. aeruginosa* against *P. pulmonarius* (CP-16: ≥136.64 μg mL^{-1}, CP-799: 126.14 μg mL^{-1}). The most effective MIC recorded for each mushroom species and strain was as follows: *P. levis* (CP-30: 3.33 μg mL^{-1}); *P. ostreatus* (CP-50: 7.83 μg mL^{-1}, CP-800: 36.38 μg mL^{-1}); *P. pulmonarius* (CP-16: 68.32 μg mL^{-1}, CP-799: 63.07 μg mL^{-1}); and *P. tuber-regium* (CP-182: 6.03 μg mL^{-1}).

Minimal bactericidal concentration

Minimum bactericidal concentration of mushroom extracts against clinical bacteria studied is shown in Table 6. There were only two *Pleurotus* species whose standardised extracts showed bactericidal activity: *P. ostreatus* (CP-800) and *P. tuber-regium* (CP-182). The extract from *P. ostreatus* (CP-800) exerted bactericidal effect at a MBC of 36.38 μg mL^{-1} on *Stenotrophomonas* spp., while at a MBC of 145.52 μg mL^{-1} on *Streptococcus agalactiae*, *Escherichia coli*, *Bacillus subtilis* and *Pseudomonas aeruginosa*. *Pleurotus ostreatus* (CP-800) did not have bactericidal effect on *Salmonella typhi*, *Staphylococcus aureus* and *Listeria monocytogenes*. The most effective MBC of the extract was 24.12 μg mL^{-1} recorded in *P. tuber-regium* (CP-182), whereas that from *P. ostreatus* (CP-800) was 36.38 μg mL^{-1}. The extract from *P. tuber-regium* had bactericidal effect on all bacteria studied, at a MBC of 24.12 μg mL^{-1} on *S. typhi* and *P. aeruginosa*; at a MBC of 48.23 μg mL^{-1} on *S. agalactiae*, *Stenotrophomonas* spp., *B. subtilis*, *S. aureus* and *L. monocytogenes*; and at 96.46 μg mL^{-1} on *E. coli*.

Correlation between experimental data

A regression analysis was performed to show the correlation coefficient (*r*) between assays studied. Significant correlations were found between the pH of

Table 5 Minimal inhibitory concentration (MIC) of standardised hydroalcoholic extracts from *Pleurotus* species against eight clinically relevant bacterial species

		MIC (μg mL^{-1})					
		P. levis	*P. ostreatus*		*P. pulmonarius*		*P. tuber-regium*
Code	Bacteria	CP-30	CP-50	CP-800	CP-16	CP-799	CP-182
CPB-1	*Salmonella typhi*	>106.56*	125.32	145.52	136.64	126.14	24.12
CPB-4	*Streptococcus agalactiae*	13.32	7.83	72.76	136.64	63.07	6.03
CPB-7	*Stenotrophomonas* spp.	106.56	62.66	36.38	68.32	63.07	24.12
CPB-8	*Escherichia coli*	>106.56*	>125.32*	72.76	>136.64*	126.14	48.23
CPB-9	*Bacillus subtilis*	3.33	125.32	72.76	68.32	126.14	48.23
CPB-10	*Staphylococcus aureus*	26.64	125.32	72.76	68.32	63.07	12.06
CPB-11	*Listeria monocytogenes*	106.56	>125.32*	72.76	68.32	126.14	24.12
CPB-13	*Pseudomonas aeruginosa*	>106.56*	>125.32*	72.76	>136.64*	126.14	12.06

*Mushroom extract was not active at the concentration studied.

Table 6 Minimal bactericidal concentration (MBC) of standardised hydroalcoholic extracts from *Pleurotus* species against eight clinically relevant bacterial species

		MBC (μg mL^{-1})					
		P. levis	*P. ostreatus*		*P. pulmonarius*		*P. tuber-regium*
Code	Species	CP-30	CP-50	CP-800	CP-16	CP-799	CP-182
CPB-1	*Salmonella typhi*	–	–	–	–	–	24.12
CPB-4	*Streptococcus agalactiae*	–	–	145.52	–	–	48.23
CPB-7	*Stenotrophomonas* spp.	–	–	36.38	–	–	48.23
CPB-8	*Escherichia coli*	–	–	145.52	–	–	96.46
CPB-9	*Bacillus subtilis*	–	–	145.52	–	–	48.23
CPB-10	*Staphylococcus aureus*	–	–	–	–	–	48.23
CPB-11	*Listeria monocytogenes*	–	–	–	–	–	48.23
CPB-13	*Pseudomonas aeruginosa*	–	–	145.52	–	–	24.12

mushroom extracts and ABTS scavenging activity ($r = 0.83$, $P < 0.0001$; Fig. 3a), as well as an inverse relationship between the pH and total phenolic content ($r = -0.90$, $P < 0.0001$; Fig. 3b). The yield of fresh mushrooms and the protein concentration in mushroom extracts were also significantly correlated ($r = 0.86$, $P < 0.0001$; Fig. 3c). The concentration (dry weight) of the mushroom extract was significantly correlated with total phenolic content ($r = 0.84$, $P < 0.0001$; Fig. 3d), whereas it was inversely correlated with the ABTS scavenging activity ($r = -0.92$, $P < 0.0001$; Fig. 3e). There was a highly significant correlation between the ABTS scavenging activity and total phenolic content of mushroom extracts ($r = 0.87$, $P < 0.0001$; Fig. 3f).

Discussion

Pleurotus species and strains identified molecularly showed variation in antioxidant and antibacterial properties, using the same cultivation method, extraction procedure and experimental conditions. Basidiocarps had different initial antioxidant activity ranging from 3900.8 μmol of TE g^{-1} to 24661.6 μmol of TE g^{-1}. Mushroom yield was significantly correlated ($r = 0.86$, $P < 0.0001$) with the protein concentration of *Pleurotus* extracts. After extraction, the concentration (dry weight) of standardised mushroom extracts varied 33.7% among species and strains, while conductivity and resistance fluctuated 50.3%, indicating that their composition and functional properties were different. This was confirmed by the spectrophotometric visualisation of mushroom extracts, which clearly showed differing absorbance patterns. A high correlation between antioxidant activity and conductivity was interesting, not previously reported, and involved total phenolic content ($r = 0.78$, $P < 0.0001$) and ORAC ($r = 0.70$, $P < 0.0001$) assays.

The concentration (dry weight) of mushroom extracts was correlated ($r = 0.84$, $P < 0.0001$) and inversely correlated ($r = -0.92$, $P < 0.0001$) with total phenolic content and ABTS scavenging activity, respectively. Likewise, the pH of mushroom extracts was correlated ($r = 0.83$, $P < 0.0001$) with ABTS scavenging activity. Extracts from *Pleurotus* species (EC_{50} values) had better antioxidant activity than the standards in the β-carotene–linoleic acid analysis (0.16 – >5.0 μg mL^{-1}), but were equivalent or below standards studied in the DPPH (0.49–1.68 μg mL^{-1}) and ABTS (0.24–0.50 μg mL^{-1}) assays of scavenging activity. Data were similar to previous research on cultivated mushrooms. Lower antioxidant activity (10 mg mL^{-1}) against the β-carotene–linoleic acid was reported by Vamanu (2012), assessing ethanolic extracts from the mycelium of *P. ostreatus*. Gursoy *et al.* (2009) used methanolic extracts of wild mushrooms, *Morchella* spp., and also found low antioxidant activity (4.5 mg mL^{-1}) against the β-carotene–linoleic acid. These studies using ethanolic or methanolic solvents extracted less antioxidant compounds than the hydroalcoholic solvent reported in this research. *Pleurotus ostreatus* has also been studied using modifications of the techniques, and these data are given as a reference. Smolskaitė *et al.* (2015) found a DPPH antioxidant activity of 0.08–1.52 μM TE g^{-1} dw in *P. ostreatus* using different solvents (cyclohexane, dichloromethane, methanol and water), while ABTS antioxidant activity of 0.10–0.80 μM TE g^{-1} dw. A DPPH scavenging activity of 0.82–4.07 mmol TE/100 g fw was reported in methanolic extracts of *P. ostreatus* (Koutrotsios *et al.*, 2017). Other mushroom genera and species have also been studied. The DPPH radical scavenging activity varied from 0.91 to 1.51 g L^{-1} in water and alkali extraction polysaccharides from the sclerotium of *P. tuber-regium* (Wu *et al.*, 2014). Kozarski *et al.* (2011) described variable scavenging ability on DPPH radicals of hot-water extracted polysaccharides (EC_{50} values) from *Agaricus bisporus* (2.0 mg extract mL^{-1}), *A. brasiliensis* (0.27 mg extract mL^{-1}), *Ganoderma lucidum* (<0.1 mg

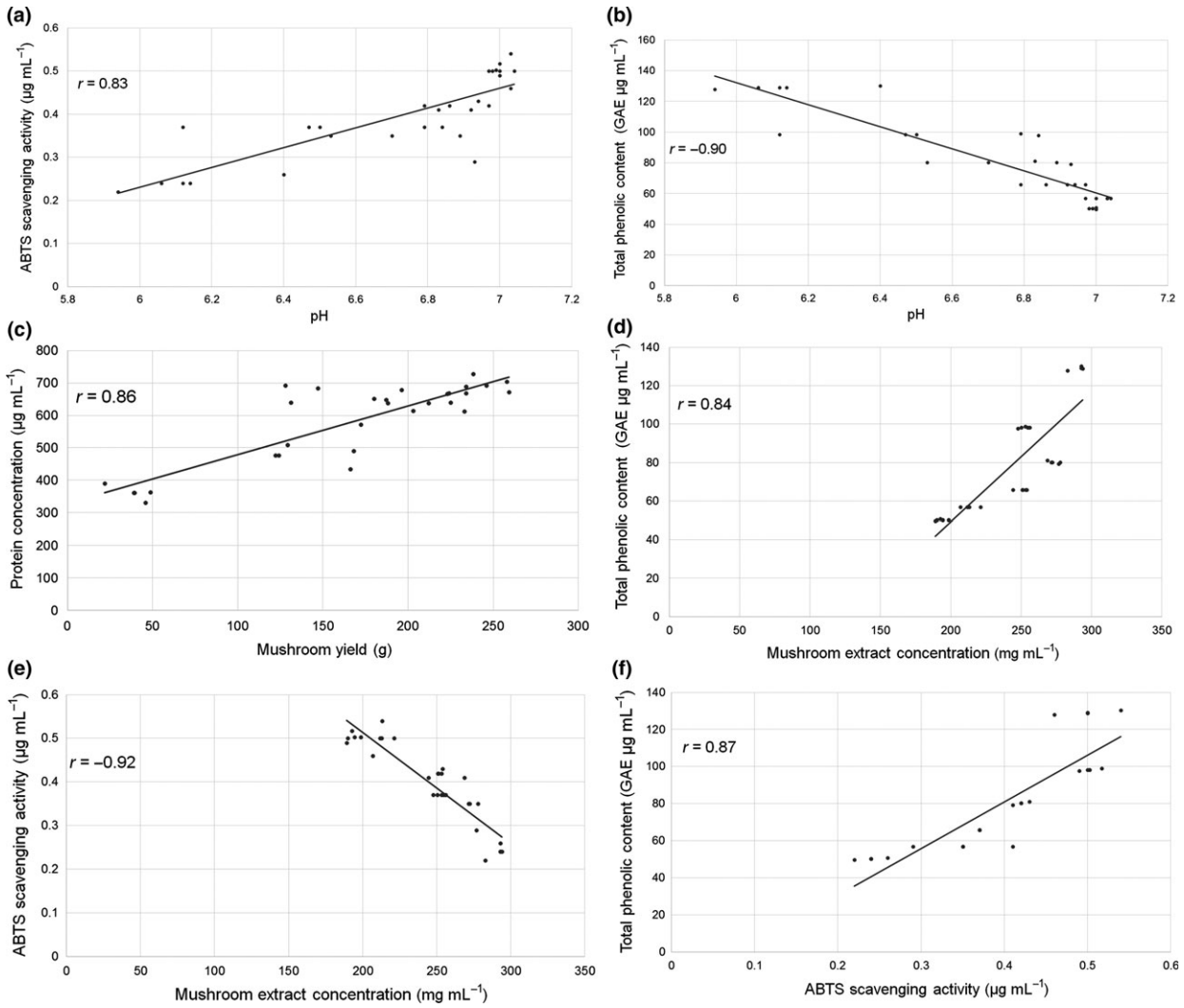

Figure 3 Regression analysis between assays and variables of mushroom extracts studied. (a) Correlation between the pH and ABTS free radical scavenging activity ($r = 0.83$, $P < 0.0001$). (b) Inverse correlation between the pH and total phenolic content ($r = -0.90$, $P < 0.0001$). (c) Correlation between fresh mushroom yield and protein concentration ($r = 0.86$, $P < 0.0001$). (d) Correlation between the concentration (dry weight) of mushroom extracts and total phenolic content ($r = 0.84$, $P < 0.0001$). (e) Inverse correlation between the concentration (dry weight) of mushroom extracts and ABTS free radical scavenging activity ($r = -0.92$, $P < 0.0001$). F: Correlation between ABTS free radical scavenging activity and total phenolic content ($r = 0.87$, $P < 0.0001$).

extract mL^{-1}) and *Phellinus linteus* (<0.1 mg extract mL^{-1}). These polysaccharides with different levels of purification show lower DPPH radical scavenging activity than crude hydroalcoholic extracts of *Pleurotus* species studied, indicating that other compounds are also involved in the scavenging activity.

Total phenolic content of *Pleurotus* species was low to moderate, ranging from 50.32 to 129.06 GAE µg mL^{-1}, and was inversely correlated ($r = -0.90$, $P < 0.0001$) with the pH of mushroom extracts. Previous research work has mainly focused on methanolic extracts from wild and cultivated mushrooms. Yang *et al*. (2002) reported greater content of total phenols in *P. ostreatus* (15.7 mg g^{-1}) and *P. cystidiosus* (10.24 mg g^{-1}), while Gogavekar *et al*. (2014) found a total phenol content of 52.20 mg g^{-1} in *P. pulmonarius* (= *P. sajor-caju*). Koutrotsios *et al*. (2017) assessed basidiocarps of sixteen strains of *P. ostreatus* and found a total phenolic content of 1.27–8.62 mg GAE/100 g fw. Other mushroom species also showed greater content of total phenols (Mau *et al*., 2002), for example *Ganoderma lucidum* (47.25–55.96 mg g^{-1}) and *G. tsugae* (51.28 mg g^{-1}). Wild mushrooms, such as several species of *Morchella*, contained high content of

total phenolics (12.36–25.38 μg GAEs mg^{-1} of extract), according to Gursoy *et al.* (2009). Other type of extracts using different solvents (cyclohexane, dichloromethane, methanol and water) were studied by Smolskaitė *et al.* (2015), and a total phenolic content of 4.26–5.67 mg GAE g^{-1} dw was recorded for *P. ostreatus* and 4.21–4.64 mg GAE g^{-1} dw for *Agaricus bisporus*. Dubost *et al.* (2007) used ethanolic extracts and found a total phenolic content of 4.27 mg GAE g^{-1} in *P. ostreatus*, 4.32 mg GAE g^{-1} in *L. edodes* and 8.0 mg GAE g^{-1} in *A. bisporus*. A 60% ethanol extract from the sclerotium of *P. tuber-regium* (Lin *et al.*, 2014) contained high total phenolic content (420 GAE μg mg^{-1} of extract) and showed DPPH free radical scavenging activity (1 mg mL^{-1}) and ABTS radical scavenging activity (5 mg mL^{-1}). In comparison with hydroalcoholic extracts studied, total phenols seem to be extracted more efficiently by other polar and nonpolar solvents reported previously, such as methanol, ethanol, cyclohexane, dichloromethane and water.

There was a highly significant correlation ($r = 0.87$, $P < 0.0001$) between ABTS scavenging activity and total phenolic content of mushroom extracts. This linear correlation showed that increasing scavenging activity corresponded to increasing content of total phenolic compounds. The maximum ABTS scavenging activity is thus associated with the greatest total phenolic content of mushroom extracts studied, indicating that phenols are major antioxidant components. These results were in agreement with previous correlation reports in *P. ostreatus*, *P. cystidiosus* and *P. tuber-regium* (Yang *et al.*, 2002; Lin *et al.*, 2014; Smolskaitė *et al.*, 2015; Koutrotsios *et al.*, 2017), suggesting a prevalent trend within the genus *Pleurotus*. The ORAC assay also showed high antioxidant activity varying from 3316.0 to 34646.0 μmol of TE mL^{-1}, in comparison with other mushrooms and conventional foods (Dubost *et al.*, 2007; Avila-Nava *et al.*, 2017), such as *P. ostreatus* (55.34 μmol of TE g^{-1}), *Lentinula edodes* (62.67 μmol of TE g^{-1}), *Agaricus* mushrooms (86.33–138.33 μmol of TE g^{-1}), tomato (268.5 μmol of TE mL^{-1}), corn flour (245.1 μmol of TE mL^{-1}), chia seed (315.0 μmol of TE mL^{-1}) and pumpkin seed (402.2 μmol of TE mL^{-1}). Smolskaitė *et al.* (2015) reported the ORAC antioxidant activity of 1.73–115 μM of TE g^{-1} dw in extracts of *P. ostreatus* using different solvents (cyclohexane, dichloromethane, methanol and water). Hydroalcoholic extracts used in the ORAC assay of this study extracted antioxidant compounds more efficiently than other solvents previously reported, such as water–acetone, cyclohexane, dichloromethane and methanol.

A comparative analysis indicated that the strain CP-800 of *P. ostreatus* from West Africa showed remarkable antioxidant and antimicrobial properties, as follows: (i) the greatest concentration (dry weight) of the mushroom extract (291.04 mg mL^{-1}) and conductivity (12101.68 μS cm^{-1}); (ii) the lowest resistance (54.0 Ω) and pH (6.13); (iii) the most effective concentration (EC_{50}) for scavenging activity in the ABTS assay (0.24 μg mL^{-1}); (iv) the greatest antioxidant activity in the ORAC assay (34646.0 μmol of TE mL^{-1}); (v) bacteriostatic activity against all bacteria studied; (vi) bactericidal activity against five of eight bacteria studied; and (vii) the greatest protein concentration in the extract (696.56 μg mL^{-1}).

In general, there was no correlation between antioxidant and antimicrobial properties of mushroom extracts. The strain CP-182 of *P. tuber-regium* from West Africa showed the lowest antioxidant activity in the ORAC assay (3316.0 μmol of TE mL^{-1}), whereas it was the sole strain that exerted the most potent bacteriostatic and bactericidal activity against all Gram-positive and Gram-negative bacteria tested. *Pleurotus tuber-regium* also had the lowest extract yield (192.92 mg mL^{-1}), total phenolic content (50.32 GAE μg mL^{-1}), protein concentration (281.16 μg mL^{-1}) and absorbance after comparative spectrophotometric visualisation of mushroom extracts, in comparison with the other species studied.

Different strains of the same species showed variation according to parameters studied. This was the case of strains CP-50 (North America × Eastern Europe) and CP-800 (West Africa) of *P. ostreatus*, which varied significantly in mushroom yield (141.8–236.6 g), concentration (dry weight) of the standardised extract (250.64–291.04 mg mL^{-1}), conductivity (6057.28–12101.68 μS cm^{-1}), resistance (54.0–108.20 Ω), pH (6.13–6.90), protein concentration (386.93–696.56 μg mL^{-1}), scavenging and antioxidant activity in the DPPH (0.63–1.05 μg mL^{-1}), ABTS (0.24–0.42 μg mL^{-1}), β-carotene–linoleic (0.17–0.34 μg mL^{-1}), and ORAC (8199.2–34646.0 μmol of TE mL^{-1}) assays, total phenolic content (65.76–129.06 GAE μg mL^{-1}), minimal inhibitory concentration (against *Streptococcus agalactiae*, *Stenotrophomonas* spp., *Escherichia coli*, *Listeria monocytogenes* and *Pseudomonas aeruginosa*) and minimal bactericidal concentration (against *S. agalactiae*, *Stenotrophomonas* spp., *E. coli*, *Bacillus subtilis* and *P. aeruginosa*). Similar effect, but to a lesser extent, was also observed in the strains CP-16 (South-East Asia) and CP-799 (West Africa) of *P. pulmonarius*, which varied significantly in mushroom antioxidant activity according to the ORAC assay (basidiocarps: 9555.2–24661.6 μmol of TE g^{-1}), conductivity (7280.08–9969.72 μS cm^{-1}), resistance (67.0–91.40 Ω), antioxidant activity in the ORAC (extracts: 4455.2–6496.8 μmol of TE mL^{-1}) assay, total phenolic content (80.19–98.36 GAE μg mL^{-1}) and minimal inhibitory concentration (against *S. agalactiae*, *E. coli*, *B. subtilis*, *L. monocytogenes* and *P. aeruginosa*).

The case of *P. levis* (CP-30) from North America was interesting, as there are no previous reports on its functional properties. Mushroom yield in *P. levis* was similar to that of *P. ostreatus* and *P. pulmonarius*, but showed the lowest mushroom antioxidant activity (basidiocarps: 3900.8 μmol of TE g^{-1}). In comparison with the other species and strains studied, its standardised mushroom extract had the lowest conductivity (6015.94 μS cm^{-1}), the highest resistance (108.80 Ω) and pH (7.01), as well as a high protein concentration (689.20 μg mL^{-1}). The extract of *P. levis* showed low to moderate scavenging and antioxidant activity in the DPPH, ABTS, β-carotene-linoleic acid and ORAC assays, as well as the total phenolic content. Bacteriostatic activity was recorded against Gram-positive and Gram-negative bacteria, including *S. agalactiae*, *Stenotrophomonas* spp., *Staphylococcus aureus* and *L. monocytogenes*. The highest inhibition was recorded on *B. subtilis* (MIC: 3.33 μg mL^{-1}), a Gram-positive bacterium, as compared to the rest of species and strains studied. However, *P. levis* did not show any bactericidal activity, which was also the case for the strains CP-50 of *P. ostreatus*, and CP-16 and CP-799 of *P. pulmonarius*.

Antimicrobial (bacteria and moulds) properties have been found in 158 mushroom species belonging to eighty-eight genera, including *P. eryngii*, *P. eryngii* var. *ferulae*, *P. ostreatus*, *P. pulmonarius* (= *P. sajor-caju*) and *Lentinus squarrosulus* (Shen *et al*., 2017). In these *Pleurotus* species, antibacterial properties have been reported against *Bacillus* spp., *Clostridium perfringens*, *Staphylococcus* spp., *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Salmonella* spp. This study showed that *Pleurotus* strains and species studied exhibited differing antibacterial properties. The greatest bacteriostatic or bactericidal activities against Gram-positive and Gram-negative bacteria were found in *P. tuber-regium* (CP-182) from West Africa, followed by *P. ostreatus* (CP-800) from West Africa and *P. levis* (CP-30) from North America. *Pleurotus tuber-regium* is the only species within the genus that produces a sclerotium, rounded mass of hyphae capable of resisting extreme environmental conditions. It is possible that such specialised adaptation has allowed the accumulation of bioactive compounds with antibacterial properties in *P. tuber-regium*. *Streptococcus agalactiae*, a Gram-positive bacterium, showed a high degree of susceptibility to a low concentration (MIC: 1–10 μg mL^{-1}) of standardised mushroom extracts from *P. ostreatus* (CP-50) and *P. tuber-regium* (CP-182), whereas *B. subtilis*, another Gram-positive bacterium, to the extract from *P. levis*. All tested Gram-positive and Gram-negative bacteria showed varying degrees of susceptibility to regular (MIC: 11–100 μg mL^{-1}) or high (MIC: >101 μg mL^{-1}) concentrations of extracts studied. *Salmonella typhi*, a Gram-negative bacterium, was the least susceptible bacterial species to extracts from *Pleurotus* species assessed. Lower antibacterial activity was reported by Vamanu (2012) assessing ethanolic extracts from the mycelium of *P. ostreatus* against *Bacillus cereus* (MIC: 12.5 mg mL^{-1}), *Candida* spp. (MIC: 1.25 mg mL^{-1}), *E. coli* (MIC: 1.25 mg mL^{-1}), *Listeria innocua* (MIC: 20 mg mL^{-1}), *P. aeruginosa* (MIC: 12.5 mg mL^{-1}) and *S. aureus* (MIC: 12.5 mg mL^{-1}). Adebayo *et al*. (2012) reported antibacterial activity of polysaccharides from *P. pulmonarius* against *E. coli*, *P. mirabis*, *K. pneumoniae*, *S. aureus*, *S. typhi* and *Shigella* spp. Methanolic and protein acidic extracts of *P. ostreatus*, *P. eryngii* and *P. nebrodensis* have also been reported to show antibacterial effects against *B. cereus*, *Candida albicans*, *E. coli*, *P. aeruginosa*, *S. aureus* and *S. epidermidis* (Schillaci *et al*., 2013; Smolskaitė *et al*., 2015).

Antioxidant and antibacterial properties of *Pleurotus* species studied, as well as those from other mushrooms, increasingly encourage the development of mushroom products for the food industry (Pérez Armendáriz *et al*., 2015; Lu *et al*., 2016; Rašeta *et al*., 2016; Wang *et al*., 2016; Bach *et al*., 2017), such as high-quality functional foods, dietary supplements, extracts, purified extracts, capsules, tablets and beverages.

Conclusions

In this study, standardised mushroom hydroalcoholic extracts from *Pleurotus* species, molecularly identified, showed valuable antioxidant and antimicrobial properties for human diet. Strains studied varied in the extract yield, conductivity, as well as antioxidant and antibacterial properties, using the same cultivation method, extraction procedure and experimental conditions. There was no correlation between antioxidant and antibacterial properties of mushroom extracts. The maximum ABTS scavenging activity was correlated with the greatest total phenolic content of mushroom extracts studied, indicating that phenols are major antioxidant components. The pH and concentration (dry weight) of extracts affected ABTS scavenging activity and the total phenolic content. Gram-positive and Gram-negative bacteria showed differing degrees of susceptibility to *Pleurotus* extracts. Remarkable antioxidant and antibacterial properties were found in *P. levis* and *P. tuber-regium* compared to the other species studied that are cultivated commercially, which can be considered for future manufacturing of mushroom products. *Pleurotus levis* represents a novel source of bioactive compounds within the genus. Further analyses are needed to determine chemical characteristics of bioactive compounds involved, as well as their effects on different *in vivo* models and clinical trials.

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Conflict of interest

The authors have declared that no competing interests exist.

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