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ORIGINAL PAPER

Lentinus crinitus basidiocarp stipe and pileus: chemical composition, cytotoxicity and antioxidant activity

Míria B. D. Bertéli[1] · Olavo B. Q. Oliveira Filho[1] · Janyeli D. S. Freitas[1] · Wanessa C. Bortolucci[1] · Gustavo R. Silva[2] · Zilda C. Gazim[1] · Francislaine A. R. Lívero[2] · Evellyn C. W. Lovato[3] · Juliana S. Valle[1] · Giani A. Linde[1] · Lillian Barros[4] · Filipa S. Reis[4] · Isabel C. F. R. Ferreira[4] · Luzia D. Paccola-Meirelles[1] · Nelson B. Colauto[1]

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Abstract

Lentinus crinitus is a wild fungus, which produces mushrooms consumed by some Amazonian Indians. Besides, it is recognized for its diverse biological activities and biotechnological applications. However, there are few reports with limited information on basidiocarp chemical composition and cytotoxicity. Our study determined and evaluated the chemical composition, cytotoxicity, and antioxidant activity of *L. crinitus* pileus and stipe separately. Chromatographic methods were used to evaluate basidiocarp chemical composition. Cytotoxicity was verified using a cell culture from porcine liver and against a panel of human tumor cells from different models. Antioxidant activity was assessed by different in vitro methods. The pileus had higher levels of protein, ash, tocopherols, and organic acids, mainly malic acid, than the stipe. The stipe revealed higher contents of carbohydrates, energy, soluble sugars, and phenolic acids, mostly *p*-hydroxybenzoic acid. *L. crinitus* basidiocarp has mainly trehalose as soluble sugar, and less than 1% fat being ~60% polyunsaturated fatty acids (mostly linoleic and oleic acids), and ~13% saturated fatty acids (mostly palmitic acid). *L. crinitus* revealed high antioxidant activity for most methods and no cytotoxic activity against tumor and non-tumor cells. *L. crinitus* basidiocarp can be considered a functional food with applicability in food, cosmetic, and pharmaceutical industries.

✉ Nelson B. Colauto
nelsonbcolauto@gmail.com

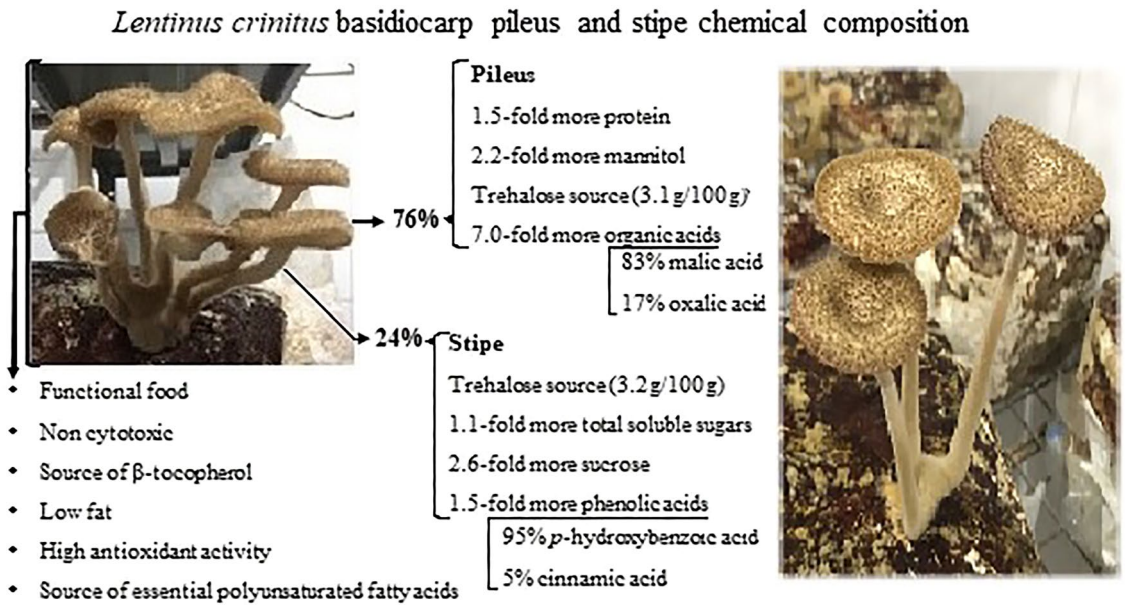
[1] Laboratory of Molecular Biology, Graduate Program in Biotechnology Applied to Agriculture, Universidade Paranaense, Praça Mascarenhas de Moraes, 4282, Umuarama, PR, P.O. Box 224, 87.502-210, Brazil

[2] Laboratory of Preclinical Research of Natural Products, Graduate Program in Medicinal Plants and Phytotherapics in Basic Attention, Graduate Program in Animal Science with Emphasis on Bioactive Products, Universidade Paranaense, Umuarama, PR, Brazil

[3] Laboratory of Neurosciences, Graduate Program in Medicinal Plants and Phytotherapics in Basic Attention, Universidade Paranaense, Umuarama, PR, Brazil

[4] Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, Bragança 5300-253, Portugal

Graphic abstract



Keywords Basidiocarp · *β*-Tocopherol · Linoleic acid · Malic acid · *p*-Hydroxybenzoic acid · Trehalose

Introduction

Mushrooms are prized for their taste, nutritional value, and bioactive compounds used for health and well-being [1–5] but just some of them are cultivated in industrial scale worldwide such as *Pleurotus*, *Lentinula*, *Auricularia*, *Agaricus*, *Flammulina*, *Coprinus*, *Agrocybe*, and *Volvariella* genera [6–13].

Lentinus crinitus (L.) Fr. (Basidiomycota) is a saprotrophic wild fungus growing on decaying tree trunks [14, 15] with a pantropical and neotropical distribution [16]. It is an important part of a regular diet of ethnic groups from the Amazon such as the *Yanomami* (*Yąnomamö*) Indians, and it is boiled in water or roasted in banana leaves before eating [14]. This author also reported that the *Yanomami* Indians from *Tototobi* village have two words for eating, one for meat and one for other foods. The word for meat consumption is also applied to mushrooms, and they are supposed to consider this protein source equivalent to meat and eat it, despite being tough and leathery.

Lentinus crinitus has broad ligninolytic activity, producing several enzymes such as laccases and proteases [17–20]; high antioxidant potential [21]; having been reported as a producer of antimicrobial [22, 23], antibiotic [15], and antitumoral [24, 25] compounds. In addition, it is simultaneously able to bioaccumulate lithium in the mycelial biomass, which makes it an option as a functional food [26, 27]. *L. crinitus* also has been used for degradation and discoloration of textile dyes [28, 29] and bioremediation of contaminated soils with chemicals such as organochlorines [30] and dichlorophenoxyacetic acid (2,4-D) [31].

Mushroom production can be a low environmental impact and profitable activity, mainly for Brazilian rural farmers. To promote mushroom consumption and production of *L. crinitus*, it is necessary to evaluate its biological and cytotoxic activities. There are few studies on *L. crinitus* chemical composition [23, 32, 33] and no reports were found on the cytotoxicity activity of this mushroom. Moreover, *L. crinitus* stipe has a higher toughness than the pileus, and can be considered a by-product of mushroom production. Both pileus and stipe could have different chemical compositions [34] and, therefore, they can be used for different purposes. Though, no reports comparing the chemical composition and biological activity of *L. crinitus* pileus and stipe have been found.

Despite *L. crinitus* biological activities, biotechnological applications, and ethnomycological studies, no reports on its basidiocarp chemical composition and cytotoxic activity have been found. Thus, the objective of this study was to determine and evaluate the chemical composition and antioxidant and cytotoxic activities of *L. crinitus*

basidiocarp, cultivated in agro-industrial residues, comparing the results obtained for the pileus and stipe.

Materials and methods

Biological material

Lentinus crinitus (L.) Fr. U9-1 strain from the culture collection of the Laboratory of Molecular Biology of the Paranaense University, GenBank accession numbers MG211674, was used. The strain was registered under the code A04E776 in the National System of Genetic Patrimony Management and Associated Traditional Knowledge (SisGen, its acronym in Portuguese). The cryopreserved fungus at − 86 ºC, according to Linde et al. [35], Zaghi Jr et al. [36] and Tanaka et al. [19], was transferred to malt extract agar medium (39 g/L; MEA) and kept at 28 °C in the dark to recover mycelial vigor. Mycelia from the colony edge with homogenous branching and without sectoring were used as inoculum. For mushroom (basidiocarp) production, the cultivation substrate consisted of sugarcane bagasse and rice husk (1:1), according to Colauto and Eira [37] and Machado et al. [32], and substrate carbon-to-nitrogen (C/N) was 48. Each substrate was kept at 28 °C until complete colonization (30 days) without room ventilation, the top part of the cultivation bag was opened, the room temperature reduced to 18 °C for 24 h (thermal shock), and ventilation was started to reduce carbon dioxide but relative humidity was kept at 80% throughout cultivation period [37]. Basidiocarps were harvested daily when the pileus border was flat, indicating the end of basidiocarp growth and the beginning of basidiocarp senescence. The basidiocarps ($n = 512$) were dehydrated in an oven with air circulation at 60 °C until constant mass. Basidiocarp pileus and stipe were separated, ground in an industrial blender, homogenized, and kept in separate Falcon tubes at − 20 °C.

Biological material and production and processing of basidiocarps

Nutritional value of basidiocarp pileus and stipe

The proximate composition (protein, fat, ash, and carbohydrates content) of the samples was determined according to standard procedures [38]. 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 powdered sample with petroleum ether, using a Soxhlet apparatus; ash content was determined by incineration at 600 ± 15 ºC. Total carbohydrates were calculated by difference [total carbohydrates (g/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, of 25 October 2011, on the Provision of Food Information to Consumers [39], as: Energy [(kcal/100 g; dry basis) = $4 \times (g_{protein} + g_{carbohydrates}) + 9 \times (g_{fat})$].

Hydrophilic compounds

Soluble sugars The soluble sugars present in *L. crinitus* basidiocarp pileus and stipe were analyzed by high-performance liquid chromatography (HPLC) using a refraction index (RI) detector. This methodology was conducted according to a previously described methodology [40]. The results were expressed in g per 100 g (dry basis).

Organic acids The organic acid profile of the studied samples was determined following a procedure previously optimized and described by Barros et al. [41]. The analysis was performed by ultra-fast liquid chromatography (UFLC) coupled to a photodiode array detector (PDA) following the previously referred procedure. The organic acids were quantified by comparison of the peak area recorded at 215 nm (245 nm for ascorbic acid) with calibration curves obtained from commercial standards of each compound. The results were expressed in mg per 100 g (dry basis).

Other organic acids

Phenolic acids and related compounds The phenolic acid determination was performed by an UPLC system coupled to a PDA and a mass detector (LC-DAD-ESI/MSn), according to a previously described procedure [42]. The phenolic compounds were identified by comparing their retention times, UV–Vis and mass spectra with those obtained with standard compounds, when available. Otherwise, compounds were tentatively identified, comparing the obtained information with available data reported in the literature. For quantitative analysis, a calibration curve for each available phenolic standard was constructed based on the UV–Vis signal. For the identified phenolic compounds to which a commercial standard was not available, the quantification was performed through the calibration curve of another compound from the same phenolic group [42]. The results were expressed as µg per 100 g (dry basis).

Lipophilic compounds

Fatty acids The fatty acids profile of the samples was determined by gas–liquid chromatography with flame ionization detection (GC-FID)/capillary column as described previously by Reis et al. [43]. The identification of the different fatty acids was made by comparison of the relative retention times of FAME (fatty acid methyl esters) peaks from

samples with standards. The results were expressed in the relative percentage of each fatty acid.

Tocopherols The methodology used to determine the tocopherol composition was according to Heleno et al. [44]. The analysis was performed using the same HPLC system described for soluble sugar, 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).

Bioactivity evaluation of basidiocarp pileus and stipe

Extract preparation

For each dried sample of basidiocarp pileus or stipe, 1 g (as described in "Biological material") was extracted with 30-mL ethanol under magnetic stirring for 1 h at room temperature ($n = 3$). Then, each residue was re-extracted maintaining the same operational conditions. The extracts were mixed and evaporated at 40 ºC in a rotary evaporator (Büchi R-210, Flawil, Switzerland) to remove alcohol and be lyophilized. Each lyophilized extract was re-dissolved at 8 mg/mL in autoclaved distilled water to assess the cytotoxic activity. Subsequently, each solution was diluted successively to obtain the concentration necessary to perform the experimental study.

Cytotoxic activity of basidiocarp pileus and stipe in human tumor cell lines and non-tumor cells

Four human tumor cell lines were used, namely HeLa (cervical carcinoma), HepG2 (hepatocellular carcinoma), MCF7 (breast adenocarcinoma), and NCI-H460 (non-small cell lung cancer). Cells were routinely maintained as adherent cell cultures. Cells were treated for 48 h with the diluted extract solutions [45]. The adherent cells were fixed by adding cold 10% trichloroacetic acid (TCA, 100 mL) and incubated for 60 min at 4 ºC. Plates were then washed with deionized water and dried; sulforhodamine B solution (0.1% in 1% acetic acid, 100 mL) 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 [46]. The same procedure described above for the SRB assay was performed for the growth inhibition. The results were also expressed as GI_{50} values.

Antioxidant activity of basidiocarp pileus and stipe

To obtain the methanolic extract, 1 g basidiocarp pileus or stipe (dry basis) was homogenized with 10-mL methyl alcohol in Falcon tubes. The mixture was kept at 60 °C for 45 min, and centrifuged at 6000 g at 5 °C for 10 min. The supernatant was considered the crude extract. The total antioxidant capacity of *L. crinitus* extracts was evaluated by 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) free radical sequestration method. The inhibitory concentration to reduce 50% of free radicals (IC_{50}) in a sample was determined from a correlation among absorbance and sample concentrations. All assays were performed in triplicate [47, 48].

The antioxidant activity was also evaluated by the ferric reducing antioxidant power (FRAP) method. The antioxidant activity of each reaction was calculated against a standard ferrous sulfate curve (2000 µM) and as positive control 800 µM trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used [49]. All assays were performed in triplicate.

The antioxidant activity of basidiocarp pileus or stipe extract was also evaluated by co-oxidation of β-carotene/linoleic acid (BCLA) method, according to Mattos et al. [50]. The reaction was maintained at 40 °C for 120 min and the absorbance was measured at 470 nm (SpectraMax Plus^{384} Microplate Reader), every 5 min, from 0 to 120 min. Trolox (100 µg/mL) was used as a positive control. The results were expressed as the absorbance reduction along the reaction time. The β-carotene bleaching rate was calculated according to the following equation:

$$R = \ln (a/b)/t \quad (1)$$

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

Parameter	Basidiocarp		*p* value
	Pileus	Stipe	
Ash (g/100 g)	4.29 ± 0.07	2.66 ± 0.02	< 0.001
Proteins (g/100 g)	14.4 ± 0.3	9.5 ± 0.1	< 0.001
Fat (g/100 g)	0.52 ± 0.03	0.55 ± 0.02	0.02
Carbohydrates (g/100 g)	80.8 ± 0.3	87.3 ± 0.2	< 0.001
Energy (kcal/100 g)	385.5 ± 0.4	392.15 ± 0.03	< 0.001

Values expressed as arithmetic mean ± standard deviation (dry basis) expressed as g/100 g, except for energy expressed as kcal/100 g

p value indicates significant differences by the Student's *t* test ($p \leq 0.05$; $n = 9$)

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

Compound	Basidiocarp		*p* value
	Pileus	Stipe	
Sugars (g/100 g)			
Mannitol	0.18 ± 0.001	0.08 ± 0.02	< 0.001
Sucrose	0.260 ± 0.007	0.69 ± 0.02	< 0.001
Trehalose	3.13 ± 0.07	3.2 ± 0.3	0.528
Total soluble sugars	3.57 ± 0.06	3.97 ± 0.50	0.039
Organic acids (mg/100)			
Oxalic acid	165 ± 1	tr	–
Malic Acid	801 ± 12	137 ± 1	< 0.001
Fumaric acid	0.0425 ± 0.0006	tr	–
Total organic acids	966 ± 15	137 ± 1	< 0.001
Phenolic acids (µg/100 g)			
p-Hydroxybenzoic acid	537 ± 4	791 ± 3	< 0.001
Cinnamic acid	81.2 ± 0.2	38.2 ± 0.5	< 0.001
Total phenolic acids	537 ± 4	791 ± 3	< 0.001

Values expressed as arithmetic mean ± standard deviation (dry basis)

tr trace

p value indicates significant differences by the Student's *t* test ($p \leq 0.05$; $n = 9$)

where R is the bleaching rate of *β*-carotene in the mixture; ln is the natural log; a is the absorbance in zero time; b is the absorbance in t time ($t = 0, 5, 10, \ldots 120$ min).

The antioxidant activity was calculated according to the percentage of inhibition in relation to the control, using the following equation:

$$\text{Antioxidant activity} = \left[\left(R_{\text{control}} - R_{\text{sample}}\right)/R_{\text{control}}\right] \times 100 \quad (2)$$

where R_{control} and R_{sample} were the bleaching rates of *β*-carotene in the mixture without the antioxidant (R_{control}) and with basidiocarp pileus or stipe extract (R_{sample}) [51].

Statistical analysis

Three dried samples of *L. crinitus* pileus or stipe (as described in "Biological material") were used and all assays were carried out in triplicate ($n = 9$). Results were expressed as arithmetic mean values and standard deviation. The results of each parameter were compared by means of the Student's *t* test to determine the significant difference between samples ($p \leq 0.05$). This analysis was carried out using SPSS software program (IBM SPSS software, Armonk, NY, USA). Antioxidant activity statistical analysis was determined by Tukey's test ($p \leq 0.05$).

Results

The whole basidiocarp has an average natural pileus:stipe proportion of 76:24 (mass:mass) based on the assay cultivation conditions. The basidiocarp is leathery (tough) when harvested at the senescence phase, as found in our study, but it is tender when harvested at the beginning of the fructification (young phase). The pileus that is tenderer than the stipe had 1.5- and 1.6-fold higher proteins and ashes than the stipe, respectively (Table 1). The pileus also had sevenfold more organic acids than the stipe and the major ones were malic and oxalic acids (Table 2). The pileus had 5.8-fold more malic acid than the stipe and more oxalic acid than that stipe, which has only traces (Table 2). Malic acid represents 83 and 100% of total organic acids in the pileus and the stipe, respectively (Table 2).

The stipe presented a higher content of carbohydrates and energy than the pileus, but fat content was the same (Table 1). The stipe also had 1.1-fold higher total soluble sugars than the pileus, mainly sucrose that was 2.6-fold higher than the pileus, but mannitol was 2.2-fold lower than the pileus (Table 2). Trehalose content was the same for the pileus and the stipe, and it represents 88 and 82% of the total soluble sugar content, respectively (Table 2). The stipe had also 1.5-fold higher phenolic acids than the pileus and the major acids were *p*-hydroxybenzoic and cinnamic acids (Table 2). The stipe had 1.5-fold more *p*-hydroxybenzoic acid than the pileus, but the cinnamic acid was 2.1-fold higher in the pileus than the stipe (Table 2). *p*-Hydroxybenzoic acid represents almost 100% of the phenolic acids in the pileus and the stipe (Table 2).

The lipid fractions of basidiocarp pileus and stipe presented similar compositions with 21 fatty acids identified by GC analyses (Table 3). Polyunsaturated fatty acids were the predominant class (~ 63%), followed by saturated fatty acids (~ 23%), and monounsaturated fatty acids (~ 14%) (Table 3). The amount of each lipophilic acid was similar in the pileus and the stipe (Table 3). The major compounds in the pileus and the stipe were C18 series such as the linoleic (~ 60%) and oleic (~ 13%) acids and C16 series such as the palmitic (~ 13%) acid (Table 3). The basidiocarp pileus and stipe presented important unsaturated fatty acids such as arachidic, eicosenoic, *cis*-11,14-eicosadienoic, *cis*-11,14,17-eicosatrienoic, and heneicosanoic acids (~ 0.2% each) from the C20 series, and behenic (~ 2.5%) acid from the C22:0 series, which are important essential fatty acids even in small amounts. In addition, basidiocarp pileus had β-tocopherol 1.2-fold higher than the stipe (Table 3).

The basidiocarp pileus and stipe extracts presented low antioxidant activity by DPPH• method of ~ 6000- and ~ 12,000-fold lower than the control quercetin (Table 4). However, the pileus and stipe extracts had high antioxidant

Table 3 Chemical composition in the lipophilic extract (fatty acids, relative percentage, and tocopherols) of *Lentinus crinitus* basidiocarp pileus and stipe

Compound	Basidiocarp		*p* value
	Pileus (%)	Stipe (%)	
Caproic acid (C6:0)	0.264 ± 0.001	0.317 ± 0.001	< 0.001
Caprylic acid (C8:0)	0.317 ± 0.005	0.189 ± 0.007	< 0.001
Capric acid (C10:0)	0.66 ± 0.02	0.1229 ± 0.0009	< 0.001
Lauric acid (C12:0)	0.546 ± 0.004	0.463 ± 0.003	< 0.001
Myristic acid (C14:0)	1.23 ± 0.02	1.33 ± 0.01	< 0.001
Pentadecanoic acid (C15:0)	0.919 ± 0.008	1.77 ± 0.03	< 0.001
Palmitic acid (C16:0)	13.40 ± 0.03	12.80 ± 0.07	< 0.001
Palmitoleic acid (C16:1)	0.216 ± 0.009	0.360 ± 0.002	< 0.001
Heptadecanoic acid (C17:0)	0.420 ± 0.007	0.77 ± 0.01	< 0.001
Stearic acid (C18:0)	2.94 ± 0.02	2.23 ± 0.01	< 0.001
Oleic acid (C18:1n9)	13.5 ± 0.4	12.65 ± 0.04	< 0.001
Linoleic acid (C18:2n6)	59.9 ± 0.4	61.80 ± 0.03	< 0.001
α-Linolenic acid (C18:3n3)	2.04 ± 0.03	1.20 ± 0.04	< 0.001
Arachidic acid (C20:0)	0.2448 ± 0.0008	0.197 ± 0.002	< 0.001
Eicosenoic acid (C20:1)	0.2974 ± 0.0005	0.118 ± 0.002	< 0.001
cis-11,14-Eicosadienoic acid (C20:2)	0.326 ± 0.009	0.28 ± 0.01	< 0.001
cis-11,14,17-Eicosatrienoic acid and heneicosanoic acid (C20:3n3 + C21:0)	0.271 ± 0.004	0.51 ± 0.02	< 0.001
Behenic acid (C22:0)	2.31 ± 0.05	2.452 ± 0.001	< 0.001
Erucic acid (C22:1n9)	0.053 ± 0.002	0.058 ± 0.002	< 0.001
Lignoceric acid (C24:0)	nd	0.393 ± 0.006	–
Total saturated fatty acids (% total fatty acids)	23.23 ± 0.04	23.0 ± 0.1	0.001
Total monounsaturated fatty acids (% total fatty acids)	14.3 ± 0.3	13.18 ± 0.04	< 0.001
Total polyunsaturated fatty acids (% total fatty acids)	62.5 ± 0.4	63.74 ± 0.07	< 0.001
Tocopherols (μg/100 g)			
β-Tocopherol	491 ± 4	394 ± 3	< 0.001

Values expressed as arithmetic mean ± standard deviation (dry basis)

nd not detected

p value indicates significant differences by the Student's *t* test ($p \leq 0.05$; $n = 9$)

Table 4 Antioxidant activity from extract of *Lentinus crinitus* basidiocarp pileus and stipe by free radical reduction method 2,2-diphenyl-1-picrylhydrazyl (DPPH•) and ferric reducing antioxidant power (FRAP)

Parameter	Quercetin	Trolox	Basidiocarp	
			Pileus	Stipe
DPPH• (IC_{50} in mg/mL)	0.02 ± 0.10a	na	99 ± 2b	197 ± 4c
FRAP (μmol Fe^{+2}/g of sample)	na	10.5 ± 0.9c	35.4 ± 0.1a	26.1 ± 0.1b

Values expressed as arithmetic mean ± standard deviation (dry basis). Different letters in the same row indicate significant differences by the Tukey test ($p \leq 0.05$; $n = 3$)

na not applicable, IC_{50} half maximal inhibitory concentration

activity by FRAP method of 3.4- and 2.5-fold higher, respectively, than the control trolox (Table 4). The pileus and stipe extracts at 100 mg/mL protected 56 and 42% of the β-carotene against oxidation, respectively. These values are equivalent to 62 and 47% of trolox protection activity (control), respectively (Fig. 1). The antioxidant activity of the basidiocarp pileus and stipe by FRAP, a polar aqueous method, and BCLA, a nonpolar method, are in agreement with the phenolic and the organic polar acids, and the nonpolar tocopherol, natural antioxidants found in *L. crinitus* of our study (Table 4 and Fig. 1).

The basidiocarp pileus and stipe extracts were not effective to inhibit the growth of tumor and non-tumor cells at a concentration higher than 300 μg/mL against HepG2 and

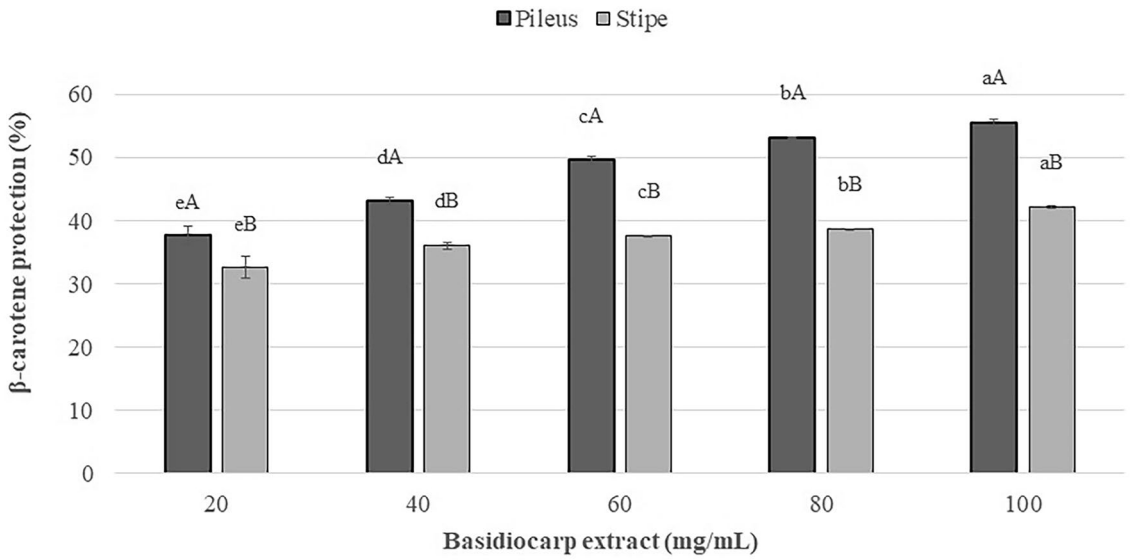


Fig. 1 Antioxidant activity of the extract of *Lentinus crinitus* basidiocarp pileus and stipe by the cooxidation of *β*-carotene/linoleic acid (BCLA) method. Values expressed as arithmetic mean ± standard deviation (dry basis). Different upper-case letters between basidiocarp pileus and stipe, at same basidiocarp extract concentration, and different lower-case letters among basidiocarp pileus and/or stipe, at different basidiocarp extract concentrations, indicate statistical difference by Tukey's test ($p \leq 0.05$). Positive control trolox at 0.2 mg/mL = 89.80 ± 4.13% *β*-carotene protection

Table 5 Cytotoxic activity of the methanolic extracts of *Lentinus crinitus* basidiocarp pileus and stipe and positive control ellipticine against human tumor cell lines and non-tumor cells

Cell line	Basidiocarp		*p* value	Ellipticine (GI_{50} μg/mL)
	Pileus (GI_{50} μg/mL)	Stipe (GI_{50} μg/mL)		
Tumor cell				
HepG2	303 ± 6	336.3 ± 0.5	< 0.001	3.2 ± 0.5
MCF7	> 400	> 400	–	0.91 ± 0.04
NCI-H460	> 400	> 400	–	1.42 ± 0.01
HeLa	> 400	> 400	–	1.1 ± 0.2
Non-tumor cell				
PLP2	> 400	> 400	–	2.06 ± 0.03

Values expressed as arithmetic mean ± standard deviation (dry basis). GI_{50} = extract concentration corresponding to 50% growth inhibition activity

MCF7 breast adenocarcinoma, *NCI-H460* lung carcinoma, *HeLa* cervical carcinoma, *HepG2* hepato cellular carcinoma

p value indicates significant differences by the Student's *t* test ($p \leq 0.05$; $n = 9$)

higher than 400 μg/mL for MCF7, NCI-H460, HeLa, and PLP2 cells compared to the control ellipticine (3.2 μg/mL) (Table 5). This and the GI_{50} values indicate that the basidiocarp pileus and stipe had no cytotoxicity activity against tumor and non-tumor cell lines.

Discussion

The high protein and low fat and energy contents of edible basidiocarps are appropriated for using them in low caloric diets [52]. Basidiocarp pileus of *Pleurotus ostreatus* has threefold more protein content than the stipe [53–55]. Our study showed that *L. crinitus* basidiocarp pileus has 1.5-fold higher protein content than the stipe. This value is similar to those reported for most of consumed basidiocarps [52–55, 59]. The whole basidiocarp protein content of 25 basidiomycetes (without *L. crinitus*) ranged from 13.2 to 62.8 g/100 g [56], and for *Lentinus strigosus* from 18.0 to 21.6 g/100 g [57]. For *L. crinitus*, protein content of 9.8 g/100 g (value converted from 6.25 to 4.38 factor; original value was 14.0 g/100 g) was reported [33], from 10.5 to 14.4 g/100 g [58], and from 20.0 to 27.0 g/100 g [32]. Our results showed the protein content was 13.3 g/100 g (14.5 g/100 g for the pileus and 9.5 g/100 g for the stipe), considering 76:24 (mass:mass) proportion that could vary according to carbon dioxide level in the cultivation room. It indicates that the protein content in edible basidiocarps varies, but our results are like those found in the literature for *L. crinitus*. However, it is below the range reported by Chang and Miles [59] that is from 19 to 35 g/100 g for edible basidiocarps and some other foods such as soybean (39.1 g/100 g) and in milk (25.2 g/100 g), but higher than rice (7.3 g/100 g) and equivalent to wheat (13.2 g/100 g). Nevertheless, the protein content reported for basidiocarps must be compared with some caution because it may vary due to lack of moist

sample control and information of the conversion factor, which should be 4.38 instead of 6.25 (generally used for other foods), mainly because basidiocarps have high non-protein nitrogen content [56, 59].

Our study showed that trehalose represents more than 80% of the total soluble sugars in the basidiocarp with similar amounts in the pileus and the stipe. Trehalose is the main carbohydrate in fungus, mainly because the glucose by active transport is converted to trehalose [59], and according to Kalač [56], basidiocarps have an average of 3.92 g/100 g, which is a close value found in our study for *L. crinitus* (3.2 g/100 g). Trehalose migrates from mycelia to basidiocarps, and protects the cells against stresses such as desiccation, temperature, and oxygen pressures [60, 61]. This protection mechanism seems to be related to malondialdehyde inhibition, a free radical naturally produced by lipid peroxidation with mutagenic and carcinogenic activities, and it could explain the capacity of *L. crinitus* enzymatic production [19] and mycelial biomass growth [62] under extreme conditions of temperature and pH.

Species belonging to the genus *Pleurotus* contain carbohydrates ranging from 47 to 82 g/100 g whereas *Agaricus bisporus* has 60 g/100 g; these water-soluble polysaccharides have been reported to inhibit tumors [59]. Thus, the high carbohydrate (87 g/100 g) content, mainly trehalose (3.2 g/100 g) from *L. crinitus* of our study, has several applications such as functional food and cosmetic and therapeutic applications [63–65]. Trehalose has been obtained in relatively small amounts by extraction from natural sources, chemical synthesis, microbial fermentation, and enzymatic conversion from maltose, but with low yield even using genetic engineering techniques [64, 66, 67]. According to Martirosyan and Singh [68], functional foods are natural or processed foods that contain known or unknown biologically active compounds at non-toxic amounts, and that provide a health benefit for the prevention, management, or treatment of a disease. *L. crinitus* basidiocarp, which is already regularly consumed by the *Yanomami* Indians, can be a source of trehalose, as the fungus is robust and can easily grow in several lignocellulolytic substrates [58].

The fat content of commercial basidiocarps ranges from 0.6 to 3.1 g/100 g (*A. bisporus*, *Auricularia* spp., *Flammulina velutipes*, *Lentinula edodes*, *Pholiota nameko*, *Pleurotus* spp., *Tremella fuciformis*, and *Volvariella volvacea*) [59] and of wild basidiocarps from 0.4 to 3.8 g/100 g (*Cortinarius glaucopus*, *Fistulina hepatica*, *Hygrophoropsis aurantiaca*, *Hypholoma capnoides*, *Laccaria laccata*, *Lactarius salmonicolor*, *Lepista inversa*, *Russula delica*, *Suillus mediterraneensis*, and *Tricholoma imbricatum*) [69]. In our study, *L. crinitus* basidiocarp showed 0.52 and 0.55 g/100 g fat content for the pileus and the stipe, respectively. Silva Neto et al. [33] reported 1.5 g/100 g of fat content for *L. crinitus*. This indicates that *L. crinitus* basidiocarp is an excellent alternative for low calorie diets. According to Chang and Miles [59], the energy value of the main cultivated basidiocarps ranged from 261 to 392 kcal/100 g, and *L. crinitus* basidiocarp, in our study, had 385 kcal/100 g in the pileus and 392 kcal/100 g in the stipe, which is similar to the main commercial basidiocarps. In addition to the reduced fat and caloric content, the fatty acids of *L. crinitus* basidiocarp in our study had a high nutritional value since the unsaturated fatty acid amount is threefold higher than the saturated ones. The linoleic acid (ω-6) represents more than 60% fatty acids in *L. crinitus* basidiocarp in our study, and this polyunsaturated essential fatty acid is one of two essential ones to human diet. The linoleic acid represents 28–76% of the total lipids in commercial basidiocarps, and has been identified as the main substance with antimutagenic activity in *Agaricus blazei* and *Grifola frondosa* [59]. In the cell metabolism, the linoleic acid is the precursor of the arachidonic acid that promotes the production of inflammation mediators such as eicosanoids, prostaglandins, thromboxanes, and leukotrienes [70]. Thus, *L. crinitus* basidiocarp is associated with a healthy diet due to the low caloric value and a high polyunsaturated fatty acid content.

The hydrophilic fraction of *L. crinitus* basidiocarp, specifically organic acids, had malic (801 mg/100 g in the pileus) and oxalic (165 mg/100 g in the pileus) acids as major compounds. Organic acids in basidiocarps are related to taste and flavor formation such as malic acid that varies from 15 to 700 mg/100 g for *Cantharellus cibarius* [71], 217 mg/100 g for *Agaricus brasiliensis* [72], and 2200 mg/100 g for *Polyporus squamosus* [73]. Oxalic acid has been reported with 76 mg/100 g *P. squamosus* [73] and 115 mg/100 g for *A. brasiliensis* [72]. It indicates that the malic and oxalic acids in our study are among the highest levels found in the literature. The main phenolic acid found in our study was *p*-hydroxybenzoic acid, found in the pileus (537 µg/100 g) and stipe (791 µg/100 g) of *L. crinitus* basidiocarp. The *p*-hydroxybenzoic acid content has been reported from 0 to 23.9 mg/100 g (average of 3.4 mg/100 g) among 16 wild basidiocarps [74], and from 0.5 to 2.4 mg/100 g (average of 1.3 mg/100 g) among eight commercial basidiocarps [75]. This indicates that the *p*-hydroxybenzoic acid in our study is among the lowest levels found in the cited literature in this paragraph. Cinnamic acid is a precursor of other complex phenolic compounds, mainly found in several plants, and with anticancer, antituberculosis, antimalarial, antifungal, antimicrobial, antiatherogenic, and antioxidant activities, and also used to alter the potency, permeability, solubility or other parameters of drugs [76]. Phenolic compounds could improve chemical stability and shelf life of foods and cosmetics as they are strong antioxidants [77]. However, the phenolic acid content of *L. crinitus* basidiocarp in our study may explain its high antioxidant activity by the FRAP method, as the phenolic acids can chelate metals or

displace the electrical charge stabilizing free radicals [78, 79]. In addition, as far as we know, this is the first report concerning the organic and phenolic acid contents of *L. crinitus* basidiocarp pileus and stipe.

Different basidiocarp compounds are associated with antioxidant activity [80], but tocopherols and phenolic compounds are the most important studied ones, although the organic acids have been associated with the antioxidant activity of *A. brasiliensis*, mainly for BCLA method [72]. Our results showed that *L. crinitus* basidiocarp pileus and stipe have high antioxidant activity by the FRAP and BCLA methods and high amounts of antioxidant compounds such as malic and *p*-hydroxybenzoic acids and *β*-tocopherol. Reis et al. [81] reported *β*-tocopherol contents of 48.2 and 1.61 µg/100 g, respectively, for *Pleurotus eryngii* and *A. bisporus*. In our study, *L. crinitus* pileus and stipe had ~ tenfold more β-tocopherol (491.0 and 394.0 µg/100 g, respectively) than cited *P. eryngii* and *A. bisporus*. In general, the basidiocarp antioxidant activity can be attributed to the ability to donate hydrogen, chelate metals, and its effectiveness as good scavengers of superoxide and free radicals [82]. Our results showed that *L. crinitus* pileus and stipe have 2.4- and 1.5-fold more antioxidant activities than the positive control trolox, reinforcing that this basidiocarp is a functional food.

L. crinitus basidiocarp pileus and stipe had very low cytotoxicity against HepG2 and no cytotoxicity against MCF7, NCI-H460, and HeLa tumor cell lines. Cytotoxicity activity for several cell lines was reported for the panepoxydone-compound isolated from *L. crinitus* [24, 83–87]. In addition, there were no cytotoxicity against PLP2 non-tumor cells—a mandatory cytotoxicity test [88]—showing that this basidiomycete might be used in diets as it is already done by the Amazonian Indians in Brazil [14].

Conclusions

The basidiocarp of *L. crinitus* has a pileus:stipe proportion of 76:24 (mass:mass). The pileus has high levels of protein, ash, tocopherols, and organic acids, mainly malic and oxalic acids. The stipe has a high content of carbohydrates, energy, soluble sugars, and phenolic acids, mostly *p*-hydroxybenzoic acid. Basidiocarp pileus or stipe has trehalose as the main soluble sugar and less than 1% fat being ~ 60% polyunsaturated fatty acids, mostly linoleic and oleic acids, and ~ 13% saturated fatty acids, mostly palmitic acid. It has high antioxidant activity by FRAP and BCLA methods, but low antioxidant activity by DPPH• method; it has no cytotoxic activity against tumor and non-tumor cells. The basidiocarp pileus and stipe of *L. crinitus* are functional foods with antioxidant activity, and sources of protein, polyunsaturated fatty acids, malic acid, *p*-hydroxybenzoic acid, trehalose, and tocopherols. The basidiocarp has no cytotoxicity, enabling its use in the food, cosmetic, and pharmaceutical industries.

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Declarations

Conflict of interest The authors declare no conflict of interest.

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