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Basidiocarp structures of *Lentinus crinitus*: an antimicrobial source against foodborne pathogens and food spoilage microorganisms

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Abstract

Lentinus crinitus basidiocarps are an alternative to antimicrobials, but the stipe (24% basidiocarp) is discarded even with potential antimicrobial activity. This study evaluated the antimicrobial activity of *L. crinitus* basidiocarp pileus and stipe extracts against foodborne pathogens and food spoilage microorganisms. Basidiocarps of *L. crinitus* were grown in sugarcane bagasse and rice husks and the pileus and stipe methanolic extract was analyzed by broth microdilution method for antimicrobial activity against eight bacteria and eight fungi. The minimum bactericidal concentration values for pileus and stipe ranged from 0.40 to 0.50 mg mL^{-1}, for streptomycin from 0.10 to 0.50 mg mL^{-1}, and for ampicillin from 0.40 to 1.20 mg mL^{-1}. The minimum fungicidal concentration values for pileus and stipe ranged from 0.06 to 0.60 mg mL^{-1}, for bifonazole from 0.20 to 0.25 mg mL^{-1}, and for ketoconazole from 0.30 to 3.50 mg mL^{-1}. Extracts had bacteriostatic, bactericidal, fungistatic and fungicidal activity against all microorganisms, but with greater efficiency and specificity for some microorganisms. Both pileus and stipe are promising and sustainable alternatives for use in food, agricultural, and pharmaceutical industries.

Keywords Antibacterial activity · Antifungal activity · Basidiocarp stipe · Mushroom · Polyporaceae

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Introduction

Food safety is generally considered the primary concern in food systems because microbial contamination can cause 30 to 50% losses in the food chain (Krepker et al. 2017; Fung et al. 2018; Niaz et al. 2019). Thus, products with high quality and safety levels are required and the addition of food antimicrobials has become widespread in the food industry (Baptista et al. 2020). In general, synthetic preservatives, including many salts and organic acids such as propionates, benzoates, sulfites, nitrites, chlorides, nisin, natamycin, sorbic, citric, tartaric, and ascorbic acids, potassium sorbate, and lactate have been officially applied as food antimicrobials and recognized by regulatory agencies (Kalpana and Rajeswari 2019).

There are concerns about adding synthetic antimicrobials (Ayaz et al. 1980) and antioxidants in foods, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) due to adverse effects on human health associated with degenerative diseases (Suh et al. 2005). There are also nutritional concerns because some synthetic antimicrobials can induce vitamin degradation such as thiamin deterioration by sulfides (Arioglu-Tuncil et al. 2020). Therefore, the use of synthetic food additives is under the rigid control of regulatory agencies and consequently the demand for and use of antimicrobials from natural sources has increased not only due to their safety and low toxicity on health but also due to consumers' insecurity about chemical additives (Bensid et al. 2020). Natural antimicrobial compounds include lysozyme from egg white, polyphenols from plants, lactoperoxidase from milk, chitosan from shrimp shells, and bacteriocins from lactic acid bacteria (Msagati 2013). However, some of them have not been approved as food antimicrobials, probably due to the need and cost of extensive studies to establish the safety of a new antimicrobial agent to regulatory agencies (Beales and Smith 2004). In addition, many natural resources, such as some fungi or parts of their structure, are still poorly recognized with antimicrobial function.

Lentinus crinitus is an edible wild basidiomycete that usually grows on decaying tree trunks (Silva and Gilbertone 2006). It has pantropical occurrence, being distributed in almost all Brazilian and South American biomes (Coimbra and Welch 2018), and appreciated by Amazonian Indians, such as the *Yanomami* (Vargas-Isla et al. 2013). The entire basidiocarp has a bactericidal activity on *Bacillus cereus*, *Enterobacter cloacae*, *Escherichia coli*, *Listeria monocytogenes*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, *Salmonella enterica*, and *Staphylococcus aureus*, with minimum bactericidal concentration (MBC) values from 0.248 to 0.604 mg mL^{-1}. Moreover, the entire basidiocarp has minimum fungicidal concentration (MFC) values from 0.0601 to 0.6004 mg mL^{-1} on *Trichoderma viride*, *Penicillium verrucosum*, *Penicillium ochrochloron*, *Penicillium funiculosum*, *Aspergillus versicolor*, *Aspergillus ochraceus*, *Aspergillus niger*, and *Aspergillus fumigatus*. In addition, *L. crinitus* entire basidiocarp extracts did not show cytotoxicity with GI$_{50}$ values higher than 400 µg mL^{-1} (Bertéli et al. 2021a).

Despite one report on the antimicrobial and cytotoxic activities of *L. crinitus* entire basidiocarp (Bertéli et al. 2021a), there are no antimicrobial data for separated pileus and stipe from this basidiocarp. The stipe represents 24% of *L. crinitus* basidiocarp mass (Bertéli et al. 2021b). It is fibrous, difficult to chew, and generally discarded in the basidiocarp production of *L. crinitus*; however, it could be a source of antimicrobial compounds. Thus, this study evaluated the antimicrobial activity of the extract of *L. crinitus* basidiocarp (pileus and stipe) against foodborne pathogens and food spoilage microorganisms.

Materials and methods

Biological material

Lentinus crinitus (L.) Fr. (Basidiomycota: Polyporales) U9-1 strain was from the Culture Collection of the Laboratory of Molecular Biology at Paranaense University, identified by sequencing the internal transcribed spacers of ribosomal DNA, deposited on the GenBank database (MG211674) ((Marim et al. 2018), and registered in Brazil (A04E776) in the *Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado* (SisGen).

The fungus was cultivated in 2% malt extract agar (MEA) at 25 ± 1 °C, for seven days, and colonies with mycelial growth vigor and without sectoring were selected as inoculum for wheat grains. Wheat grains were cooked in ultrapure water at 90 ºC for 40 min, excess water was removed, and calcium carbonate (1 g) was added to every 100 g grains. Grains were transferred to polypropylene bags, autoclaved (121 °C for 1 h and 30 min), and after reaching room temperature, MEA disks with growing mycelium were loaded into the bags of grains for incubation (25 ± 1 °C) up to full colonization (Tanaka et al. 2013). The colonized wheat grains were added to the cultivation substrates as inoculum.

Basidiocarp production

Sugarcane bagasse from a sugarcane processing mill and rice husks from rice mill were used with 1:1 (volume:volume) proportion to compose the cultivation substrate with 10 replicates. Each replicate was a polypropylene bag with 2 kg of cultivation substrate autoclaved for 1 h and 30 min at 121 °C. Each bag was added of 20 g of wheat grains colonized by the fungus, thermosealed, and stored in a cultivation room at 27 ± 1 °C with 80% air humidity until full substrate colonization. The upper part of the bags was opened after 30 days and the ambient temperature reduced for 24 h to 18 ± 1 °C (thermal shock). The basidiocarp was daily harvested when the edge of the pileus was flat, indicating growth completion and onset of the senescence process. The basidiocarps were dried in an oven with air circulation for 24 h at 60 °C, pileus and stipe were detached and ground in mortar with pistil to obtain grain size ≤ 0.35 mm (48 mesh), and stored at -20 °C for subsequent analyses.

Methanolic extraction of basidiocarp pileus and stipe

Dry samples (1 g) of each basidiocarp part were extracted with 30 mL aqueous methanol (4:1; volume:volume) with magnetic shaking (150 rpm at 25 °C for 1 h), and then filtered (Whatman® filter paper grade 4) to obtain the hydromethanolic extract. The extraction was repeated with the same volume of solvent, the obtained extracts blended, the methanol rotaevaporated, and the remaining water frozen and lyophilized (Melgar et al. 2018). After lyophilization, the extracts were dissolved again in 50 mL L^{-1} dimethyl sulfoxide (DMSO) solution in distilled water at 100 mg mL^{-1} to perform the antimicrobial activity assay. These solutions were successively diluted in order to obtain the necessary concentrations for the experimental study.

Antibacterial activity of the basidiocarp pileus and stipe extract

The antibacterial activity of *L. crinitus* basidiocarp (pileus and stipe) extracts was assessed against six ATCC bacterial strains such as *Escherichia coli* (Migula) Castellani and Chalmers 35218™, *Listeria monocytogenes* (Murray et al.) Pirie 35152™, *Micrococcus luteus* (Schroeter) Cohn 10240™, *Pseudomonas aeruginosa* (Schroeter) Migula 27853™, *Staphylococcus aureus* subsp. *aureus* Rosenbach 6538™, and *Salmonella enterica* subsp. *enterica* (ex Kauffmann and Edwards) Le Minor and Popoff serovar Typhimurium 13311™, and two bacterial clinical isolates as *Bacillus cereus* Frankland and Frankland, and *Enterobacter cloacae* (Jordan) Hormaeche and Edwards. The method of broth microdilution in microtiter plates (96 wells) was applied (Hanel and Raether 1988; Espinel-Ingroff 2001). Bacterial suspensions standardized at 1.0×10^5 CFU mL^{-1} in sterile saline (0.85%) were stored at 4 °C as inoculum prior to use. The extract solubilized with DMSO (50 mL L^{-1}) and polysorbate-80 (1 mg mL^{-1}) was dispensed into wells with 100 µL Luria-Bertani (LB) culture medium according to Miller (1972). Subsequently, bacterial suspensions at 1.0×10^5 CFU mL^{-1} per well were added in the medium. The mixtures were transferred to microplates and incubated on a shaking station (at 37 °C for 24 h at 160 rpm). The lowest extract concentrations that completely inhibits the growth of bacteria, observed under optical microscope, was determined as the minimum inhibitory concentration (MIC). Different concentrations of dissolved extracts were used in the range from 0.1 to 10 mg mL^{-1}. MBC was determined by subcultivation of 2 µL from the treated samples in microtiter plates containing 100 µL LB broth per well (Miller 1972) in 24 h incubation. The lowest concentration without microbial growth was determined as MBC, representing 99.5% of the original inoculum death. The optical density for every well was determined by Microplate Manager 4.0 software (microplate absorbance system from Bio-Rad Laboratories) at 655 nm and compared with the positive control and the blank solution. The positive controls were 1 mg mL^{-1} of the antibiotics streptomycin (Sigma P7794) or ampicillin (Panfarma) in sterile saline and the negative control was a 50 mL L^{-1} DMSO solution.

Antifungal activity of the basidiocarp pileus and stipe extract

The antifungal activity of basidiocarp (pileus and stipe) extracts was assessed against six ATCC fungal strains such as *Aspergillus fumigatus* Fresenius 1022™, *Aspergillus niger* van Tieghem 6275™, *Aspergillus versicolor* (Vuillemin) Tiraboschi 11730™, *Penicillium ochrochloron* Biourge 90288™, *Talaromyces funiculosus* (Thom) Samson et al. 8725™ (synonym = *Penicillium funiculosum* Thom), and *Trichoderma virens* (Miller et al.) von Arx 9645™, and two fungal food isolate as *Aspergillus ochraceus* Wilhelm and *Penicillium aurantiogriseum* Dierckx (synonym = *Penicillium verrucosum* var. *cyclopium* (Westling) Samson, Stolk & Hadlok). Microbial cultures kept at 4 °C on 20 g L^{-1} malt agar were monthly subcultured (Booth 1971). The antifungal activity was assessed by the modified microdilution method with microplates of 96 wells (Hanel and Raether 1988; Espinel-Ingroff 2001). The spores were obtained by surface washing of the Petri dishes with a sterile solution of saline (0.85%) with polysorbate-80 (0.1%). The suspension of spore was set with sterile 0.85% saline to about 1.0×10^5 conidia mL^{-1} in 100 µL per well (final volume) and kept at 4 °C before use. Contamination-free inoculum dilutions were verified by cultivation on MEA. Pileus and stipe extracts were dissolved in DMSO (50 mL L^{-1}) with 1 mg mL^{-1} polysorbate-80 starting from concentration of 1 mg mL^{-1} and added to MEA containing the inoculum. Microplates were shaken at 160 rpm for 72 h at 28 °C. The lowest extract concentrations without visible growth under optical microscope were defined as the MIC. The MFC was determined by using 2 µL from the treated wells, which were transferred to the culture medium and kept in microtiter plates with 100 µL broth per well for 72 h at 28 °C. The lowest concentration without visible microbial growth was determined as MFC, representing 99.5% death of the original inoculum. The negative control was DMSO solution (50 mL L^{-1}) and the positive controls were the commercial fungicides bifonazole (Srbolek) and ketoconazole (Zorkapharma, Šabac, Serbia).

Statistical analysis

Antimicrobial assays were performed in triplicate for each microorganism and the results showed the arithmetic mean (± standard deviation) examined by one-way analysis of variance and Tukey's honestly significant difference (HSD) test with $\alpha = 0.05$.

Results

L. crinitus pileus and stipe extracts showed bacteriostatic activity against all bacteria assessed (Table 1). Pileus MIC values ranged from 0.20 to 0.40 mg mL^{-1}, and stipe from 0.125 to 0.40 mg mL^{-1}, and positive controls from 0.04 to 0.25 mg mL^{-1} for streptomycin and 0.25 and 0.75 mg mL^{-1} for ampicillin (Table 1).

Compared to ampicillin control, pileus had MIC values on average 22% lower against *B. cereus*, *S. aureus* and *E. coli*; and 73% lower against *P. aeruginosa*. When compared to streptomycin control, the average pileus MIC values were 52% higher against *M. luteus*, *E. cloacae*, *E. coli*, *S. enterica*; 100% against *B. cereus* and *L. monocytogenes*, 400% against *S. aureus*, and equal against *P. aeruginosa* (Table 1). Compared to ampicillin control, stipe had MIC values on average 20% lower against *B. cereus* and 70% against *L. monocytogenes* and *P. aeruginosa*, equal against *E. coli* and *S. enterica*, and 47% higher against *M. luteus*, *S. aureus*, and *E. cloacae*; when compared to streptomycin control, the average values were 38% lower against *L. monocytogenes*, equal against *P. aeruginosa*, and 82% higher against *B. cereus*, *M. luteus*, *E. cloacae*, *E. coli*, *S. enterica* and 900% against *S. aureus* (Table 1). The pileus and stipe MIC values were equal against *B. cereus*, *M. luteus*, *P. aeruginosa*, and *S. enterica*, but pileus had average value 33% lower than that of stipe against *S. aureus*, *E. cloacae*, and *E. coli*, and 220% higher against *L. monocytogenes*.

Pileus and stipe showed bactericidal activity against all bacteria assessed (Table 1). Pileus and stipe MBC values ranged from 0.40 to 0.50 mg mL^{-1}, and controls ranged from 0.10 to 0.50 mg mL^{-1} for streptomycin and from 0.40 to 1.20 mg mL^{-1} for ampicillin (Table 1). Compared to ampicillin control, pileus had MBC values on average 24% lower against *E. cloacae*, *E. coli*, *S. enterica*, and 67% against *P. aeruginosa*, equal against *B. cereus*, *L. monocytogenes*, and *M. luteus*, and 25% higher against *S. aureus*; when compared to streptomycin control, the average values were 40% higher against *L. monocytogenes*, *M. luteus*, *E. cloacae*, *E. coli*, *P. aeruginosa*, 100% against *B. cereus*, and 400% against *S. aureus*, and equal against *S. enterica* (Table 1). When compared to ampicillin control, stipe had MBC values on average 33% lower against *S. enterica* and 67% against *P. aeruginosa*, equal against *B. cereus*, *L. monocytogenes*, *M. luteus*, *E. cloacae*, and *E. coli*, and 25% higher against *S. aureus*; when compared to streptomycin control, the average values were 53% higher against *L. monocytogenes*, *M. luteus*, *E. cloacae*, *E. coli*, *P. aeruginosa*, 100% against *B. cereus*, and 400% against *S. aureus*, and equal against *S. enterica* (Table 1). Pileus and stipe MBC values were equal against *B. cereus*, *L. monocytogenes*, *M. luteus*, *S. aureus*, *P. aeruginosa*, and *S. enterica*, but pileus had average value 20% lower than stipe against *E. cloacae* and *E. coli*.

This result indicates that pileus is as efficient as ampicillin control against *B. cereus*, *L. monocytogenes*, and *M. luteus*, and more efficient against *E. cloacae*, *E. coli*, *S. enterica* and especially against *P. aeruginosa*. When compared to streptomycin control, pileus was equal against *S. enterica* and less efficient mainly against *S. aureus*. Stipe is as efficient as ampicillin control against *B. cereus*, *L. monocytogenes*, *M. luteus*, *E. cloacae*, *E. coli*, and more efficient against *S. enterica* and especially against *P. aeruginosa*. When compared to streptomycin, stipe was as efficient against *S. enterica* and less efficient against *S. aureus*. Pileus and stipe have similar efficiency against *B. cereus*, *L. monocytogenes*, *M. luteus*, *S. aureus*, *P. aeruginosa*, and *S. enterica*, but pileus is more efficient against *E. cloacae* and *E. coli*, both Gram-negative.

Table 1 Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *Lentinus crinitus* basidiocarp pileus and stipe extract and positive controls streptomycin and ampicillin

Bacterium	Pileus (mg mL^{-1})	Stipe (mg mL^{-1})	Streptomycin (mg mL^{-1})	Ampicillin (mg mL^{-1})
	MIC **MBC**	**MIC** **MBC**	**MIC** **MBC**	**MIC** **MBC**
Bacillus cereus	0.20±0.02[b]	0.20±0.02[b]	0.10±0.003[a]	0.25±0.04[c]
	0.40±0.03[b]	0.40±0.06[b]	0.20±0.06[a]	0.40±0.03[b]
Enterobacter cloacae	0.30±0.03[b]	0.40±0.06[c]	0.20±0.02[a]	0.25±0.03[ab]
	0.40±0.05[b]	0.50±0.03[c]	0.30±0.04[a]	0.50±0.04[c]
Escherichia coli	0.30±0.04[b]	0.40±0.06[c]	0.20±0.02[a]	0.40±0.02[c]
	0.40±0.02[b]	0.50±0.05[c]	0.30±0.01[a]	0.50±0.06[c]
Listeria monocytogenes	0.40±0.02[c]	0.125±0.08[a]	0.20±0.03[b]	0.40±0.01[c]
	0.50±0.03[b]	0.50±0.03[b]	0.30±0.01[a]	0.50±0.02[b]
Micrococcus luteus	0.30±0.05[b]	0.30±0.05[b]	0.20±0.03[a]	0.25±0.06[ab]
	0.40±0.06[b]	0.40±0.06[b]	0.30±0.01[a]	0.40±0.01[b]
Pseudomonas aeruginosa	0.20±0.02[a]	0.20±0.02[a]	0.20±0.002[a]	0.75±0.03[b]
	0.40±0.06[b]	0.40±0.05[b]	0.30±0.00[a]	1.20±0.20[c]
Salmonella enterica	0.40±0.06[b]	0.40±0.03[b]	0.25±0.02[a]	0.40±0.02[b]
	0.50±0.03[a]	0.50±0.05[a]	0.50±0.02[a]	0.75±0.02[b]
Staphylococcus aureus	0.20±0.01[b]	0.40±0.06[c]	0.04±0.002[a]	0.25±0.06[b]
	0.50±0.03[c]	0.50±0.0.03[c]	0.10±0.002[a]	0.40±0.01[b]

*Averages followed by different letters in the same row for MIC or MBC differ by Tukey's HSD (honestly significant difference) test ($p \leq 0.05$)

Pileus and stipe showed fungistatic activity against all fungi assessed (Table 2). Pileus and stipe MIC values ranged from 0.03 to 0.50 mg mL^{-1} and for positive controls from 0.03 to 0.50 mg mL^{-1} for bifonazole and from 0.20 to 3.50 mg mL^{-1} for ketoconazole (Table 2).

Compared to ketoconazole control, pileus had MIC values on average 15% lower against *P. aurantiogriseum*, 92% against *A. ochraceus*, *P. ochrochloron*, and *T. virens*, and 25% higher against *A. fumigatus* and *A. niger*, 150% against *A. versicolor* and *T. funiculosum*; when compared to bifonazole control, the average values were 68% lower against *P. ochrochloron* and *T. virens*, and 68% higher against *A. fumigatus*, *A. ochraceus*, *A. niger*, *P. aurantiogriseum*, 150% against *T. funiculosum*, and 400% against *A. versicolor* (Table 2). When compared to ketoconazole control,

stipe had MIC values on average 38% lower against *A. niger*, *T. funiculosum*, 95% against *A. ochraceus*, *P. ochrochloron*, and *T. virens*, and 75% higher against *A. versicolor*, *P. aurantiogriseum*, and 150% against *A. fumigatus*; when compared to bifonazole control, the average values were 38% lower against *A. niger*, *T. funiculosum*, *P. ochrochloron*, *T. virens*, and 73% against *A. ochraceus*, and 244% higher against *A. fumigatus*, *A. versicolor*, and *P. aurantiogriseum* (Table 2). Pileus and stipe MIC values were equal against *P. ochrochloron*, but pileus had average value 56% lower than stipe against *A. fumigatus*, *P. aurantiogriseum*, and *T. virens*, and 43% higher against *A. versicolor*, 100% against *A. niger*, 300% against *T. funiculosum*, and 525% against *A. ochraceus*.

Pileus and stipe showed fungicidal activity against all fungi assessed (Table 2). Pileus and stipe MFC values ranged from 0.06 to 0.60 mg mL^{-1} and for controls, from 0.20 to 0.25 mg mL^{-1} for bifonazole and from 0.30 to 3.50 mg mL^{-1} for ketoconazole (Table 2). Compared to ketoconazole control, pileus had MFC values on average 17% lower against *P. aurantiogriseum*, 88% against *A. ochraceus*, *P. ochrochloron*, and *T. virens*, and 20% higher against *A. fumigatus*, *A. niger*, *A. versicolor*, and *T. funiculosum*; when compared to bifonazole control, the average values were 60% lower against *P. ochrochloron* and *T. virens*, and 25% higher against *P. aurantiogriseum*, 178% against *A. fumigatus*, *A. ochraceus*, *A. niger*, *A. versicolor*, and *T. funiculosum* (Table 2). Compared to ketoconazole control, stipe had MFC values on average 94% lower against *A. ochraceus*, *P. ochrochloron*, and *T. virens*, equal against *A. versicolor*, and 20% higher against *A. fumigatus*, *A. niger*, *T. funiculosum*, and 67% against *P. aurantiogriseum*; when compared to bifonazole control, the average values were 53% lower against *A. ochraceus*, *P. ochrochloron*, and *T. virens*, and 168% higher against *A. fumigatus*, *A. niger*, *A. versicolor*, *T. funiculosum*, and *P. aurantiogriseum* (Table 2). Pileus and stipe MFC values were equal against *A. fumigatus*, *A. niger*, *T. funiculosum*, and *P. ochrochloron*, but pileus had average value 51% lower that stipe against *P. aurantiogriseum* and *T. virens*, and 20% higher against *A. versicolor* and 733% against *A. ochraceus*.

The results indicate that pileus is more efficient compared to ketoconazole control mainly against *A. ochraceus*, *P. ochrochloron*, and *T. virens*, but when compared to bifonazole control, pileus is more efficient against *P. ochrochloron* and *T. virens* and less efficient mainly against *A. fumigatus*, *A. ochraceus*, *A. niger*, *A. versicolor*, and *T. funiculosum*. Stipe is as efficient as ketoconazole control against *A. versicolor* and more efficient against *A. ochraceus*, *P. ochrochloron*, and *T. virens*; even when compared to bifonazole control; stipe is more efficient against *A. ochraceus*, *P. ochrochloron*, and *T. virens*, and less efficient

Table 2 Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of *Lentinus crinitus* basidiocarp pileus and stipe extract and positive controls bifonazole and ketoconazole

Fungus	Pileus (mg mL^{-1}) MIC MFC	Stipe (mg mL^{-1}) MIC MFC	Bifonazole (mg mL^{-1}) MIC MFC	Ketoconazole (mg mL^{-1}) MIC MFC
Aspergillus fumigatus	0.25 ± 0.03^b 0.60 ± 0.06^c	0.50 ± 0.02^c 0.60 ± 0.03^c	0.15 ± 0.02^a 0.20 ± 0.00^a	0.20 ± 0.02^{ab} 0.50 ± 0.03^b
Aspergillus ochraceus	0.25 ± 0.01^c 0.50 ± 0.02^c	0.04 ± 0.002^a 0.06 ± 0.003^a	0.15 ± 0.03^b 0.20 ± 0.02^b	1.50 ± 0.20^d 2.00 ± 0.30^d
Aspergillus niger	0.25 ± 0.03^b 0.60 ± 0.03^c	0.125 ± 0.08^a 0.60 ± 0.04^c	0.15 ± 0.04^{ab} 0.20 ± 0.06^a	0.20 ± 0.02^{ab} 0.50 ± 0.01^b
Aspergillus versicolor	0.50 ± 0.02^d 0.60 ± 0.03^c	0.35 ± 0.08^c 0.50 ± 0.06^b	0.10 ± 0.01^a 0.20 ± 0.03^a	0.20 ± 0.06^b 0.50 ± 0.02^b
Penicillium aurantiogriseum	0.17 ± 0.06^{ab} 0.25 ± 0.03^{ab}	0.35 ± 0.04^c 0.50 ± 0.03^c	0.10 ± 0.02^a 0.20 ± 0.03^a	0.20 ± 0.03^b 0.30 ± 0.02^b
Penicillium ochrochloron	0.09 ± 0.002^a 0.125 ± 0.08^a	0.09 ± 0.003^a 0.125 ± 0.06^a	0.20 ± 0.01^b 0.25 ± 0.06^b	2.50 ± 0.30^c 3.50 ± 0.60^c
Talaromyces funiculosum	0.50 ± 0.05^c 0.60 ± 0.006^c	0.125 ± 0.02^a 0.60 ± 0.06^c	0.20 ± 0.02^b 0.25 ± 0.06^a	0.20 ± 0.02^b 0.50 ± 0.06^b
Trichoderma virens	0.03 ± 0.001^a 0.06 ± 0.003^a	0.09 ± 0.005^b 0.125 ± 0.06^b	0.15 ± 0.03^c 0.20 ± 0.01^c	1.00 ± 0.20^d 1.00 ± 0.20^d

*Averages followed by different letters in the same row for MIC or MFC differ by Tukey's HSD (honestly significant difference) test ($p \le 0.05$)

against *A. fumigatus*, *A. niger*, *A. versicolor*, *T. funiculosum*, and *P. aurantiogriseum*. Pileus and stipe have similar efficiency mainly against *P. ochrochloron*; however, pileus is more efficient against *P. aurantiogriseum* and *T. virens*, and stipe is more efficient against *A. ochraceus*.

Discussion

L. crinitus basidiocarp pileus and stipe extract showed broad antimicrobial activity. For the extract of the entire basidiocarp of *L. crinitus*, MIC values from 0.12 to 0.499 mg mL^{-1} are reported (Bertéli et al. 2021a), which were similar to those of our study for the same bacteria. Regarding antifungal activity, pileus and stipe showed broad spectrum of action against all fungi assessed. The antifungal activity of the extract of the entire basidiocarp of *L. crinitus* had MIC values ranging from 0.0399 to 0.499 mg mL^{-1} (Bertéli et al. 2021a) against the same fungal strains of our study. However, our study is the first report on the antimicrobial activity of pileus and stipe detached from *L. crinitus* basidiocarp.

The stipe antimicrobial activity was similar to that of pileus to control a wide diversity of bacteria and fungi. Stipe had MIC 3.2 times greater than pileus against *L. monocytogenes*, the pathogenic agent of meningitis and listeriosis, which can lead to death (Koopmans et al. 2018); the MFC value was 8.3 times higher against *A. ochraceus*, a known producer of highly cytotoxicity mycotoxins such as ochratoxins among many others (Bräse et al. 2009). Stipe represents about 24% of the total *L. crinitus* basidiocarp mass and smaller amount of proteins, ashes, organic acids as oxalic and malic acid, and phenolic acids such as *p*-hydroxybenzoic compared to pileus (Bertéli et al. 2021b). The chemical composition, cytotoxicity, and antioxidant activity results for the basidiocarp (pileus and stipe apart) of our study were published in Bertéli et al. (2021b) as the samples, the batch, and the assay were the same.

Phenolics are important antioxidant compounds produced by mushrooms such as *p*-hydroxybenzoic acid, which has also antimicrobial activity (Manuja et al. 2013; Naczk and Shahidi 2006). In *L. crinitus* basidiocarp, *p*-hydroxybenzoic was reported at 537±4 µg 100 g^{-1} in the pileus and at 791±3 µg 100 g^{-1} in the stipe (Bertéli et al. 2021b); p-hydroxybenzoic acid from *Ganoderma lucidum* basidiocarp has MIC from 0.003 to 0.12 mg mL^{-1} against *A. fumigatus*, *A. versicolor*, *A. ochraceus*, *A. niger*, *T. viride*, *P. funiculosum*, *P. ochrochloron*, and *P. verrucosum* (Heleno et al. 2013), which suggests that this phenolic acid is the main compound involved in the antimicrobial activity of *L. crinitus* basidiocarp stipe. The stipe antimicrobial activity provides potential uses of this residue as a natural source of antimicrobial compounds to be added to foods. *L.*

crinitus basidiocarp stipe – although consumed by Amazon Indians – is the most fibrous part of the basidiocarp (Li et al. 2018; Prance 1984) and a residue from mushroom cultivation. *L. crinitus* basidiocarp stipe extracts could be added to foods and cosmetics as adjuvants for microbial control. *L. crinitus* basidiocarp stipe and pileus showed reduced cytotoxicity against HepG2 and no cytotoxicity against MCF7, NCI-H460, and HeLa tumor cell lines (Bertéli et al. 2021b). Furthermore, the antimicrobial activity of *L. crinitus* basidiocarp stipe is greater than or equal to the main antimicrobials added in foods and cosmetics, such as sodium sulfite and potassium metabisulfite, which had, for the same microorganisms of our study, MIC values from 1.00 to 3.97 mg mL^{-1} and from 0.505 to 2.01 mg mL^{-1}, respectively, and MBC values from 0.497 to 4.04 mg mL^{-1} and from 0.502 to 2.02 mg mL^{-1}, respectively (Bertéli et al. 2021a). Compared to results of our study – with the same strains – the stipe extract presented lower MIC values from 0.125 to 0.40 mg mL^{-1} and MBC values from 0.40 to 0.50 mg mL^{-1}. Regarding antifungal activity – for the same fungal strains – MIC values reported for sodium sulfite and potassium metabisulfite were from 1.01 to 2.03 mg mL^{-1} and from 0.500 to 2.01 mg mL^{-1}, respectively; MFC values ranged from 2.01 to 4.04 mg mL^{-1} for sodium sulfite and potassium metabisulfite (Bertéli et al. 2021a). Again, compared to results of our study – for the same fungal strains – stipe extract presented lower MIC values from 0.04 to 0.50 mg mL^{-1} and MFC from 0.06 to 0.60 mg mL^{-1}. Several synthetic food preservatives such as sulfites – widely used in foods – had been related with undesirable effects on human health such as allergies (Pisoschi et al. 2018). Thus, natural compounds with antimicrobial activity such as *L. crinitus* basidiocarp pileus and stipe are natural alternatives for use in food preservation with nutraceutical properties.

Conclusions

The extracts of *L. crinitus* basidiocarp-pileus and basidiocarp-stipe have bactericide and fungicide activity against all the studied microorganisms. Pileus and stipe are as efficient as ampicillin control against *B. cereus*, *L. monocytogenes*, and *M. luteus*, and against *E. cloacae* and *E. coli* for stipe only. Pileus and stipe are more efficient than ampicillin control against *S. enterica* and *P. aeruginosa*, and against *E. cloacae* and *E. coli* for pileus only. Compared to streptomycin control, pileus and stipe are equally effective against *S. enterica* and less effective mainly against *S. aureus*. Compared to ketoconazole control, pileus and stipe are more efficient against *A. ochraceus*, *P. ochrochloron*, and *T. virens*, and against *P. aurantiogriseum* for pileus only, and equal to against *A. versicolor* for stipe only. Pileus and stipe are

more efficient than bifonazole control against *P. ochrochloron* and *T. virens*, and against *A. ochraceus* for stipe only. Both *L. crinitus* basidiocarp pileus and stipe have antimicrobial activity against foodborne pathogens and food spoilage microorganisms and are promising alternatives for use in the food, agricultural, and pharmaceutical industries.

Author contributions Míria Benetati Delgado Bertéli: formal analysis, investigation, methodology. Mariana Martins Magalhães de Souza: formal analysis and writing. Lillian Barros: methodology, writing - review & editing. Isabel C. F. R. Ferreira: methodology, writing - review & editing. Jasmina Glamočlija: methodology, writing - review & editing. Marina Soković: methodology, writing - review & editing. Douglas Cardoso Dragunski: methodology, writing - review & editing. Juliana Silveira do Valle: writing - review & editing. Ederlan de Souza Ferreira: writing - review & editing. Laise Cedraz Pinto: writing - review & editing. Carolina Oliveira de Souza: writing - review & editing. Suelen Pereira Ruiz: writing - review & editing. Giani Andrea Linde: conceptualization, writing - review & editing, supervision. Nelson Barros Colauto: conceptualization, writing - review & editing, supervision.

Declarations

Conflict of interest The authors declared that they have no conflicts of interest in this work.

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