Food & Function

ROYAL SOCIETY OF CHEMISTRY

PAPER

View Article Online
View Journal | View Issue

Cite this: *Food Funct.*, 2021, **12**, 6780

Antimicrobial activity, chemical composition and cytotoxicity of *Lentinus crinitus* basidiocarp

Míria Benetati Delgado Bertéli, [a] Lillian Barros, [b] Filipa S. Reis, [b] Isabel C. F. R. Ferreira, [b] Jasmina Glamočlija, [c] Marina Soković, [c] Juliana Silveira do Valle, [a] Giani Andrea Linde, [a] Suelen Pereira Ruiz [a] and Nelson Barros Colauto *[a]

Lentinus crinitus (L.) Fr. (Basidiomycota: Polyporales) is a wild mushroom with several biotechnological applications; however, there are few studies on its chemical composition and antimicrobial activity. Therefore, this study aims to evaluate the chemical composition, cytotoxicity, and antimicrobial activity of *L. crinitus* basidiocarp. For that, its nutritional value (AOAC procedures) and its composition in some hydrophilic and lipophilic compounds (chromatographic techniques) were assessed. Moreover, the potential hepatotoxic effects were evaluated using a primary cell culture obtained from porcine liver, and its growth inhibitory capacity was also evaluated against four human tumour cell lines (spectrophotometric assays). The antimicrobial activity was evaluated by microdilution against eight bacteria and fungi. The basidiocarp has a high content of carbohydrates and, therefore, a relatively high energetic value. It is also rich in soluble sugars, β-tocopherol, phenolic acids, mainly *p*-hydroxybenzoic acid, and organic acids, mainly malic acid. *L. crinitus* did not show cytotoxicity in non-tumour cells, but it did not inhibit the growth of human tumour cell lines either. The basidiocarp has a wide antimicrobial activity, inhibiting the growth of different species of bacteria and fungi. It showed minimum bactericidal and fungicidal concentration values similar to or lower than those verified by commercial antibiotics or food additives used as preservatives. The antimicrobial activity was more evident against *Listeria monocytogenes*, *Salmonella enterica*, and *Penicillium ochrochloron*, followed by *Aspergillus ochraceus* and *Trichoderma viride*, when compared to the controls. The results obtained in this study showed that *L. crinitus* basidiocarp has great potential to be used by the industry without toxicity risks.

Received 2nd March 2021,
Accepted 6th May 2021

DOI: 10.1039/d1fo00656h

rsc.li/food-function

1. Introduction

Basidiomycota represents the second largest phylum of the kingdom fungi with more than 30 000 identified species[1] and is one of the greatest unexploited resources of natural compounds.[2] Edible mushrooms are highly nutritional and healthy[3] with reported bioactivities such as antitumoral, immunostimulant, antioxidant, antimicrobial, hypocholesterolemic, and hepatoprotector activities, among others.[4–6]

Lentinus crinitus (L.) Fr. (Basidiomycota: Polyporales) is a pantropical and neotropical[7] mushroom commonly found in decomposing tree trunks.[8] The basidiocarps are consumed as a source of protein by indigenous people from *Yanomami* and *Txicão* tribes in Brazil, *Uitoto*, *Muinane*, and *Andoke* in Colombia, Hotï in Venezuela, and the rural population of Loreto in Peru.[9] There are some reports on its antioxidant potential,[10] metal bioaccumulation capacity,[11,12] and high enzyme production capacity (*i.e.* laccase,[13] protease,[14] and xylanase[15]). We can find some studies regarding the nutritional potential of this mushroom, namely its content in ashes, fat, protein, and total carbohydrates.[16–18] To the best of our knowledge, the antimicrobial potential of *L. crinitus* basidiocarp has been reported just against *Fusarium* sp. conidium sporulation.[19] However, there are no detailed reports on its chemical characterization, cytotoxicity, or antimicrobial activity against foodborne pathogens.

Foodborne diseases are one of the major burdens to public health, caused by the consumption of contaminated foods with pathogenic microorganisms or their toxins.[20,21] The main contaminants of food are bacteria[20] and mycotoxins, resulting in average costs of 100 billion dollars per year for developing countries.[22] Some of these pathogenic microorganisms

[a]*Paranaense University, Molecular Biology Laboratory, 87502-210 Umuarama, PR, Brazil. E-mail: nelsonbcolauto@gmail.com; Tel: +55 (44)991391112*
[b]*Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal*
[c]*Mycological Laboratory, Mycology, Department of Plant Physiology, Institute for Biological Research "Siniša Stanković" – National Institute of Republic of Serbia, University of Belgrade, Bulevar despota Stefana 142, 11000 Belgrade, Serbia*

include *Pseudomonas aeruginosa*, a multi-drug resistant bacterium,[23] *Listeria monocytogenes*, which develops multidrug resistance,[24] and *Bacillus cereus* which causes food intoxication.[25] Therefore, these are relevant bacteria that cause foodborne diseases to be controlled with new compounds. Several synthetic chemical compounds, such as nitrates, benzoates, sulfites, and sorbates are applicable to foods for their preservation. However, some of these compounds have been related to undesirable effects on human health such as allergies or even carcinogenic effects.[21,26,27] Thus, the search for natural compounds as an alternative for food preservation has been one of the fields of interest of scientific research in recent years. Thus, this study aimed to evaluate the chemical composition, cytotoxicity, and antimicrobial activity of *L. crinitus* basidiocarp grown on agro-industrial residues, contributing to the food science and technology field.

2. Materials and methods

2.1 Biological material

This study used *Lentinus crinitus* (L.) Fr. (Basidiomycota: Polyporales) U9-1 strain from the Culture Collection of the Laboratory of Molecular Biology at Paranaense University, cryopreserved by the wheat grain technique,[28,29] identified by sequencing the internal transcribed spacers (ITS) of ribosomal DNA, and deposited in the GenBank database (http://www.ncbi.nlm.nih.gov/genbank/) with the accession number MG211674.[29] The strain was registered in the National System of Genetic Patrimony Management and Associated Traditional Knowledge (SisGen, its acronym in Portuguese) under the code A04E776. The fungus was cultivated in a 2% malt extract agar (MEA) substrate at 25 ± 1 °C for seven days, and the colonies without sectioning and with mycelial growth vigor were selected as the inoculum of wheat grains. Wheat grains cooked at 90 °C in ultrapure water for 40 min were used. After cooking, the excess water was removed and 1 g of calcium carbonate ($CaCO_3$) was added to every 100 g of grains. The grains were transferred to polypropylene bags and autoclaved at 121 °C for 1 h and 30 min. After cooling, MEA disks containing mycelia were transferred to the bags with grains and incubated at 25 ± 1 °C until complete colonization.[30] The colonized wheat grains were utilized as inoculum on the cultivation substrate.

2.2 Cultivation substrate for mushroom production

Sugarcane bagasse and rice husks from a sugarcane processing mill and from a rice mill, respectively, were utilized with a 1 : 1 (v : v) proportion to compose the cultivation substrate with 10 replications. Each replication was represented by a polypropylene bag with 2 kg of the cultivation substrate autoclaved for 1 h and 30 min at 121 °C. To each bag was added 20 g of wheat grains containing fungus, thermosealed, and stored in a cultivation room with temperature control at 27 ± 1 °C and 80% air humidity until complete substrate colonization. After 30 days, the top part of the bags was opened, and room temperature was reduced to 18 ± 1 °C for 24 h (thermal shock). The basidiocarp harvesting was performed daily when the pileus edge was flat, indicating growth completion and senescence process onset. The harvested basidiocarps (stipe and pileus together) were dehydrated in an air circulating oven at 60 °C for 24 h, ground in a mortar with a pestle to obtain granulometry ≤0.35 mm (48 mesh) and stored at −20 °C for subsequent analyses.

2.3 Chemical composition of the basidiocarp

2.3.1 Macronutrient composition and energetic value. The proximate composition (protein, fat, ash, and carbohydrate content) of the samples was determined according to standard procedures.[31] The crude protein content ($N \times 4.38$) of the samples was estimated by the macro-Kjeldahl method; crude fat was determined by extracting a known mass of the powdered sample with petroleum ether, using a Soxhlet apparatus; the ash content was determined by incineration at 600 ± 15 °C. Total carbohydrates were calculated by difference [total carbohydrates (g per 100 g; dry basis) = $100 - (g_{protein} + g_{fat} + g_{ash})$]. The energy was calculated according to Regulation (EC) number 1169/2011 of the European Parliament and of the Council[32] on the Provision of Food Information to Consumers, as: energy [(kcal per 100 g; dry basis) = $4 \times (g_{protein} + g_{carbohydrates}) + 9 \times (g_{fat})$].

2.3.2 Hydrophilic compounds

Soluble sugars. The dried samples (1 g) were spiked with raffinose as the internal standard (IS, 25 mg mL^{-1}) and extracted with 40 mL of 80% aqueous ethanol at 80 °C for 1 h. The resulting suspension was centrifuged (15 000*g* for 10 min). The resulting suspension was filtered and concentrated under reduced pressure (rotary evaporator Büchi R-210, Flawil, Switzerland) and defatted three times with 10 mL of diethyl ether. After concentration at ≈40 °C, the solid residues were dissolved in water to a final volume of 5 mL and filtered through 0.2 μm disposable LC nylon disk filters and transferred into an injection vial to be analyzed by high performance liquid chromatography (HPLC). The soluble sugars present in *L. crinitus* basidiocarp were analyzed by HPLC using a refraction index (RI) detector. The HPLC equipment consisted of an integrated system with a pump (Knauer, Smartline system 1000, Berlin, Germany), degasser system (Smartline manager 5000), autosampler (AS-2057 Jasco, Easton, MD, USA) and an RI detector (Knauer Smartline 2300). Data were analyzed using Clarity 2.4 software (DataApex). The chromatographic separation was achieved with a Eurospher 100-5 NH_2 column (4.6 × 250 mm, 5 mm, Knauer) operating at 30 °C (7971 R Grace oven). The mobile phase was acetonitrile : deionized water, 70 : 30 (v : v) at a flow rate of 1 mL min^{-1}. The compounds were identified by chromatographic comparisons with authentic standards. The quantification was performed using the internal standard method.[33] The results were expressed in g per 100 g (dry basis).

Organic acids. Samples (approximately 1.5 g) were extracted using 25 mL of *meta*-phosphoric acid (25 °C at 150 rpm) by stirring for 45 min and subsequently filtered through filter

paper (Whatman® grade 4). Before analysis, the sample was filtered through 0.2 μm nylon filters.[34] The organic acid profile of the studied samples was determined according to a previously described procedure.[34] The analysis was performed by ultra-fast liquid chromatography (UFLC), coupled to a photodiode array detector (PDA), following the previously mentioned procedure. The analysis was performed using a Shimadzu 20A series UFLC (Shimadzu Corporation, Kyoto, Japan). Separation was achieved on a SphereClone (Phenomenex, Torrance, CA, USA) reverse phase C18 column (5 μm, 250 mm, 4.6 mm i.d.) thermostatted at 35 °C. The elution was performed with sulfuric acid (3.6 mM) using a flow rate of 0.8 mL min^{-1}. Detection was carried out in a PDA, using 215 nm and 245 nm (for ascorbic acid) as preferred wavelengths. The found organic acids were quantified by comparing the area of their peaks recorded at 215 nm (245 nm for ascorbic acid) to calibration curves obtained from commercial standards of each compound. The results were expressed in mg per 100 g (dry basis).

Phenolic acids and related compounds. The dried samples (approximately 1 g) were extracted using 30 mL of aqueous methanol (80 : 20; v : v) by magnetic stirring (25 °C, 150 rpm) for 1 h, and subsequently filtered (Whatman® grade 4 filter paper) obtaining a hydromethanolic extract. The extraction procedure was repeated with an additional portion of the solvent, repeating the previous process. The obtained extracts were combined, the methanol was evaporated (rotary evaporator previously referred) and the residual aqueous phase was frozen and lyophilized.[35] After lyophilization, the dried extract was dissolved in aqueous methanol (20 : 80; v : v) obtaining a 20 mg mL^{-1} final concentration, filtered through a 0.2 μm nylon filter and analyzed by UFLC. The phenolic acid determination was performed using the UFLC system mentioned before for organic acid determinations. Detection was carried out in the PDA (photodiode-array detection), using 280 nm as the preferred wavelength and in a mass spectrometer (MS) connected to a HPLC system *via* the PDA cell outlet. The phenolic compounds were identified by comparing their retention times, UV-Vis and mass spectra with those obtained with standard compounds. For the quantitative analysis, a calibration curve for each available phenolic standard was constructed based on the UV-Vis signal. The results were expressed as μg per 100 g (dry basis).

2.3.3 Lipophilic compounds

Fatty acids. Fatty acids (obtained after Soxhlet extraction) were methylated with 5 mL of methanol : 95% sulfuric acid : toluene (2 : 1 : 1; v : v : v) for approximately 12 h in a bath at 50 °C and 160 rpm; to obtain phase separation, 3 mL of deionized water were added; the fatty acid methyl esters (FAME) were recovered by shaking in a vortex with 3 mL of diethyl ether, and the upper phase was passed through a microcolumn of anhydrous sodium sulfate to eliminate water. The sample was recovered in a vial with Teflon and filtered through a 0.2 μm Whatman® nylon filter.[36] The fatty acid profile of the samples was determined by gas–liquid chromatography with a flame ionization detection (GC-FID)-capillary column. The identification of the different fatty acids was made by comparison of the relative retention time of FAME peaks from samples with standards. The results were expressed in the relative percentage of each fatty acid.

Tocopherols. Extracts were prepared following a previously described procedure.[37] Before extraction, samples were added with butylhydroxytoluene (BHT, 100 μL) and spiked with tocol (250 μL) as the internal standard (IS) for further chromatographic analysis. Samples (≈500 mg) were extracted by vortex mixing with methanol (4 mL) for 1 min; subsequently, hexane (4 mL) was added and the mixture was vortexed again for one additional minute. Finally, a saturated NaCl aqueous solution (2 mL) was added and the mixture was homogenized (1 min). After that, samples were centrifuged (5 min, 4000*g*), and the upper layer, corresponding to the hexane fraction, was transferred to a 25 mL amber vial. Samples were re-extracted twice with hexane and the combined extracts were dehydrated with anhydrous sodium sulfate, taken to dryness under a nitrogen stream and stored at −20 °C. Tocopherol extracts were re-dissolved in 1 mL of *n*-hexane, filtered (0.22 μm disposable LC filter disk), and transferred to an amber injection vial for chromatographic analysis. The analysis was performed using the same HPLC system described for soluble sugars but coupled to a fluorescence detector. The tocopherol identification was performed by chromatographic comparisons with authentic standards and the quantification was based on the fluorescence signal response of each standard. The results were expressed in μg per 100 g (dry basis).

2.4 Biological activities of the basidiocarp

2.4.1 Extract preparation. The same extraction procedure described for the phenolic compounds was applied for the cytotoxicity and antimicrobial activity assays. After the lyophilization process, the obtained extracts were re-dissolved in: (i) autoclaved distilled water, at 8 mg mL^{-1}, to assess the cytotoxic activity assay; and (ii) 5 mL per 100 mL solution of DMSO in distilled water, at 100 mg mL^{-1}, to assess the antimicrobial activity assay. These solutions were diluted successively in order to obtain the concentrations necessary to perform the experimental study.

2.4.2 Cytotoxic activity. Four human tumor cell lines were used, namely HeLa (cervical carcinoma), HepG2 (hepatocellular carcinoma), MCF-7 (breast adenocarcinoma), and NCI-H460 (non-small cell lung cancer). Cells were routinely maintained as adherent cell cultures and treated for 48 h with the diluted extract solutions.[38] The adherent cells were fixed by adding cold 10% trichloroacetic acid (TCA, 100 mL) and incubated for 60 min at 4 °C. Plates were then washed with deionized water and dried; 0.1% sulforhodamine B solution (SRB, 100 mL) in 1% acetic acid was then added to each plate well and incubated for 30 min at room temperature. Unbound SRB was removed by washing with 1% acetic acid. Plates were air-dried, the bound SRB was solubilized with 10 mM tris (200 mL, pH 7.4) and the absorbance was measured at 540 nm. The results were expressed as GI_{50} values (sample concentration that inhibited 50% of the net cell growth). Ellipticine was used as a positive control. For the possible

hepatotoxicity evaluation, a culture cell obtained from porcine liver, designed as PLP2, was used.[39] The same procedure described above for the SRB assay was performed for the growth inhibition. The results were also expressed as GI_{50} values.

2.4.3 Antibacterial activity. The antibacterial activity of *L. crinitus* basidiocarp extract was tested against the following Gram-positive bacteria: *Staphylococcus aureus* subsp. *aureus* Rosenbach (ATCC 6538), *Bacillus cereus* Frankland and Frankland (clinical isolate), *Micrococcus luteus* (Schroeter) Cohn (ATCC 10240), and *Listeria monocytogenes* (Murray *et al.*) Pirie (NCTC 7973), and the following Gram-negative ones: *Pseudomonas aeruginosa* (Schroeter) Migula (ATCC 27853), *Escherichia coli* (Migula) Castellani and Chalmers (ATCC 35218), *Enterobacter cloacae* (Jordan) Hormaeche and Edwards (clinical isolate), and *Salmonella enterica* subsp. *enterica* (ex-Kauffmann and Edwards) Le Minor and Popoff serovar Typhimurium (ATCC 13311). The broth microdilution method in 96-well microtiter plates was used.[40,41] The bacterial suspensions were standardized with sterile saline solution (0.85%) until the concentration of 1.0×10^5 CFU mL^{-1}. The prepared inoculums were stored at 4 °C until their utilization. The extract was dissolved in DMSO solution (50 mL L^{-1}) containing 1 mg mL^{-1} polysorbate-80 and added to the wells containing 100 μL of LB (Luria Bertani) cultivation medium according to Miller.[42] Next, the bacterial suspensions at 1.0×10^5 CFU mL^{-1} were added until reaching the desired concentrations. The mixtures were deposited in microplates and incubated in a rotary agitator (160 rpm) for 24 h at 37 °C. The lowest concentrations of the extract that were able to completely inhibit bacterial growth, without visible growth in an optical microscope, were defined as the minimum inhibitory concentrations (MIC). The minimum bactericidal concentration (MBC) was determined by serial sub cultivation in microtiter plates containing 100 μL of LB broth[41] per well and incubated for 24 h. The lowest concentration without bacterial growth was defined as MBC, indicating 99.5% death of the original inoculum. The optical density of each well was measured at a wavelength of 655 nm using Microplate Manager 4.0 (Bio-Rad Laboratories) and compared to the blank and the positive control. The antibiotics streptomycin (Sigma P 7794) and ampicillin (Panfarma) (1 mg mL^{-1} in sterile saline solution) and the food additives sodium sulfite (E221) and potassium metabisulfite (E224)[43] from 0.001 to 3.5 mg mL^{-1} were used as positive controls, and DMSO solution (50 mL L^{-1}) was used as the negative control.

2.4.4 Antifungal activity. The antifungal activity of the basidiocarp extract was evaluated in eight fungi: *Aspergillus fumigatus* Fresenius (ATCC 1022), *Aspergillus niger* van Tieghem (ATCC 6275), *Aspergillus ochraceus* Batista et Maia (ATCC 12066), *Aspergillus versicolor* (Vuillemin) Tiraboschi (ATCC 11730), *Penicillium funiculosum* Thom (ATCC 8725), *Penicillium ochrochloron* Biourge (ATCC 90288), *Penicillium verrucosum* var. *cyclopium* (Westling) Samson, Stolk & Hadlok (food isolate), and *Trichoderma viride* Pers. (IAM 5061). The microorganisms were kept in 2% malt agar at 4 °C.[44] The modified microdilution technique using 96-well microtiter plates was used to assess the antifungal activity of the basidiocarp extract.[40,41] The fungal spores were washed from the plate surface with a sterile 0.85% saline solution containing 1 mg mL^{-1} polysorbate-80. The spore suspension was adjusted with saline solution (0.85%) until approximately 1.0×10^5 conidia per mL for the final volume of 100 μL per well and stored at 4 °C until use. The diluted inoculums were cultivated in 2% malt extract agar to prove the absence of contamination. The basidiocarp extract was diluted in DMSO solution (50 mL L^{-1}) containing 1 mg mL^{-1} polysorbate-80. Each solution with the diluted extract was added to 2% malt extract medium containing inoculum. The microplates were incubated in a rotary agitator (160 rpm) for 72 h at 28 °C. The wells with the lowest concentrations of visible extract without growth, but visible under an optical microscope, were defined as the minimum inhibitory concentration (MIC) of the extract. The minimum fungicidal concentration (MFC) was determined by a serial sub cultivation of 2 μL of evaluated fungal cultures which were dissolved in culture medium and inoculated in microtiter plates containing 100 μL of 2% malt extract broth per well for 72 h at 28 °C. The lowest concentration without visible growth under an optical microscope was defined as the MFC, indicating 99.5% of the original inoculum death. The commercial fungicides bifonazole (Srbolek) and ketoconazole (Zorkapharma, Šabac, Serbia) and the food additives sodium sulfite (E221) and potassium metabisulfite (E224)[43] from 0.001 to 3.5 mg mL^{-1} were used as positive controls, and DMSO solution (50 mL L^{-1}) was used as the negative control.

2.5 Experimental design and statistical analysis

For chemical, cytotoxic, and antimicrobial analyses, three randomly chosen samples were used, and all assays were carried out in triplicate. The results were expressed as arithmetical average ± standard deviation (SD) and an analysis of variance (ANOVA) was applied, and the typical requirements, homoscedasticity by Levene's test and normal distribution by Shapiro Wilk's test, were preliminarily performed. Welch test was applied to verify the existence of statistically significant differences. The ANOVA results were classified using Tukey HSD (honestly significant difference) test or Tamhane's T2, when homoscedasticity was verified, respectively. All statistical tests were performed at a 5% significance level using IBM SPSS Statistics for Windows, version 25 (IBM Corporation, New York, USA).

3. Results

Regarding the proximate composition, carbohydrates were the most abundant macronutrient compound present in *L. crinitus* basidiocarp (82.4 g per 100 g), followed by proteins (13.0 g per 100 g). Ashes and total fat were part of the minority compounds, obtaining an energy value of 385.4 kcal per 100 g (Table 1).

Regarding the hydrophilic compounds found in the basidiocarp, the main soluble sugar was trehalose (80%), followed

Table 1 Macronutrient composition and energetic value of dried *Lentinus crinitus* basidiocarp

Nutrient/energy	Value
Ash (g per 100 g)	4.2 ± 0.2
Proteins (g per 100 g)	13.0 ± 0.7
Fat (g per 100 g)	0.48 ± 0.03
Carbohydrates (g per 100 g)	82.4 ± 0.4
Energy (kcal per 100 g)	385.4 ± 0.8

Values expressed as arithmetic mean ± standard deviation (dry basis; $n = 3$).

Table 2 Chemical composition of the hydrophilic extract (sugars, organic acids, and phenolic acids) of *Lentinus crinitus* basidiocarp

Compound	Value
Sugar (g per 100 g)	
Mannitol	0.18 ± 0.01
Sucrose	0.73 ± 0.05
Trehalose	3.5 ± 0.2
Total soluble sugars	4.4 ± 0.3
Organic acids (mg per 100 g)	
Oxalic acid	132 ± 5
Malic acid	730 ± 4
Fumaric acid	0.035 ± 0.001
Total organic acids	863 ± 1
Phenolic acids (μg per 100 g)	
p-Hydroxybenzoic acid	724 ± 12
Cinnamic acid	105.52 ± 0.01
Total phenolic acids	724 ± 12

Values expressed as arithmetic mean ± standard deviation (dry basis; $n = 3$).

Table 3 Chemical composition of the lipophilic extract (fatty acids and tocopherols) of *Lentinus crinitus* basidiocarp

Compound	Value (%)
Caproic acid (C6:0)	0.116 ± 0.005
Caprylic acid (C8:0)	0.143 ± 0.001
Capric acid (C10:0)	0.205 ± 0.004
Lauric acid (C12:0)	0.39 ± 0.01
Myristic acid (C14:0)	1.05 ± 0.03
Pentadecanoic acid (C15:0)	1.27 ± 0.01
Palmitic acid (C16:0)	11.23 ± 0.03
Palmitoleic acid (C16:1)	0.32 ± 0.02
Heptadecanoic acid (C17:0)	0.65 ± 0.05
Stearic acid (C18:0)	2.38 ± 0.02
Oleic acid ($C18:1_{n\text{-}9}$)	9.8 ± 0.1
Linoleic acid ($C18:2_{n\text{-}6}$)	66.4 ± 0.3
α-Linolenic acid ($C18:3_{n\text{-}3}$)	1.80 ± 0.01
Arachidic acid (C20:0)	0.253 ± 0.004
Eicosenoic acid (C20:1)	0.072 ± 0.001
cis-11,14-Eicosadienoic acid (C20:2)	0.46 ± 0.01
cis-11,14,17-Eicosatrienoic acid and heneicosanoic acid ($C20:3_{n\text{-}3}$ + C21:0)	0.55 ± 0.02
Behenic acid (C22:0)	2.3 ± 0.2
Erucic acid ($C22:1_{n\text{-}9}$)	0.082 ± 0.005
Lignoceric acid (C24:0)	0.52 ± 0.01
Total saturated fatty acids (% of total fatty acids)	20.5 ± 0.2
Total monounsaturated fatty acids (% of total fatty acids)	10.29 ± 0.09
Total polyunsaturated fatty acids (% of total fatty acids)	69.2 ± 0.3
Tocopherol	
β-Tocopherol (μg per 100 g)	505 ± 32

Values expressed as arithmetic mean ± standard deviation (dry basis; $n = 3$); *cis*-11,14,17-eicosatrienoic and heneicosanoic acids had a single combined value because the chromatographic system does not allow the separation of these compounds.

by sucrose (16%) and mannitol (4%) (Table 2). The main organic acids were malic acid (84%), followed by oxalic acid (15%) and fumaric acid (<1%) (Table 2). The main phenolic acid found was *p*-hydroxybenzoic acid and a precursor of complex phenolic compounds, cinnamic acid (Table 2).

Regarding the lipophilic compounds of the basidiocarp, polyunsaturated fatty acids (PUFA) were the major ones over saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) (Table 3). Twenty fatty acids were identified in the lipid fraction of the basidiocarp and the major chemical compounds were linoleic (66.4%), palmitic (11.2%), and oleic (9.8%) acids (Table 3). Also, β-tocopherol was identified in the basidiocarp at 505 μg per 100 g (Table 3).

The basidiocarp extracts did not show cytotoxicity, since there was no growth inhibition of tumor and non-tumor cell cultures at the tested concentrations (GI_{50} values higher than 400 μg mL^{-1}; Table 4). These results indicate that *L. crinitus* is safe for human consumption although, as previously mentioned, it is mostly consumed by Amazonian Indians.[9,45]

The basidiocarp extract of *L. crinitus* presented bacteriostatic activity against all evaluated bacterial strains. The MIC values for the basidiocarp extract ranged from 0.12 to 0.499 mg mL^{-1}. For the positive controls, they ranged from 0.040 to 0.248 mg mL^{-1} for streptomycin and from 0.25 to 0.74 mg mL^{-1} for ampicillin. Regarding the commercial additives used, MIC values ranged from 1.00 to 3.97 mg mL^{-1} for E221 and from 0.505 to 2.01 mg mL^{-1} for E224 (Fig. 1). The basidiocarp extract MIC values were all equal to or lower than the food additive controls (Fig. 1). Only for *E. coli* the value obtained for the basidiocarp extract was equal to that of E224

Table 4 Cytotoxic activity of the methanolic extracts of *Lentinus crinitus* basidiocarp and positive control ellipticine against human tumor cell lines and non-tumor cells

Cell line	Basidiocarp (GI_{50} μg mL^{-1})	Ellipticine (GI_{50} μg mL^{-1})
Tumor cell		
HepG2	>400	3.2 ± 0.5
MCF7	>400	0.91 ± 0.04
NCI-H460	>400	1.42 ± 0.01
HeLa	>400	1.1 ± 0.2
Non-tumor cell		
PLP2	>400	2.06 ± 0.03

Values expressed as arithmetic mean ± standard deviation (dry basis; $n = 3$). GI_{50} = extract concentration corresponding to 50% growth inhibition activity. MCF-7 = breast adenocarcinoma, NCI-H460 = lung carcinoma, HeLa = cervical carcinoma, HepG2 = hepatocellular carcinoma, and PLP2 = primary cell culture obtained from porcine liver.

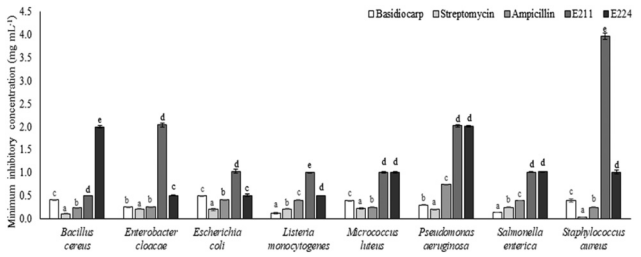

Fig. 1 Minimum inhibitory concentration (MIC) of *Lentinus crinitus* basidiocarp extract and positive controls streptomycin, ampicillin, sodium sulfite (E221), and potassium metabisulfite (E224). Different letters indicate significant differences among treatments and controls by Tukey's HSD (honestly significant difference) test ($p < 0.05$) (arithmetic mean ± standard deviation; $n = 3$).

control (Fig. 1). Regarding the ampicillin and streptomycin controls, the basidiocarp extract had similar or lower MIC values, except for *B. cereus*, *E. coli*, *M. luteus*, and *S. aureus* which showed higher values, and for *P. aeruginosa* and *E. cloacae* whose values were higher than that of streptomycin control (Fig. 1). These results suggest that the basidiocarp extract is a promising agent to control almost all the tested bacteria with higher activity than the controls.

Noticeably, compared to all controls, the basidiocarp extract was better against *L. monocytogenes*, *P. aeruginosa*, and *S. enterica*. Moreover, for these strains, the basidiocarp extract had an MIC varying from 2.7 to 4.0-fold lower than the ampicillin control, and an MIC varying from 2.5 to 10-fold lower than the food additive controls.

The basidiocarp extract of *L. crinitus* presented bactericide activity against all evaluated bacterial strains (Fig. 2). The most evident strain inhibition of the basidiocarp extract was against *E. coli*, *L. monocytogenes*, *M. luteus*, *P. aeruginosa*, *S. enterica*, and *S. aureus* with the lower MBC values compared to food additive controls (Fig. 2). The MBC values for the basidiocarp extract ranged from 0.248 to 0.604 mg mL^{-1}, and for the positive controls they ranged from 0.102 to 0.50 mg mL^{-1} for streptomycin and from 0.402 to 1.203 mg mL^{-1} for ampicillin. Regarding the food additives, the MBC values ranged from 0.497 to 4.04 mg mL^{-1} for E221, and from 0.502 to 2.02 mg mL^{-1} for E224 (Fig. 2). The basidiocarp extract MBC values were all equal to or lower than those of the food additive controls (Fig. 2). Only for *B. cereus* the basidiocarp extract revealed equal values for E221 control, and for *E. cloacae* higher values compared to those of E224 control (Fig. 2). The basidiocarp extract presented lower MBC values against *L. monocytogenes* and *S. enterica* (around 0.25 mg mL^{-1} for both), values 2.0 and 3.0-fold lower than that for the control ampicillin, respectively, and 4 to 8-fold lower than that for the food additive controls (Fig. 2). In addition, MBC values were close to the ones for the controls against *M. luteus* and *P. aeruginosa* (Fig. 2). Once again, the results obtained suggest that the basidiocarp extract is a promising agent for controlling most of the studied bacteria. Noticeably, when compared to all controls, the basidiocarp extract was better against *L. monocytogenes*, and *S. enterica* compared with all bacterial strains and controls (Fig. 2). The basidiocarp extract showed bactericidal activity against the other remaining bacteria, but with MBC values, in general, lower than those of the food additive controls and similar to those of the streptomycin and ampicillin controls (Fig. 2).

The basidiocarp extract was effective against all fungal strains showing MIC values ranging from 0.0399 to 0.499 mg mL^{-1}, and for the positive controls the MIC values ranged from 0.0999 to 0.199 mg mL^{-1} for bifonazole, and from 0.200 to 2.499 mg mL^{-1} for ketoconazole. Regarding food additives, MIC values ranged from 1.01 to 2.03 mg mL^{-1} for E211, and from 0.500 to 2.01 mg mL^{-1} for E224 (Fig. 3). The basidiocarp extract had lower MIC values for all fungal strains compared to food additive controls (Fig. 3). For the positive controls ampicillin and streptomycin, the basidiocarp extract presented MIC values that were equal to or lower than the ones for the fungal strains, except against *A. fumigatus* and *A. versicolor*, for which it had higher values (Fig. 3).

The MFC of the basidiocarp extract was effective against all fungal strains and ranged from 0.0601 to 0.6004 mg mL^{-1}, and for the positive controls it ranged from 0.198 to 0.2504 mg mL^{-1} for bifonazole, and from 0.302 to 3.49 mg mL^{-1} for ketoconazole. Regarding food additives, the MFC ranged from 2.01 to 4.04 mg mL^{-1} for E211, and from 0.502 to 2.02 mg mL^{-1} for

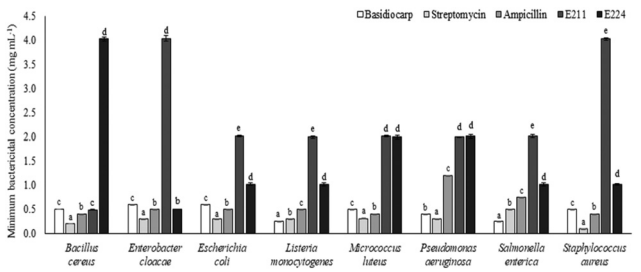

Fig. 2 Minimum bactericidal concentration (MBC) of *Lentinus crinitus* basidiocarp extract and positive controls streptomycin, ampicillin, sodium sulfite (E221), and potassium metabisulfite (E224). Different letters indicate significant differences among treatments and controls by Tukey's HSD (honestly significant difference) test ($p < 0.05$) (arithmetic mean ± standard deviation; $n = 3$).

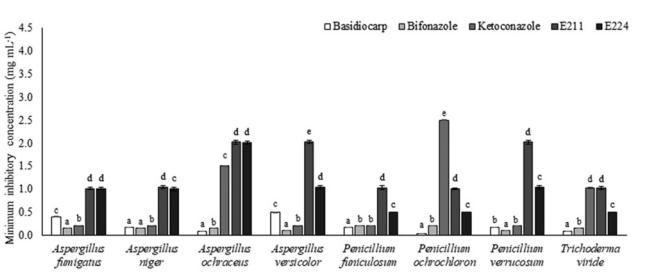

Fig. 3 Minimum inhibitory concentration (MIC) of *Lentinus crinitus* basidiocarp extract and positive controls bifonazole, ketoconazole, sodium sulfite (E221), and potassium metabisulfite (E224). Different letters indicate significant differences among treatments and controls by Tukey's HSD (honestly significant difference) test ($p < 0.05$) (arithmetic mean ± standard deviation; $n = 3$).

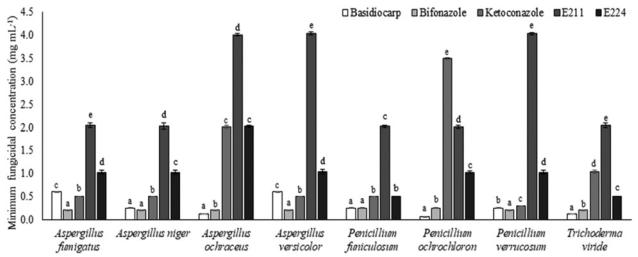

Fig. 4 Minimum fungicidal concentration (MFC) of *Lentinus crinitus* basidiocarp extract and positive controls bifonazole, ketoconazole, sodium sulfite (E221), and potassium metabisulfite (E224). Different letters indicate significant differences among treatments and controls by Tukey's HSD (honestly significant difference) test ($p < 0.05$) (arithmetic mean ± standard deviation; $n = 3$).

E224 (Fig. 4). The MFC values of the basidiocarp extract were all lower than those of the food additive controls. The basidiocarp extract presented lower MFC values against *A. ochraceus*, *P. ochrochlorum*, and *T. viride* when compared to all controls. For *A. niger* and *P. funiculosum*, the basidiocarp extract MFC values did not differ from that of bifonazole control and were only 2.0-fold lower than that of ketoconazole control. However, the values were lower for both fungal strains when compared to food additive controls, which indicates the high efficiency of the basidiocarp extract. For *A. versicolor* and *A. fumigatus*, MFC values of basidiocarp extract were higher than those of bifonazole and ketoconazole controls but still lower than those of E221 and E224 controls.

Overall, the basidiocarp extract presented bacteriostatic and bactericide activity against all the studied bacteria with better MIC and MBC values than most controls (Fig. 1 and 2), and also fungistatic and fungicide activity against all the fungi tested, with better MIC and MFC values than most controls (Fig. 3 and 4). Therefore, the *L. crinitus* basidiocarp extract has potential utilization for controlling important microorganisms in food and health.

4. Discussion

Edible mushrooms have good nutritional quality, especially proteins, fibers, and also bioactive compounds like polyphenols.[46,47] Our study showed that *L. crinitus* basidiocarp is a good source of carbohydrates and proteins, having a low-fat content, as reported for other mushrooms.[3,47,47] Thus, as all these studies suggest, mushrooms are a good option for inclusion in low-calorie diets.[49]

The protein content values of *L. crinitus* basidiocarp reported in previous studies was 9.8 g per 100 g (original value converted by 4.38 factor),[17] 10.1 g per 100 g (original value converted by 4.38 factor),[18] and 27.0 g per 100 g.[16] Our results showed that the protein content (13.0 g per 100 g) is similar to those found in the literature for this fungus. Chang and Miles[50] reported that edible mushrooms normally contain 19.0 to 35.0 g per 100 g protein (dry basis) and Kalač[3] reported that that of wild-growing mushrooms ranged from 13.2 to 62.8 g per 100 g. Therefore, we can conclude that the values obtained for the protein content of *L. crinitus* are slightly below the range reported in the literature for this type of food. However, compared to other foods, the protein levels of this mushroom are similar to the levels found in eggs (13.0 g per 100 g) and are even higher than those found in raw rice (7.3 g per 100 g) or wheat flour (9.8 g per 100 g).[51]

Trehalose is the main oligosaccharide in mushrooms ranging from 0.21 to 42.8 g per 100 g with the median from 3.8 to 4.0 g per 100 g for wild collected mushrooms,[3,52] and from 0.23 to 72.8 g per 100 g with a median of 2.63 g per 100 g for commercial cultivated mushrooms.[3] For *L. crinitus* basidiocarp, in our study, trehalose (3.5 g per 100 g) was the most abundant soluble sugar and it had a similar content compared to most basidiomycetes reported in the literature. Trehalose has effects on metabolic pathways such as modulating glucose homeostasis, improving beta cell function, reducing postprandial insulin release, and lipid profile.[53–55] In addition, trehalose migrates from mycelia to basidiocarps and protects the cells against stresses such as desiccation, temperature, and oxygen pressures.[56,57] This oligosaccharide may also be related to *L. crinitus* enzymatic activity[29] and mycelial biomass production, mainly at extreme temperatures (19 to 40 °C) and pH (2.5 to 10) conditions.[58]

Edible mushroom fat contents were reported from 1.1 to 8.3 g per 100 g.[50] For *L. crinitus* basidiocarp cultivated in a substrate based on *Theobroma grandiflorum* exocarp, the fat content ranged from 3.3 to 4.5 g per 100 g (ref. 16) and was 1.5 g per 100 g for wild *L. crinitus* basidiocarp collected in the Brazilian Cerrado.[17] In our study, the basidiocarp had a lower fat content (0.48 g per 100 g) compared with wild and cultivated *L. crinitus* basidiocarp or other mushrooms. Moreover, the *L. crinitus* basidiocarp from our study had the lowest value of total monounsaturated fatty acids (10.3% of total fatty acids) and the highest value of total polyunsaturated fatty acids (69.2% of total fatty acids), the major compound being linoleic acid ($C18:2_{n-6}$) followed by oleic acid ($C18:1_{n-9}$) and α-linolenic acid ($C18:3_{n-3}$). Oleic acid (omega-9) may reduce the risk of coronary artery diseases.[59] Linoleic acid (omega-6), considered an essential fatty acid that cannot be synthesized by the human body, is a precursor of arachidonic acid which plays an important role in inflammatory processes,[60] and has a significant role in health because it lowers cholesterol and triglycerides.[61] α-Linolenic acid is also an essential fatty acid which showed physiological benefits in humans such as brain development, anti-inflammatory activity, and cardiovascular health improvement.[62–65]

Tocopherols are known natural antioxidant compounds[66] with anti-inflammatory and anticancer properties with peroxidation prevention in cellular membrane phospholipids.[67] In our study, β-tocopherol (505 μg per 100 g) was the only vitamin identified. The majority of mushrooms (seven out of eight) studied by Barros *et al.*[49] had lower levels of β-tocopherol (3 to 193 μg per 100 g) than *L. crinitus*; only one (*Boletus edulis*) had a higher β-tocopherol content (890 μg per 100 g). Toledo

et al.[52] reported that among nine wild mushrooms, four had 12.36 to 59.03 μg per 100 g β-tocopherol but it was not detectable in five mushroom species. Wild mushrooms (fourteen out of eighteen) studied by Heleno *et al.*[37] had lower levels of β-tocopherol (7 to 386 μg per 100 g), only one (*Laccaria laccata*) showed higher levels (706 μg per 100 g) than *L. crinitus*, but it was not detected in three species. This suggests that *L. crinitus* basidiocarp has high β-tocopherol levels compared with the average values reported in the literature.

For hydrophilic compounds, malic acid has been reported with values of 130 mg per 100 g for *Agaricus bisporus* and is absent for *Agaricus brasiliensis.*[48] Oxalic acid, another hydrophilic compound, has been reported with values of 580 mg per 100 g for *A. bisporus*[48] and 115 mg per 100 g for *A. brasiliensis.*[68] This indicates that the values for malic (730 mg per 100 g) and oxalic (132 mg per 100 g) acids of our study are among the highest reported levels. These organic acids have a considerable effect on the taste and acceptability of mushrooms and can play a biological role in health due to their potential antioxidant activity.[68] In addition, oxalic acid has antibacterial activity.[69]

Phenolic compounds are one of the most important groups of secondary metabolites found in mushrooms[66] that exhibit antioxidant capacity and provide protection against disorders with anticancer, cardioprotective, anti-inflammatory, antithrombotic, and vasodilator activities.[66,70–72] The main phenolic acid found in our study was *p*-hydroxybenzoic acid (724 μg per 100 g). Other studies reported values of 10, 156, and 157 μg per 100 g for commercial mushrooms such as *Pleurotus eryngii*, *Pleurotus ostreatus*, and *Lentinula edodes*,[73] respectively, and from 469 to 2407 μg per 100 g (mean of 1304 μg per 100 g) for eight edible commercial mushrooms,[46] and from 0 μg to 23 870 μg per 100 g (mean of 3358 μg per 100 g) for wild mushrooms,[74] but it was not found for *A. bisporus* commercial mushroom.[73] This indicates that the *p*-hydroxybenzoic acid value found in *L. crinitus* basidiocarp in our study had an intermediary value among the ones reported in the literature. Since the bioactivities (*e.g.* antioxidant and antimicrobial activity) of organic acids and specifically phenolic compounds are related to the response to stress conditions (abiotic, edaphoclimatic, or production conditions),[75] this may justify the greater or lesser differences among values for different species, as well as the absence of any compound.

The antimicrobial activity of the *L. crinitus* basidiocarp extract was verified against all the studied bacterial and fungal strains, showing a broad action spectrum. The antibacterial activity of *P. ostreatus* and *L. edodes* basidiocarp extracts has been reported against *S. aureus*, *B. subtilis*, *E. coli*, *P. aeruginosa*, and *S. enterica* with MIC values varying from 2.0 to 8.0 mg mL^{-1}.[76] Methanolic extracts of *A. brasiliensis* and *A. bisporus* were tested against the same bacterial strains used in our study, revealing MIC values ranging from 0.1 to 2.30 mg mL^{-1} and 0.3 to 1.15 mg mL^{-1}, respectively, and MBC values ranging from 0.3 to 4.60 mg mL^{-1} and from 0.3 to 4.70 mg mL^{-1}, respectively.[48] *P. ostreatus*, *L. edodes*, and *A. bisporus* were tested against *E. coli* and *P. aeruginosa* showing MIC values higher than 20 mg mL^{-1};[77] and *L. edodes* was tested against *B. cereus*, *S. aureus*, and *E. coli* revealing MIC values ranging from 1.56 to 100 mg mL^{-1}.[78] Our results for the *L. crinitus* basidiocarp extract presented MIC values ranging from 13 to 200-fold higher activity than the values reported in the literature for the same bacteria with bacteriostatic activity. In addition, this extract presented MBC values ranging from 2.0 and 9.0-fold higher activity than the values reported in the literature for the same bacteria with bactericidal activity. On the other hand, for the *A. brasiliensis* ethanolic extract, the most sensitive bacteria were *S. aureus* and *B. cereus* with MIC values of 0.08 and 0.04 mg mL^{-1} and MBC of 0.20 and 0.08 mg mL^{-1}, respectively,[48] showing higher activity than those in our studies (MIC 0.40 and MBC 0.50 mg mL^{-1}) for both species, respectively. However, in our study *L. crinitus* showed antibacterial activity with MIC and MBC values, respectively, ranging from 4.2 to 9.5 and 2.0 to 6.7-fold lower than those of the food additive controls and similar to some antibiotic controls, and the mushroom extract has stronger bacteriostatic and bactericidal activities than streptomycin and ampicillin against *L. monocytogenes* and *S. enterica*.

The antifungal activity of *P. ostreatus* basidiocarp extracts expressed as the MIC value has been reported as 12 mg mL^{-1} against *A. niger* and 16 mg mL^{-1} against *P. funiculosum*, but no activity was observed against *A. fumigatus* and *T. viride.*[79] The extract of *A. brasiliensis* basidiocarp presented an MIC of 0.15 mg mL^{-1} and MFC of 1.15 mg mL^{-1} against *A. versicolor*,[48] and the 1 mg mL^{-1} hydroalcoholic extract of *L. crinitus* basidiocarp presented 50% mycelial growth inhibition of *Fusarium* sp. conidia.[19] *Ganoderma lucidum* against the same fungal strains used in our study revealed MIC values from 0.005 to 1.5 mg mL^{-1} and MFC from 0.1 to 4.5 mg mL^{-1}.[80] The basidiocarp extract of *L. crinitus*, in our study, had MIC values 3.7 to 8.8-fold lower than that of *G. lucidum* extract for the same fungal strains,[80] and for *A. versicolor*, *T. viride*, and *P. funiculosum* had 15 and 19-fold lower values of MIC and MFC than those for *L. crinitus*. Moreover, *L. crinitus* had, respectively, MIC and MFC values 2.5 to 25.2- and 3.4 to 33.6-fold lower than the ones of food additive and antibiotic controls evaluated in our study. In addition, *L. crinitus* was more effective than bifonazole and ketoconazole against *A. ochraceus*, *P. ochrochloron*, and *T. viride*. Thus, our results make evident the antifungal potential of *L. crinitus*, which presents a broad action spectrum against infectious fungi and mainly foodborne diseases related to fungi in food.

The antimicrobial activity of basidiomycetes may be related to several chemical compounds such as terpenes, organic acids, phenolic compounds, and steroids among others.[8,80–83] The acids *p*-hydroxybenzoic and cinnamic had MIC values from 0.003 to 0.12 mg mL^{-1} and from 0.007 to 0.03 mg mL^{-1}, respectively, against *A. fumigatus*, *A. versicolor*, *A. ochraceus*, *A. niger*, *T. viride*, *P. funiculosum*, *P. ochrochloron*, and *P. verrucosum.*[80] In addition, *p*-hydroxybenzoic acid has been indicated as an antimicrobial agent against bacteria and fungi.[84] Other compounds such as malic and oxalic acids are food safety additives[85] and have been reported as having antimicrobial activity. Malic acid was reported with antimicrobial activity against *E. coli*, *L. monocytogenes*,[86,87] *Salmonella enteri-*

tidis,[86] and *Salmonella gaminara.*[87] Oxalic acid was reported with antimicrobial activity against *E. coli.*[88] In our study, *L. crinitus* showed several major bioactive compounds such as *p*-hydroxybenzoic, cinnamic, β-tocopherol, linoleic, malic, and oxalic acids that may still be acting synergistically toward antimicrobial activity. The antimicrobial activity might be related to the ability of linoleic acid to break bacterial cell membranes;[89] the phenolic acid compounds to destabilize the bacterium cytoplasmic membrane, altering the plasma permeability and inhibiting extracellular microbial enzymes;[90] and the non-dissociated form of organic acid penetration into cells ensuing cytoplasm acidification.[91]

Our study suggests that *L. crinitus* basidiocarp – a mushroom mainly consumed by Amazonian ethnic groups[9] – presents nutritional and nutraceutical compounds, high antimicrobial activity, and no cytotoxicity, which makes it a potential candidate for mushroom industrial production.

5. Conclusions

L. crinitus basidiocarp has considerably high levels of carbohydrates, energy, soluble sugars, β-tocopherol, phenolic acids (mainly *p*-hydroxybenzoic acid), and organic acids (mainly malic and oxalic acids). The predominant lipophilic compounds are polyunsaturated fatty acids, mainly linoleic (66.4%), palmitic (11.2%), and oleic (9.8%) acids. The basidiocarp extract has no cytotoxic activity against human tumor cell lines HeLa, HepG2, MCF-7, and NCI-H460, as well as against PLP2 non-tumor cells. The basidiocarp extract has bacteriostatic, bactericidal, fungistatic, and fungicidal activities against all tested microorganisms with MBC and MFC values similar or in several cases significantly lower to those found in commercial antibiotics, and much lower than those found in commercial food additives. Most bactericidal activity is against *L. monocytogenes* and *S. enterica* while the fungicidal activity is mostly against *P. ochrochloron*, followed by *A. ochraceus* and *T. viride.* This study promotes the consumption of *L. crinitus* basidiocarp that is without toxicity. Its nutritional and bioactive potential has been established and compared with other edible and medicinal mushrooms. Therefore, its consumption, which is mostly made by indigenous populations, can be extended. Moreover, the basidiocarp extract of *L. crinitus* and its compounds emerge as a potential alternative to the available antimicrobials, having a broad action spectrum. However, the antimicrobial mechanism of action of *L. crinitus* extract needs to be elucidated along with the compounds responsible for the antimicrobial activity against the tested microorganisms. These results contribute to the development of new studies in which this species may be at the base of the development of new antimicrobial formulations for application in the food, pharmaceutical, and/or agriculture industries.

Conflicts of interest

There are no conflicts of interest to declare.

Acknowledgements

The authors thank Paranaense University, Fundação Araucária, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brazil (CAPES) -finance code 001-, and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the financial support and the fellowship. The authors also thank the Foundation for Science and Technology (FCT, Portugal) for financial support through national funds FCT/MCTES to CIMO (UIDB/00690/2020); national funding by FCT, PI, through the institutional scientific employment program-contract for L. Barros's contract, to the project Valor Natural for the contract of F. Reis (Mobilized Project Norte-01-0247-FEDER-024479), and the Ministry of Education, Science and Technological Development of Republic of Serbia (451-03-68/2020-14/200007).

References

1 P. M. Kirk, P. Cannon, D. Minter and J. Stalpers, *Ainsworth & Bisby's dictionary of the fungi*, CAB International, Wallingford, UK, 10th edn, 2008.
2 G. Gebreyohannes, A. Nyerere, C. Bii and D. B. Sbhatu, Determination of antimicrobial activity of extracts of indigenous wild mushrooms against pathogenic organisms, *J. Evid. Based Complementary Altern. Med.*, 2019, **2019**, 1–7.
3 P. A. Kalač, Review of chemical composition and nutritional value of wild-growing and cultivated mushrooms, *J. Sci. Food Agric.*, 2013, **93**(2), 209–218, DOI: 10.1002/jsfa.5960.
4 J. J. Zhang, Y. Li, T. Zhou, D. P. Xu, P. Zhang, S. Li and H. B. Li, Bioactivities and health benefits of mushrooms mainly from China, *Molecules*, 2016, **21**(7), 1–16, DOI: 10.3390/molecules21070938.
5 S. P. Wasser, Medicinal mushroom science: current perspectives, advances, evidences, and challenges, *Biomed. J.*, 2014, **37**(6), 345–356, DOI: 10.4103/2319-4170.138318.
6 M. B. D. Bertéli, S. H. Umeo, A. Bertéli, J. S. do Valle, G. A. Linde and N. B. Colauto, Mycelial antineoplastic activity of *Agaricus blazei*, *World J. Microbiol. Biotechnol.*, 2014, **30**(8), 2307–2313, DOI: 10.1007/s11274-014-1656-6.
7 G. T. Silva and T. B. Gibertoni, Aphyllophorales (Basidiomycota) em áreas urbanas da região metropolitana do Recife, PE, Brasil, *Hoehnea*, 2006, **33**(4), 533–543.
8 W. R. Abraham and D. Abate, Antimicrobial metabolites from *Lentinus crinitus*, *J. Antibiot.*, 1994, **47**(11), 1348–1350, DOI: 10.7164/antibiotics.47.1348.
9 R. Vargas-Isla, N. K. Ishikawa and V. Py-Daniel, Contribuições etnomicológicas dos povos indígenas da Amazônia, *Biota Amazônia*, 2013, **3**, 58–65, DOI: 10.18561/2179-5746/biotaamazonia.v3n1p58-65.
10 S. H. Umeo, G. P. N. Souza, P. M. Rapachi, D. M. Garcia, L. D. Paccola-Meirelles, J. S. Valle, N. B. Colauto and G. A. Linde, Screening of basidiomycetes in submerged cul-

tivation based on antioxidant activity, *Genet. Mol. Res.*, 2015, **14**(3), 9907–9914, DOI: 10.4238/2015.August.19.25.

11 M. G. I. Faria, J. S. Valle, A. D. Lopes, A. C. Gonçalves, D. C. Dragunski, N. B. Colauto and G. A. Linde, Bioaccumulation of lithium (Li_2CO_3) in mycelia of the culinary-medicinal oyster mushroom, *Pleurotus ostreatus* (Agaricomycetes), *Int. J. Med. Mushrooms*, 2018, **20**(9), 901–907, DOI: 10.1615/IntJMedMushrooms.2018027343.

12 M. G. I. Faria, K. V. Avelino, J. S. Valle, G. J. Silva, A. C. Gonçalves, D. C. Dragunski, N. B. Colauto and G. A. Linde, Lithium bioaccumulation in Lentinus crinitus mycelial biomass as a potential functional food, *Chemosphere*, 2019, **235**, 538–542.

13 M. F. Tavares, K. V. Avelino, N. L. Araújo, R. A. Marim, G. A. Linde, N. B. Colauto and J. S. Valle, Decolorization of azo and anthraquinone dyes by crude laccase produced by *Lentinus crinitus* in solid state cultivation, *Braz. J. Microbiol.*, 2020, **51**(1), 99–106, DOI: 10.1007/s42770-019-00189-w.

14 E. Caroline, D. M. Brito, I. Rodrigo, S. Braga, M. Francisca, S. Teixeira, I. I. Salomão and R. Martim, Production and partial characterization of aspartic proteases synthesized by *Lentinus crinitus* (L.) Fr. 1825 DPUA 1693 (Polyporaceae), *Bol. Mus. Para. Emílio Goeldi. Cienc. Nat.*, 2019, **1693**, 463–472.

15 G. Cambri, M. M. L. De Sousa, D. D. M. Fonseca, F. Marchini, J. L. M. Da Silveira and J. Paba, Analysis of the biotechnological potential of a *Lentinus crinitus* isolate in the light of its secretome, *J. Proteome Res.*, 2016, **15**(12), 4557–4568.

16 A. R. G. Machado, M. F. S. Teixeira, L. de Souza Kirsch, M. da C. L. Campelo and I. M. de Aguiar Oliveira, Nutritional value and proteases of *Lentinus citrinus* produced by solid state fermentation of lignocellulosic waste from tropical region, *Saudi J. Biol. Sci.*, 2016, **23**(5), 621–627, DOI: 10.1016/j.sjbs.2015.07.002.

17 C. M. Silva Neto, D. S. Pinto, L. A. C. Santos and F. J. S. Calaça, Bromatological aspects of *Lentinus crinitus* mushroom (Basidiomycota: Polyporaceae) in agroforestry in the cerrado, *Food Sci. Technol.*, 2019, **2061**, 1–6, DOI: 10.1590/fst.14719.

18 G. L. R. Dávila, A. W. Murillo, F. C. J. Zambrano, M. H. Suárez and A. J. J. Méndez, Evaluation of nutritional values of wild mushrooms and spent substrate of *Lentinus crinitus* (L.) Fr., *Heliyon*, 2020, **6**(3), e03502, DOI: 10.1016/j.heliyon.2020.e03502.

19 A. Figueiredo and A. C. Silva, Atividade "in vitro" de extratos de *Pycnoporus sanguineus* e *Lentinus crinitus* sobre o fitopatógeno *Fusarium* sp., *Acta Amazonica*, 2014, **44**(1), 1–8, DOI: 10.1590/S0044-59672014000100001.

20 T. Bintsis, Foodborne pathogens, *AIMS Microbiol.*, 2017, **3**(3), 529–563, DOI: 10.3934/microbiol.2017.3.529.

21 A. M. Pisoschi, A. Pop, C. Georgescu, V. Turcuș, N. K. Olah and E. Mathe, An Overview of natural antimicrobials role in food, *Eur. J. Med. Chem.*, 2018, **143**, 922–935, DOI: 10.1016/j.ejmech.2017.11.095.

22 WHO – WHO estimates of the global burden of foodborne diseases: foodborne disease burden epidemiology reference group 2007–2015. https://apps.who.int/iris/bitstream/handle/10665/199350/9789241565165_eng.pdf?sequence=1, accessed 19 march 2020.

23 Z. Pang, R. Raudonis, B. R. Glick, T. J. Lin and Z. Cheng, Antibiotic resistance in *Pseudomonas aeruginosa*: mechanisms and alternative therapeutic strategies, *Biotechnol. Adv.*, 2019, **37**(1), 177–192, DOI: 10.1016/j.biotechadv.2018.11.013. Epub 2018 Nov 27. PMID: 30500353.

24 A. N. Olaimat, M. A. Al-Holy, H. M. Shahbaz, A. A. Al-Nabulsi, M. H. A. Ghoush, T. M. Osaili, M. M. Ayyash and R. A. Holley, Emergence of antibiotic resistance in *Listeria monocytogenes* isolated from food products: a comprehensive review, *Compr. Rev. Food Sci. Food Saf.*, 2018, **17**, 1277–1292.

25 T. F. El-Arabi and M. W. Griffiths, Bacillus cereus, in *Foodborne Infections and Intoxications*, ed. G. J. Morris and P. M. London, Academic Press, 4th edn, 2013.

26 T. Sultana, J. Rana, S. R. Chakraborty, K. K. Das, T. Rahman and R. Noor, Microbiological analysis of common preservatives used in food items and demonstration of their *in vitro* anti-bacterial activity, *Asian Pac. J. Trop. Dis.*, 2014, **4**(6), 452–456, DOI: 10.1016/S2222-1808(14)60605-8.

27 R. Gyawali and S. A. Ibrahim, Natural products as antimicrobial agents, *Food Control*, 2014, **46**, 412–429.

28 H. S. Tanaka, M. B. D. Bertéli, F. A. Cordeiro, A. D. Lopes, J. S. Valle, G. A. Linde and N. B. Colauto, Semisolid culture medium improves mycelial recovery of *Agaricus subrufescens* cryopreserved in cereal grains, *Braz. J. Microbiol.*, 2019, **50**(2), 527–532, DOI: 10.1007/s42770-019-00063-9.

29 R. A. Marim, K. V. Avelino, G. A. Linde, N. B. Colauto and J. S. Valle, *Lentinus crinitus* strains respond differently to cultivation pH and temperature, *Genet. Mol. Res.*, 2018, **17**(1), gmr16039885, DOI: 10.4238/gmr16039885.

30 H. S. Tanaka, T. R. D'A. Mantovani, M. P. Santos, G. A. Linde and N. B. Colauto, Cereal grains and glycerol in *Agaricus blazei* cryopreservation, *Biosci. J.*, 2013, **29**(3), 627–633.

31 AOAC – Association of Official Analytical Chemists, *Official Methods of Analysis of AOAC International*, AOAC INTERNATIONAL, Gaithersburg, 20th edn, 2016.

32 The European Parliament and Council, (2011). Regulation (EC) No. 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the Provision of Food Information to Consumers. Official Journal of the European Union, 304, 22.11.2011: L304/18-L304/63.

33 S. Petropoulos, Â. Fernandes, C. Pereira, N. Tzortzakis, J. Vaz, M. Soković, L. Barros and I. C. F. R. Ferreira, Bioactivities, chemical composition and nutritional value of *Cynara cardunculus* L. seeds, *Food Chem.*, 2019, **289**, 404–412, DOI: 10.1016/j.foodchem.2019.03.066.

34 L. Barros, C. Pereira and I. C. F. R. Ferreira, Optimized analysis of organic acids in edible mushrooms from Portugal

by ultra fast liquid chromatography and photodiode array detection, *Food Anal. Methods*, 2013, **6**(1), 309–316.

35 B. Melgar, M. I. Dias, A. Ciric, M. Sokovic, E. M. Garcia-Castello, A. D. Rodriguez-Lopez, L. Barros and I. C. R. F. Ferreira, Bioactive characterization of *Persea americana* mill. by-products: a rich source of inherent antioxidants, *Ind. Crops Prod.*, 2018, **111**, 212–218.

36 F. S. Reis, L. Barros, A. Martins and I. C. F. R. Ferreira, Chemical composition and nutritional value of the most widely appreciated cultivated mushrooms: an inter-species comparative study, *Food Chem. Toxicol.*, 2012, **50**(2), 191–197.

37 S. A. Heleno, L. Barros, M. J. Sousa, A. Martins and I. C. F. R. Ferreira, Tocopherols composition of Portuguese wild mushrooms with antioxidant capacity, *Food Chem.*, 2010, **119**(4), 1443–1450.

38 F. S. Reis, J. C. M. Barreira, R. C. Calhelha, L. J. I. D. Griensven, A. Ćirić, J. Glamočlija, M. Soković and I. C. F. R. Ferreira, Chemical characterization of the medicinal mushroom *Phellinus linteus* (Berkeley & Curtis) Teng and contribution of different fractions to its bioactivity, *LWT – Food Sci. Technol.*, 2014, **58**(2), 478–485.

39 R. M. V. Abreu, I. C. F. R. Ferreira, R. C. Calhelha, R. T. Lima, M. H. Vasconcelos, F. Adega, R. Chaves and M. J. R. P. Queiroz, Anti-hepatocellular carcinoma activity using human HepG2 cells and hepatotoxicity of 6-substituted methyl 3-aminothieno[3,2-b]Pyridine-2- carboxylate derivatives: *in vitro* evaluation, cell cycle analysis and QSAR studies, *Eur. J. Med. Chem.*, 2011, **46**(12), 5800–5806.

40 H. Hänel and W. A. Raether, More sophisticated method of determining the fungicidal effect of water-insoluble preparations with a cell harvester, using miconazole as an example, *Mycoses*, 1988, **31**(3), 148–154.

41 A. Espinel-Ingroff, Comparison of the E-test with the NCCLS M38-P method for antifungal susceptibility testing of common and emerging pathogenic filamentous fungi, *J. Clin. Microbiol.*, 2001, **39**(4), 1360–1367.

42 J. H. Miller, *Experiments in molecular genetics*, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1972.

43 A. Garcia-Fuentes, S. Wirtz, E. Vos and H. Verhagen, Short review of sulphites as food additives, *Eur. J. Nutr. Food Saf.*, 2015, **5**(2), 113–120.

44 C. Booth, Fungal culture media, in *Methods in Microbiology*, ed. J. R. Norris and D. W. Ribbons, Academic Press, London and New York, 1971, pp. 49–94.

45 C. E. A. Coimbra and J. R. Welch, Enciclopédia dos Alimentos Yanomami (Sanöma): Cogumelos. Edited by R. M. Apiamö, J. Autuori, N. K. Ishikawa, M. S. Martins, N. Menolli Jr., C. Sanuma, L. R. Sanuma, M. Sanuma, O. I. Sanuma, & K. Tokimoto. 2016. Instituto Socioambiental, São Paulo. 108 pp., *Ethnobiol. Lett.*, 2018, **9**(2), 309–311.

46 I. Palacios, M. Lozano, C. Moro, M. D'Arrigo, M. A. Rostagno, J. A. Martínez, A. García-Lafuente, E. Guillamón and A. Villares, Antioxidant properties of phenolic compounds occurring in edible mushrooms, *Food Chem.*, 2011, **128**(3), 674–678.

47 F. S. Reis, D. Stojković, M. Soković, J. Glamočlija, A. Ćirić, L. Barros and I. C. F. R. Ferreira, Chemical characterization of *Agaricus bohusii*, antioxidant potential and antifungal preserving properties when incorporated in cream cheese, *Food Res. Int.*, 2012, **48**(2), 620–626.

48 D. Stojković, F. S. Reis, J. Glamočlija, A. Ćirić, L. Barros, L. J. L. D. Griensven, I. C. F. R. Ferreira and M. Soković, Cultivated strains of *Agaricus bisporus* and *A. brasiliensis*: chemical characterization and evaluation of antioxidant and antimicrobial properties for the final healthy product-natural preservatives in yoghurt, *Food Funct.*, 2014, **5**(7), 1602–1612.

49 L. Barros, T. Cruz, P. Baptista, L. M. Estevinho and I. C. F. R. Ferreira, Wild and commercial mushrooms as source of nutrients and nutraceuticals, *Food Chem. Toxicol.*, 2008, **46**(8), 2742–2747.

50 S. T. Chang and P. G. Miles, *Mushrooms: cultivation, nutritional value, medicinal effect, and environmental impact*, CRC, Boca Raton, 2th edn, 2004.

51 TACO – Brazilian Food Composition Table, 11, Nepa Unicamp, São Paulo, Brazil, 2011. https://www.cfn.org.br/wpcontent/uploads/2017/03/taco_4_edicao_ampliada_e_revisada.pdf.

52 C. V. Toledo, C. Barroetaveñ, Â. Fernandes, L. Barros and I. C. F. R. Ferreira, Chemical and antioxidant properties of wild edible mushrooms from native *Nothofagus* spp. forest, Argentina, *Molecules*, 2016, **21**(9), 1–15.

53 C. Arai, N. Arai, A. Mizote, K. Kohno, K. Iwaki, T. Hanaya, S. Arai, S. Ushio and S. Fukuda, Trehalose prevents adipocyte hypertrophy and mitigates insulin resistance, *Nutr. Res.*, 2010, **30**(12), 840–848.

54 C. Yoshizane, A. Mizote, M. Yamada, N. Arai, S. Arai, K. Maruta, H. Mitsuzumi, T. Ariyasu, S. Ushio and S. Fukuda, Glycemic, insulinemic and incretin responses after oral trehalose ingestion in healthy subjects, *Nutr. J.*, 2017, **16**(1), 1–6.

55 H. Yaribeygi, A. Yaribeygi, T. Sathyapalan and A. Sahebkar, Molecular mechanisms of trehalose in modulating glucose homeostasis in diabetes, *Diabetes Metab. Syndr.: Clin. Res. Rev.*, 2019, **13**(3), 2214–2218.

56 J. H. Liu, X. D. Shang, J. Y. Liu and Q. Tan, Changes in trehalose content, enzyme activity and gene expression related to trehalose metabolism in *Flammulina velutipes* under heat shock, *Microbiology*, 2016, **162**(8), 1274–1285.

57 X. M. Liu, X. L. Wu, W. Gao, J. B. Qu, Q. Chen, C. Y. Huang and J. X. Zhang, Protective roles of trehalose in *Pleurotus pulmonarius* during heat stress response, *J. Integr. Agric.*, 2019, **18**(2), 428–437.

58 I. M. Colla, O. B. Q. Oliveira Filho, J. D. S. Freitas, M. B. D. Bertéli, G. A. Linde, J. S. Valle and N. B. Colauto, Mycelial biomass cultivation of *Lentinus crinitus*, *Biosci. J.*, 2020, **36**(6), 2238–2246, DOI: 10.14393/BJ-v36n6a2020-51183.

59 Y. M. Pacheco, S. López, B. Bermúdez, R. Abia, J. Villar and F. J. G. Muriana, A meal rich in oleic acid beneficially

modulates postprandial SICAM-1 and SVCAM-1 in normotensive and hypertensive hypertriglyceridemic subjects, *J. Nutr. Biochem.*, 2008, **19**(3), 200–205.

60 K. L. Fritsche, Too much linoleic acid promotes inflammation-doesn't it?, *Prostaglandins, Leukotrienes Essent. Fatty Acids*, 2008, **79**(3–5), 173–175.

61 J. Manjunathan and V. Kaviyarasan, Nutrient composition in wild and cultivated edible mushroom, *Lentinus tuberregium* (Fr.) Tamil Nadu., India, *Int. Food Res. J.*, 2011, **18**(2), 809–811.

62 U. N. Das, Essential fatty acids – a review, *Curr. Pharm. Biotechnol.*, 2006, **7**, 467–482.

63 A. Leaf, Omega-3 fatty acids and prevention of arrhythmias, *Curr. Opin. Lipidol.*, 2007, **18**, 31–34.

64 K. Kyu-Bong, A. N. Yoon, S. K. Hyung, A. W. Hayes and L. Byung-Mu, α-Linolenic acid: nutraceutical, pharmacological and toxicological evaluation, *Food Chem. Toxicol.*, 2014, **70**, 163–178.

65 N. Blondeau, R. H. Lipsky, M. Bourourou, M. W. Duncan, P. B. Gorelick and A. M. Marini, Alpha-linolenic acid: an omega-3 fatty acid with neuroprotective properties-ready for use in the stroke clinic?, *BioMed. Res. Int.*, 2015, 519830.

66 I. Ferreira, L. Barros and R. Abreu, Antioxidants in wild mushrooms, *Curr. Med. Chem.*, 2009, **16**(12), 1543–1560.

67 Q. Jiang, Natural forms of vitamin E: metabolism, antioxidant, and anti-inflammatory activities and their role in disease prevention and therapy, *Free Radical Biol. Med.*, 2014, **72**, 76–90.

68 A. E. S. S. Carvajal, E. A. Koehnlein, A. A. Soares, G. J. Eler, A. T. A. Nakashima, A. Bracht and R. M. Peralta, Bioactives of fruiting bodies and submerged culture mycelia of *Agaricus brasiliensis* (*A. blazei*) and their antioxidant properties, *LWT – Food Sci. Technol.*, 2012, **46**(2), 493–499.

69 A. M. Kwak, I. K. Lee, S. Y. Lee, B. S. Yun and H. W. Kang, Oxalic acid from *Lentinula edodes* culture filtrate: antimicrobial activity on phytopathogenic bacteria and qualitative and quantitative analyses, *Mycobiology*, 2016, **44**(4), 338–342.

70 C. Manach, A. Mazur and A. Scalbert, Polyphenols and prevention of cardiovascular diseases, *Curr. Opin. Lipidol.*, 2005, **16**(1), 77–84.

71 M. E. Valverde, T. Hernández-Pérez and O. Paredes-López, Edible mushrooms: improving human health and promoting quality life, *Int. J. Microbiol.*, 2015, **2015**, 376387, DOI: 10.1155/2015/376387.

72 B. Muszyńska, A. Grzywacz-Kisielewska, K. Kała and J. Gdula-Argasińska, Anti-inflammatory properties of edible mushrooms: a review, *Food Chem.*, 2018, **243**, 373–381.

73 F. S. Reis, A. Martins, L. Barros and I. C. F. R. Ferreira, Antioxidant properties and phenolic profile of the most widely appreciated cultivated mushrooms: a comparative study between *in vivo* and *in vitro* samples, *Food Chem. Toxicol.*, 2012, **50**(5), 1201–1207.

74 L. Barros, M. Dueñas, I. C. F. R. Ferreira, P. Baptista and C. Santos-Buelga, Phenolic acids determination by HPLC-DAD-ESI/MS in sixteen different Portuguese wild mushrooms species, *Food Chem. Toxicol.*, 2009, **47**(6), 1076–1079.

75 M. Naczk and F. Shahidi, Phenolics in cereals, fruits and vegetables: occurrence, extraction and analysis, *J. Pharm. Biomed. Anal.*, 2006, **41**, 1523–1542.

76 H. M. H. Chowdhury, K. Kubra and R. R. Ahmed, Screening of antimicrobial, antioxidant properties and bioactive compounds of some edible mushrooms cultivated in Bangladesh, *Ann. Clin. Microbiol. Antimicrob.*, 2015, **14**(1), 1–6.

77 O. Taofiq, S. A. Heleno, R. C. Calhelha, M. J. Alves, L. Barros, M. F. Barreiro, A. M. González-Paramás and I. C. F. R. Ferreira, Development of mushroom-based cosmeceutical formulations with anti-inflammatory, anti-tyrosinase, antioxidant, and antibacterial properties, *Molecules*, 2016, **21**(10), 1–12.

78 F. Bach, A. A. F. Zielisnk, C. V. Helm, G. M. Maciel, A. C. Pedro, A. P. Stafussa, S. Ávila and C. W. I. Haminiuk, Bio compounds of edible mushrooms: *in vitro* antioxidant and antimicrobial activities, *LWT – Food Sci. Technol.*, 2019, **107**, 214–220.

79 J. Cilerdzic, M. Stajic, J. Vukojevic, I. Milovanovic and N. Muzgonja, Antioxidant and antifungal potential of *Pleurotus ostreatus* and *Agrocybe cylindracea* basidiocarps and mycelia, *Curr. Pharm. Biotechnol.*, 2015, **16**(2), 179–186.

80 S. A. Heleno, I. C. F. R. Ferreira, A. P. Esteves, A. Ćirić, J. Glamočlija, A. Martins, M. Soković and M. J. R. P. Queiroz, Antimicrobial and demelanizing activity of *Ganoderma lucidum* extract, *p*-hydroxybenzoic and cinnamic acids and their synthetic acetylated glucuronide methyl esters, *Food Chem. Toxicol.*, 2013, **58**, 95–100.

81 M. Alves, I. C. F. R. Ferreira, J. Dias, V. Teixeira, A. Martins and M. Pintado, A Review on antimicrobial activity of mushroom (Basidiomycetes) extracts and isolated compounds, *Planta Med.*, 2012, **78**(16), 1707–1718.

82 A. M. Younis, F. S. Wu and H. H. El Shikh, Antimicrobial activity of extracts of the oyster culinary medicinal mushroom *Pleurotus ostreatus* (higher basidiomycetes) and identification of a new antimicrobial compound, *Int. J. Med. Mushrooms*, 2015, **17**(6), 579–590.

83 R. M. R. Dulay, L. A. Miranda, J. S. Malasaga, S. P. Kalaw, R. G. Reyes and C. T. Hou, Antioxidant and antibacterial activities of acetonitrile and hexane extracts of *Lentinus tigrinus* and *Pleurotus djamour*, *Biocatal. Agric. Biotechnol.*, 2017, **9**, 141–144.

84 R. Manuja, S. Sachdeva, A. Jain and J. A. Chaudhary, Comprehensive review on biological activities of *p*-hydroxy benzoic acid and its derivatives, *Int. J. Pharm. Sci. Rev. Res.*, 2013, **22**(2), 109–115.

85 P. Shukla, Food Additives from an organic chemistry perspective, *MOJ Bioorg. Org. Chem.*, 2017, **1**(3), 70–79.

86 R. M. Raybaudi-Massilia, J. Mosqueda-Melgar and O. Martín-Belloso, Antimicrobial activity of malic acid against *Listeria monocytogenes*, *Salmonella enteritidis* and *Escherichia coli*, O157:H7 in apple, pear and melon juices, *Food Control*, 2009, **20**(2), 105–112.

87 S. Eswaranandam, N. S. Hettiarachchy and M. G. Johnson, Antimicrobial activity of citric, lactic, malic, or tartaric acids and nisin-incorporated soy protein film against *Listeria monocytogenes*, *Escherichia coli*, O157:H7, and *Salmonella gaminara*, *J. Food Sci.*, 2004, **69**(3), 79–84.

88 V. K. Chaturvedi, S. Agarwal, K. K. Gupta, P. W. Ramteke and M. P. Singh, Medicinal mushroom: boon for therapeutic applications, *3 Biotech.*, 2018, **8**, 1–20.

89 J. Y. Lee, Y. S. Kim and D. H. Shin, Antimicrobial synergistic effect of linolenic acid and monoglyceride against *Bacillus cereus* and *Staphylococcus aureus*, *J. Agric. Food Chem.*, 2002, **50**, 2193–2199.

90 A. Borges, C. Ferreira, M. J. Saavedra and M. Simões, Antibacterial activity and mode of action of ferulic and gallic acids against pathogenic bacteria, *Microb. Drug Resist.*, 2013, **19**, 256–265.

91 N. Beales, Adaptation of microorganisms to cold temperatures, weak acid preservatives, low pH, and osmotic stress: a review, *Compr. Rev. Food Sci. Food Saf.*, 2004, 1–20.

