Current Research in Environmental & Applied Mycology (Journal of Fungal Biology)
10(1): 85–95 (2020) ISSN 2229-2225
www.creamjournal.org
Article
Doi 10.5943/cream/10/1/9

Screening of antimicrobial substances from mushrooms (Agaricales) of southern Brazil

Rosenberger MG[1], Paulert R[2] and Cortez VG[3]

[1] *Department of Biosciences, Federal University of Paraná, Rua Pioneiro, 2153, Palotina, 85950-000, Paraná, Brazil*
[2] *Department of Agronomic Sciences, Federal University of Paraná, Rua Pioneiro, 2153, Palotina, 85950-000, Paraná, Brazil*
[3] *Department of Biodiversity, Federal University Federal of Paraná, Rua Pioneiro, 2153, Palotina, 85950-000, Paraná, Brazil*

Rosenberger MG, Paulert R, Cortez VG 2020 – Screening of antimicrobial substances from mushrooms (Agaricales) of southern Brazil. Current Research in Environmental & Applied Mycology (Journal of Fungal Biology) 10(1), 85–95, Doi 10.5943/cream/10/1/9

Abstract

Mushrooms are a source of bioactive substances due to the synthesis of secondary metabolites, which are useful in the search of new substances for pharmaceutical use. The aim of this study was to evaluate extracts with antimicrobial potential of native South Brazilian mushrooms against pathogenic bacteria and yeast. Basidiomata of 14 mushroom species were collected, dried, grounded and extracted with methanol in the Soxhlet system. The antimicrobial activity was tested by agar diffusion and direct bioautography methods against *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans*. Six extracts inhibited the tested microorganisms, showing moderate sensitivity in the agar diffusion test. On the other hand, using the bioautography method, nine extracts presented activity and revealed ten antimicrobial substances, of which five are indicative of terpenes. Among the species investigated, to our knowledge, this is the first report of antimicrobial activity of *Simocybe tucumana* and *Mycena euspeirea.*

Key words – antibacterial activity – basidiomycetes – *Mycena euspeirea* – natural products – *Simocybe tucumana*

Introduction

Mushrooms (Phylum Basidiomycota) naturally produce numerous substances with bioactive properties such as antitumoral, antidiabetic, immunomodulatory, antioxidant, trypanocide, leishmanicide, anti-inflammatory, antiviral and antimicrobial (Rosa et al. 2009, Alves et al. 2012, 2013, Ajith & Janardhanan 2015). The research on new antimicrobial substances is highly necessary due to the emergence of resistant bacterial strains and new opportunistic species (Magiorakos et al. 2012, Korzeniewska et al. 2013, Krupodora et al. 2016).

Many substances with antimicrobial activity were obtained from basidiomycetes, but most studies evaluated only the antimicrobial activity of the total/crude extracts, without testing the isolated compounds (Krüzselyi et al. 2016, Rosenberger et al. 2018). The use of methodologies that verify antimicrobial properties of isolated substances is important to verify profiles of such compounds (Krüzselyi et al. 2016). The direct bioautography test combined with thin-layer

Submitted 4 September 2019, Accepted 25 February 2020, Published 27 February 2020
Corresponding Author: Marina Giombelli Rosenberger – e-mail – rosenberger.mg@gmail.com

chromatography (TLC) is widely used to screen compounds with antimicrobial activity (Moricz & Ott 2017). In this technique, the chromatogram containing separated substances is introduced into a biodetection process (Krüzselyi et al. 2016, Moricz & Ott 2017), and subjected to a colorimetric analysis to detect the inhibition of microbial growth (Valgas et al. 2007). Furthermore, for the detection of terpene derivatives, TLC plates are made with sulfuric anisaldehyde and are visualized as purple/pink spots (Santos et al. 2014) since the antibiotics naturally produced by mushrooms are mainly secondary metabolites, especially terpenes (Alves et al. 2012, Shen et al. 2017).

South America represents a poorly explored natural reservoir of species with possible uses, including basidiomycetes. Most studies of the antimicrobial activity of mushrooms (Agaricales) are from Chile, Brazil, and Uruguay and utilized crude extracts. Therefore, screening of basidiomycetes for antimicrobial activity is important in exploring the potential of these organisms in the continent (Rosenberger et al. 2018).

Thus, due to the properties of the mushrooms belonging to Phylum Basidiomycota and the growing interest in the discovery of new antimicrobial substances, the aim of this study was to verify the antimicrobial potential of basidiomycetes extracts as well to verify the profiles of the bioactive compounds.

Materials & Methods

Collection and Dehydration of Basidiomata

Basidiomata were collected in Palotina, State of Paraná, South Brazil, from October 2016 to May 2017. After collecting, mushrooms were dried in a chamber with forced air circulation at 40°C until they reached constant weight (Carvalho et al. 2012). After drying, basidiomata were stored in paper bags for further extraction and voucher specimens were preserved at the Herbarium of Federal University of Paraná, *Campus* Palotina (HCP). The fourteen mushrooms species were: *Calvatia rugosa*, *Coprinopsis* sp., *Crinipellis siparunae*, *Leucoagaricus* sp., *Leucocoprinus* cf. *brebissonii*, *L. venezuelanus*, *Leucopaxillus gracillimus*, *Marasmius haematocephalus*, *Mycena euspeirea*, *Pleurotus opuntiae*, *Psathyrella candolleana*, *Psathyrella* sp., *Simocybe tucumana*, and *Xeromphalina tenuipes* (Fig. 1).

Preparation of Extracts

Basidiomata were ground until a fine powder was obtained. The material extraction was carried out with methanol, in the Soxhlet system, during ca. 8 hours for each species, comprising five complete cycles in the extractor (Figueiredo & Silva 2014, Ajith & Janardhanan 2015). Methanol was evaporated in a rotary evaporator under vacuum at 45°C. Extraction yields were calculated for each mushroom. The crude methanolic extracts were placed into glass vials and stored at 4°C for further use.

Test microorganisms

Bacillus cereus ATCC 11778, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Staphylococcus aureus* ATCC 25923 and *Candida albicans* ATCC 10231 were used in the biological tests. All strains were first cultured in Brain Heart Infusion broth for 18 hours and afterward, transferred in Petri dishes containing Mueller-Hinton agar and incubated in a growth chamber at 35°C, from 18 to 24 hours, to obtain active colonies.

Agar Diffusion Test

The well-variant of agar diffusion method was used for determination of antimicrobial activity. Crude extracts were previously solubilized with dimethylsulfoxide (DMSO) (Klaus et al. 2015) and four concentrations (1,2; 2,5; 5 and 10 mg mL^{-1}) of each extract were tested. Bacterial inoculum was prepared using the isolated colonies placed in a 0.9% saline solution, comparing turbidity to the 0.5 tube of McFarland scale, which corresponds to the concentration 1×10^{8} CFU mL^{-1} (Jorgensen & Ferraro 2009).

With a sterile swab, bacterial suspension was inoculated over entire surface of Petri dishes containing Mueller-Hinton agar. In each plate, with the aid of a sterile cutter, six wells were made for testing the four concentrations, negative and positive controls. For bacteria tests, the positive control was gentamicin (0.1 mg mL^{-1}; Younes et al. 2014) and for *C. albicans* nystatin (10 mg mL^{-1}; Shakibaie et al. 2015) was used. Negative control was sterile distilled water plus DMSO. In each well, 40 µL of the extract concentrations or controls were added. Plates were incubated at 35°C and reading performed among 16-18 hours after incubation, by measuring inhibition halos in millimeters (CLSI 2012).

Fig. 1 – Basidiomata of mushrooms collected from South Brazil and screened for antimicrobial property. A *Calvatia rugosa*. B *Coprinopsis* sp. C *Crinipellis siparunae*. D *Leucocoprinus* cf. *brebissonii*. E *Leucopaxillus gracillimus*. F *Mycena euspeirea*. G *Pleurotus opuntiae*. H *Psathyrella candolleana*. I *Simocybe tucumana*.

Thin-layer Chromatography (TLC)

Separation of the substances present in the crude methanolic extracts was performed through TLC. Crude extracts were solubilized with 10 µl of methanol and applied with a capillary in the aluminum chromato-sheets 7×5 cm (silica gel G60 F_{254}, Merck). For mobile phase, the solvent system was ethyl acetate:hexane (1:1). Plates were developed at a distance of 6 cm and the solvent was evaporated at room temperature. After drying, stains were observed in ultraviolet (UV) light at 254 nm and revealed with sulfuric anisaldehyde to detect terpenes (Jesionek et al. 2013, Santos et al. 2014, Krüzselyi et al. 2016). The retention factor (R_f), ratio between the distance traveled by the substance and the distance traveled by the mobile phase, was calculated (Valgas et al. 2007).

Direct Bioautography

Chromatographic run was performed as described above. However, for use in the bioautography, the test plate was not developed with sulfuric anisaldehyde (Valgas et al. 2007, Balouiri et al. 2016). In addition, methanolic crude extracts were applied in the chromatographic plates as a single point and directly used in the biological test (without running in a mobile phase).

Bacterial inoculum of *B. cereus*, *E. coli*, *P. aeruginosa*, and *S. aureus* was prepared using the isolated colonies in a 0.9% saline solution, as described for diffusion method.

TLC with separated substances and the not runned plates were submerged in the bacterial suspension for eight seconds. Later, plates were transferred to another sterile Petri dish containing sterilized moist cotton. Plates were incubated for 16–18 hours at 35°C. To visualize the activity, bioautograms were sprayed with a solution of nitro-tetrazolium blue chloride and re-incubated for 3-4 hours at 35°C. The zones of inhibition were observed, measured and compared to the R_f values. For each extract, four plates were used for biological tests (one plate for each bacteria).

Results

Antimicrobial activity using agar diffusion test

Among the 14 methanolic crude extracts from mushrooms collected in South Brazil and evaluated by the diffusion test, only six species exhibited inhibitory effects and the results obtained are shown in Table 1.

Three mushroom extracts inhibited the growth of *B. cereus*, namely: *Calvatia rugosa*, *Leucoagaricus* sp. and *Psathyrella* sp. Major inhibition halo of *B. cereus* was observed with *C. rugosa* extract and 10.0 mg mL^{-1} was most active concentration. The second most effective extract against *B. cereus* was obtained from *Psathyrella* sp. (10.0 mg mL^{-1}), followed by the extract of *Leucoagaricus* sp.

The crude extract of *Leucocoprinus* cf. *brebissonii* was the only one able to inhibit *E. coli*, whose larger inhibition halo was observed under concentration of 10.0 mg mL^{-1} (Table 1, Fig. 2B).

Table 1 Inhibition halo averages (mm) of fungal methanolic extracts against microorganisms.

Concentration	**10.0 mg mL^{-1}**	**5.0 mg mL^{-1}**	**2.5 mg mL^{-1}**	**1.2 mg mL^{-1}**
Bacillus cereus				
Calvatia rugosa	6.3	2.0	0.0	1.3
Leucoagaricus sp.	1.8	2.7	2.7	0.5
Psathyrella sp.	2.8	1.8	2.0	1.2
Gentamicin	11.0			
Escherichia coli				
Leucocoprinus cf. *brebissonii*	2.0	1.5	1.0	1.0
Gentamicin	10.0			
Pseudomonas aeruginosa				
Crinipellis siparunae	2.0	1.5	0.0	0.0
Leucocoprinus cf. *brebissonii*	3.0	3.0	1.0	0.0
Gentamicin	7.0			
Staphylococcus aureus				
Crinipellis siparunae	2.0	2.0	0.0	0.0
Leucocoprinus cf. *brebissonii*	1.0	1.0	0.0	0.5
Leucocoprinus venezuelanus	2.1	1.8	1.0	0.0
Xeromphalina tenuipes	1.7	1.7	1.2	1.0
Gentamicin	11.0			
Candida albicans				
Psathyrella candolleana	3.8	0.0	0.0	0.0
Nystatin	7.0			

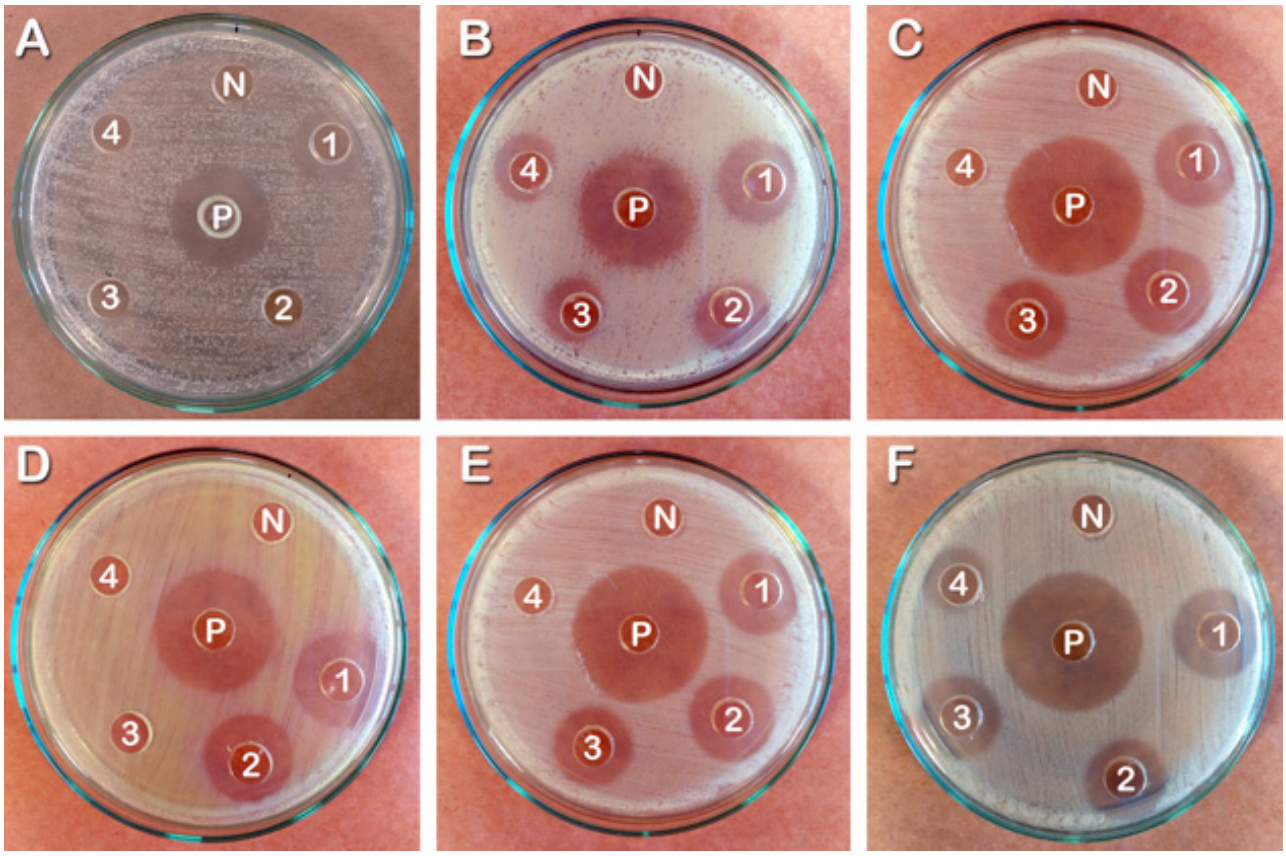

Fig. 2 – Agar diffusion test against pathogenic microrganisms using methanolic extracts from basidiomycetes collected from South Brazil. A Extract of *P. candolleana* against *C. albicans*. B Extract of *L.* cf. *brebissonii* against *E. coli*. C Extract of *L.* cf. *brebissonii* against *P. aeruginosa*. D Extract of *C. siparuanae* against *P. aeruginosa*. E Extract of *L. venezuelanus* against *S. aureus*. F Extract of *X. tenuipes* against *S. aureus*. P: positive control (gentamicin or nystatin); N: negative control (water and DMSO). Extract concentration 1: 10.0 mg mL^{-1}; 2: 5.0 mg mL^{-1}; 3: 2.5 mg mL^{-1}; 4: 1.2 mg mL^{-1}.

Two mushroom extracts inhibited growth of *P. aeruginosa*: *Leucocoprinus* cf. *brebissonii* and *C. siparunae*. The larger inhibition halo observed for these bacteria was obtained by the extract of *Leucocoprinus* cf. *brebissonii* at the concentrations of 5.0 and 10.0 mg mL^{-1} (Table 1, Fig. 2C). Second largest inhibition halo of *P. aeruginosa* was observed by the extract of *C. siparunae* (10.0 mg mL^{-1}) (Fig. 2D).

It is important to note that only the extract of *P. candolleana* inhibited the growth of *C. albicans*, exhibiting activity under the concentration of 10.0 mg mL^{-1} (Table 1, Fig. 2A).

Thin-layer Chromatography and Direct Bioautography

The solvent mixture (ethyl acetate and hexane 1:1) used as mobile phase was able to separate substances present in the methanolic crude extracts, except for the *Leucoagaricus* sp. extract, which showed no visible substances.

Bioautographs performed against *B. cereus* revealed that only the extract of *M. euspeirea* presented antimicrobial activity. The substance responsible for such activity had retention factor (Rf) of 0.56 (Fig. 3B). However, unlike to isolated substances, crude extract of *M. euspeirea* did not present antimicrobial activity against *B. cereus*, when applied at one point on the chromatographic plate.

For *E. coli*, the bioautographic tests did not detect any substance or extract with antimicrobial activity, since no inhibition halo was observed.

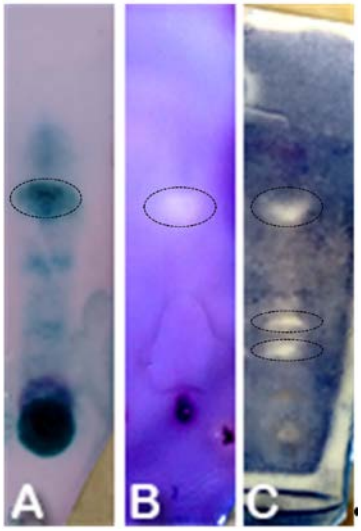

Fig. 3 – Antimicrobial activity evaluation of *Mycena euspeirea* methanolic extract. A Thin-layer chromatography revealed with sulfuric anisaldehyde. B Bioautogram against *B. cereus*. C bioautogram against *S. aureus*.

Analyzing the antibiotic activity against *S. aureus*, the bioautograms showed that extracts of *Coprinopsis* sp., *Psathyrella* sp., *M. euspeirea* and *X. tenuipes* contain substances with inhibitory activity. The extract of *M. euspeirea* presented three substances inhibiting the bacterial growth (Fig. 3C). Only one substance isolated from *Coprinopsis* sp. extract inhibited the *S. aureus* (Fig. 4).

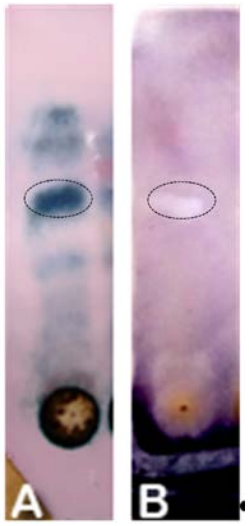

Fig. 4 – Antimicrobial activity evaluation of *Coprinopsis* sp. methanolic extract. A Thin-layer chromatography revealed with sulfuric anisaldehyde. B Bioautogram against *S. aureus* revealed with nitro-tetrazolium blue chloride.

Inhibition halos against *S. aureus* were observed in the bioautograms containing the crude extracts applied at one point (not run with the mobile phase). Six extracts obtained from *Coprinopsis* sp., *Psathyrella* sp., *M. euspeirea*, *Leucocoprinus* cf. *brebissonii*, *L. venezuelanus* and *S. tucumana* showed inhibition halos, revealing antibacterial activity against this Gram-positive bacterium. However, for crude extract of *X. tenuipes* no antibiotic activity was observed.

For not-run bioautograms it was observed that extracts of *Coprinopsis* sp., *M. euspeirea*, *M. haematocephalus*, and *P. candolleana* presented inhibition halos, revealing that crude extracts of the mushrooms showed antibacterial activity against *P. aeruginosa*. Among the developed and tested bioautograms only the extracts of *S. tucumana* (Fig. 5B) and *X. tenuipes* presented isolated

substances with antibacterial activity against *P. aeruginosa*. In contrast, not-run bioautograms of *S. tucumana* and *X. tenuipes* not showed inhibition halos against this bacterium.

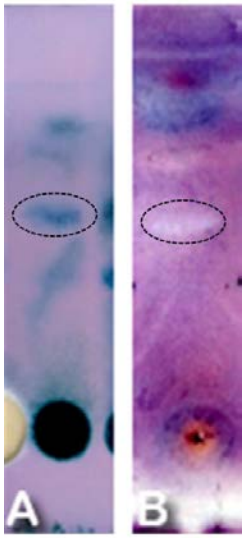

Fig. 5 – Antimicrobial activity evaluation of *Simocybe tucumana* methanolic extract. A Thin-layer chromatography revealed with sulfuric anisaldehyde. B Bioautogram against *Pseudomonas aeruginosa* revealed with nitro-tetrazolium blue chloride.

Discussion

Species of *Calvatia* are widely studied from the biotechnological point of view, presenting medicinal properties, as antitumor, anticancer, antiviral, antibacterial and antifungal activities (Coetzee & Van Wyk 2009). Ethanolic extracts of *Calvatia excipuliformis* basidiomata demonstrated activity against bacteria *B. subtilis*, *E. coli*, *Micrococcus luteus*, *P. aeruginosa* and *Staphylococcus epidermidis* (Nowacka et al. 2014). Ethanolic extract of *Calvatia* sp. inhibited growth of *E. coli* and *S. aureus*, and the aqueous extract inhibited only *S. aureus* (Bala et al. 2011). These results corroborate with our study and thus, methanolic extract of *C. rugosa* inhibited the growth of *B. cereus* when tested at a concentration from 10,0; 5,0 and 1,2 mg mL^{-1} (inhibition halo 6,3; 2,0 and 1,3 mm, respectively).

The culture broth of *Crinipellis schevczenkovi* presented activity against *B. subtilis*, *E. coli* and *S. aureus* using the agar diffusion method (Krupodora et al. 2016), as we verified for the extract of the native basidiomata of *C. siparunae*. On the other hand, the four diterpenes isolated from *C. stipitaria* did not show activity against *E. coli*, *B. subtilis*, *Bacillus pumilus* and *S. aureus* (Li & Shen 2010).

Antibacterial potential of the species of *Leucoagaricus* also has been widely investigated. The crude extract of *Leucoagaricus* sp. showed antibacterial activity against *E. coli*, *Klebsiella pneumoniae* and *Enterococcus* sp. (Deshmukh et al. 2014). In a study with *L. gongylophorus*, the culture broth inhibited the growth of *E. coli*, but did not present activity versus *B. subtilis*, *P. aeruginosa*, *Salmonella typhimurium* and *S. aureus* (Dighe & Agate 2000). Ethanolic extract of *L. leucothites* inhibited the growth of several bacteria, such as *B. cereus*, *E. coli*, *Listeria monocytogenes*, *P. aeruginosa*, *Proteus vulgaris*, *S. aureus*, *Salmonella enteritidis*, *Yersinia enterocolitica*, and *Shigella sonnei* (Aslim & Ozturk 2011). Extracts of *L. pudicus* also inhibited a wide variety of species of bacteria being: *B. subtilis*, *E. coli*, *Enterobacter aerogenes*, *S. typhimurium*, *S. aureus* and *Staphylococcus epidermidis* (Yamaç & Bilgili 2006). We observed antimicrobial activity of the extract of the *Leucoagaricus* sp. only against *B. cereus*.

Ethyl acetate extracts of *Leucocoprinus* cf. *longistriatus* and *Leucocoprinus* sp. were tested against many different species of bacteria and did not present inhibitory activity (Rosa et al. 2003).

On the contrary, we verified antibacterial action of the methanolic extracts of *Leucocoprinus* sp. and *Leucocoprinus* cf. *brebissonii*.

Several species of *Mycena* have already been tested for antimicrobial potential, such as *M.* cf. *alcalina*, *M. aurantiomarginata*, *M. leucogala*, *M. maculata* (Suay et al. 2000), *M. pura* (Antonyuk et al. 2010, Aqueveque et al. 2010), *M. hialinotricha* (Reinoso et al. 2013) among others.

The present study forms the first report of antibacterial activity related to *Mycena euspeirea*. When the substances of the crude extract were separated by TLC, the active substance (R_f 0.56) was indicative of terpene (Fig. 3A). Besides the antibacterial activity, another interesting feature of *M. euspeirea* is bioluminescence, which evidence the rich secondary metabolism of this species (Desjardin et al. 2008).

Antimicrobial activity studies also have been done using extracts from *Psathyrella* species. Extracts (both aqueous and ethanolic) obtained from the basidiomata of the *Psathyrella* sp. showed activity against *E. coli* and *S. aureus* (Bala et al. 2011). The extract of the *Psathyrella* sp. inhibited the growth of *B. subtilis* and *Escherichia faecalis*, but did not inhibit the growth of *E. coli*, *P. aeruginosa* and *S. aureus* (Reinoso et al. 2013). Chloroform extract of *P. candolleana* inhibited the growth of *Moraxella catarrhalis* and *Streptococcus pneumoniae*, on the other hand, the extract of *P. piluliformis* but did not present activity against any tested bacteria (Liktor-Busa et al. 2016). Culture filtrate of *P. atroumbonata* showed inhibitory activity against *E. coli* and *S. aureus* (Ayodele & Idoko 2011).

The antimicrobial activity, against *S. aureus*, of the extracts of *Psathyrella* sp. and *P. candolleana* was observed in the agar diffusion method and in the bioautographic test. The results of this study showed that *Psathyrella* sp. extract has a bioactive substance with R_f 0.54 with a visible pinkish colour after the use of sulfuric anisaldehyde, being indicative of belonging to the terpene group. Terpenes are widely recognized as antimicrobial compounds (Greay & Hammer 2015). Research indicates that these compounds compromise the integrity and function of the cell membrane of microorganisms (Greay & Hammer 2015).

Methanolic extract of *S. tucumana* also presented a substance with antibacterial potential and can be indicative of a terpene (Fig. 5A). This is the first report of antibacterial activity of *S. tucumana* and also seems to be the first for the genus *Simocybe*.

Ethyl acetate extract of *Xeromphalina tenuipes* showed no inhibitory activity against the tested bacteria according to a previous study (Rosa et al. 2003). In contrast to data obtained for substances, characterized as sesquiterpenes, isolated from *Xeromphalina* sp., which presented antibacterial and antifungal activity (Liermann et al. 2010). We observed that the extract of *X. tenuipes* showed a substance with antibacterial potential (R_f 0.51), being indicative of terpene too. Antibacterial activity of the crude methanolic extract of *X. tenuipes* was also observed in the agar diffusion test.

Antifungal activity of basidiomycete extracts against *C. albicans* has been investigated. Methanolic extract of *P. atroumbonata* did not present inhibitory activity when tested against *C. albicans* (Jonathan et al. 2008). The extracts of *Irpex lacteus* and *Oudemansiella canarii* inhibited the growth of this yeast (Rosa et al. 2003). Here, the extract of native basidiomata of *P. candolleana* showed activity against *C. albicans.*

When the inhibition halos of the extracts were compared with positive control (gentamicin or nystatin), we verified that extracts presented smaller halos, indicating discrete antibacterial activity. This fact may be associated with the amount of substances present, since the crude extract were used.

Many reasons may be associated with environmental and nutritional factors, since these conditions may alter the metabolism of the mushrooms, leading to variations in the production of their secondary metabolites. Thus, mushrooms from different ecosystems and mushrooms grown on different substrates can produce distinct antimicrobial substances (Aqueveque et al. 2010, Shen et al. 2017). The bioprospection of basidiomycetes aiming the antimicrobial potential is important for the search for new substances and given the extent of Brazilian mycobiota, it should be expected that promising substances with antimicrobial properties can be discovered.

Conclusion

Of the 14 mushroom methanolic extracts, six have compounds potentially useful in inhibiting the tested microorganisms, although they presented moderate sensitivity in the agar diffusion test.

On the other hand, when using the bioautography method, we observed that nine crude extracts and ten substances (belonging to five mushrooms species) showed antimicrobial properties. Among these substances, five of them are indicative of terpenes, which are widely recognized as antimicrobial substances. However, the identification of the bioactive compounds and the study of mechanisms of actions are further necessary.

Among the species investigated, to our knowledge, this is the first report of antimicrobial activity of *Simocybe tucumana* and *Mycena euspeirea* extracts.

Acknowledgements

We thank the financial support of Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Processo 483455/2013-3), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Universidade Federal do Paraná (UFPR).

References

Ajith TA, Janardhanan KK. 2015 – Medicinal Mushroom Cracked-Cap Polypore, *Phellinus rimosus* (Higher Basidiomycetes) Attenuates Acute Ethanol-Induced Lipid Peroxidation in Mice. International Journal of Medicinal Mushrooms, 17(11), 1061–1067.

Alves MJ, Ferreira ICFR, Dias J, Teixeira V et al. 2012 – A review on antimicrobial activity of mushroom (Basidiomycetes) extracts and isolated compounds. Planta Medica, 78(16), 1707–1718.

Alves MJ, Ferreira ICFR, Dias J, Teixeira V et al. 2013 – A review on antifungal activity of mushroom (Basidiomycetes) extracts and isolated compounds. Current Topics in Medicinal Chemistry, 13(21), 2648–2659.

Antonyuk VO, Nemchenko OO, Tymchuk IV, Danileuchenko VV, Stoika RS. 2010 – Studies of hemolytical and antimicrobical action of *Amanita virosa* Secr. and *Mycena pura* (Fr.) Kumm. poisonous mushrooms lectins. Biopolymers and Cell, 26(1), 29–35.

Aqueveque P, Anke T, Saéz K, Silva M, Becerra J. 2010 – Antimicrobial activity of submerged cultures of Chilean Basidiomycetes. Planta Medica, 76(15), 1787–1791.

Aslim B, Ozturk S. 2011 – Phenolic composition and antimicrobial and antioxidant activities of *Leucoagaricus leucothites* (Vittad.) Wasser. Journal of Medicinal Food, 14(11), 1419–1424.

Ayodele SM, Idoko ME. 2011 – Antimicrobial activities of four wild edible mushrooms in Nigeria. International Journal of Science and Nature, 2(1), 55–58.

Bala N, Aitken EA, Fechner N, Cusack A, Steadman KJ. 2011 – Evaluation of antibacterial activity of Australian basidiomycetous macrofungi using a high-throughput 96-well plate assay. Pharmaceutical Biology, 49(5), 492–500.

Balouiri M, Sadiki M, Ibnsouda SK. 2016 – Methods for in vitro evaluating antimicrobial activity: A review. Journal of Pharmaceutical Analysis, 6(2), 71–79.

Carvalho CSM, Aguiar LVB, Sales-Campos C, Minhoni MTA, Andrade MCN. 2012 – Bromatological characterization of *Pleurotus ostreatus* grown in different residues from different banana cultivars. Interciencia, 37(8), 621–626.

CLSI. 2012 – Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard – Eleventh Edition. CLSI document M02-A11. Wayne, PA: Clinical and Laboratory Standards Institute.

Coetzee JC, Van Wyk AE. 2009 – The genus *Calvatia* (‘Gasteromycetes’, Lycoperdaceae): A review of its ethnomycology and biotechnological potential. African Journal of Biotechnology, 8(22), 6007–6015.

Desjardin DE, Oliveira AG, Stevani CV. 2008 – Fungi bioluminescence revisited. Photochemical & Photobiological Sciences, 7(2), 170–182.

Deshmukh L, Kumar S, Aharwal RP, Rajak RC, Sandhu SS. 2014 – Study on *in-vitro* antibacterial activity of mushroom collected from Jabalpur region. International Journal of Pharmacy and Pharmaceutical Sciences, 6(9), 143–146.

Dighe S, Agate AD. 2000 – Antibacterial activity of some Indian mushrooms. International Journal of Medicinal Mushrooms, 2(2), 141–150.

Figueiredo Á, Silva AC. 2014 – Activity "*in vitro*" of extracts from *Pycnoporus sanguineus* and *Lentinus crinitus* on the pathogen *Fusarium* sp. Acta Amazonica, 44(1), 1–8.

Greay SJ, Hammer KA. 2015 – Recent developments in the bioactivity of mono- and diterpenes: anticancer and antimicrobial activity. Phytochemistry Reviews, 14(1), 1–6.

Jesionek W, Grzelak EM, Majer-Dziedzic B, Choma IM. 2013 – Thin-layer chromatography – direct bioautography for the screening of antimicrobial properties of plant extracts. Journal of Planar Chromatography, 26(2), 109–113.

Jonathan SG, Kigigha LT, Ohimain E. 2008 – Evaluation of the inhibitory potentials of eight higher Nigerian fungi against pathogenic microorganisms. African Journal of Biomedical Research, 11(2), 197–202.

Jorgensen JH, Ferraro MJ. 2009 – Antimicrobial susceptibility testing: a review of general principles and contemporary practices. Clinical Infectious Diseases, 49(11), 1749–1755.

Klaus A, Kozarski M, Vunduk J, Todorovic N et al. – 2015 Biological potential of extracts of the wild edible Basidiomycete mushroom *Grifola frondosa*. Food Research International, 67, 272–283.

Korzeniewska E, Korzeniewska A, Harnisz M. 2013 – Antibiotic resistant *Escherichia coli* in hospital and municipal sewage and their emission to the environment. Ecotoxicology and Environmental Safety, 91, 96–102.

Krupodora TA, Barshteyn VY, Zabeida EF, Pokas EV. 2016 – Antibacterial Activity of Macromycetes Mycelia and Culture Liquid. Microbiology and Biotechnology Letters, 44(3), 246–253.

Krüzselyi D, Vetter J, Ott PG, Móricz ÁM. 2016 – Investigation of antibacterial components of button mushroom (*Agaricus bisporus*) by direct bioautography and HPLC–DAD–MS. Journal of Liquid Chromatography & Related Technologies, 39(5-6), 298–302.

Li YY, Shen YM. 2010 – Four novel diterpenoids from *Crinipellis* sp. 113. Helvetica Chimica Acta, 93(11), 2151–2157.

Liermann JC, SchüFfler A, Wollinsky B, Birnbacher J et al. 2010 – Hirsutane-type sesquiterpenes with uncommon modifications from three basidiomycetes. Journal of Organic Chemistry, 75(9), 2955–2961.

Liktor-Busa E, Kovács B, Urbán E, Hohmann J, Ványolós A. 2016 – Investigation of Hungarian mushrooms for antibacterial activity and synergistic effects with standard antibiotics against resistant bacterial strains. Letters in Applied Microbiology, 62(6), 437–443.

Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y et al. 2012 – Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clinical Microbiology and Infection, 18(3), 268–281.

Moricz AM, Ott PG. 2017 – Screening and Characterization of Antimicrobial Components of Natural Products Using Planar Chromatography Coupled with Direct Bioautography, Spectroscopy and Mass Spectrometry: A Review. Current Organic Chemistry, 21(18), 1861–1874.

Nowacka N, Nowak R, Drozd M, Olech M et al. 2014 – Analysis of phenolic constituents, antiradical and antimicrobial activity of edible mushrooms growing wild in Poland. LWT - Food Science and Technology, 59(2), 689–694.

Reinoso R, Cajas-Madriaga D, Martinez M, San Martin A et al. 2013 – Biological activity of macromycetes isolated from Chilean subantarctic ecosystems. Journal of the Chilean Chemical Society, 58(4), 2016–2019.

Rosa LH, Machado KM, Rabello AL, Souza-Fagundes EM et al. 2009 – Cytotoxic, immunosuppressive, trypanocidal and antileishmanial activities of Basidiomycota fungi present in Atlantic Rainforest in Brazil. Antonie Van Leeuwenhoek, 95(3), 227–237.

Rosa LH, Machado KMG, Jacob CC, Capelari M et al. 2003 – Screening of Brazilian basidiomycetes for antimicrobial activity. Memórias do Instituto Oswaldo Cruz, 98(7), 967–974.

Rosenberger MG, Paulert P, Cortez VG. 2018 – Studies of the Antimicrobial Activity of Mushrooms (Agaricales) from South America. International Journal of Medicinal Mushrooms, 20(11), 1065–1074.

Santos DS, Oberger JV, Niero R, Wagner T et al. 2014 – Seasonal phytochemical study and antimicrobial potential of *Vetiveria zizanioides* roots. Acta Pharmaceutica, 64(4), 495–501.

Shakibaie M, Mohazab NS, Mousavi SAA. 2015 – Antifungal activity of selenium nanoparticles synthesized by bacillus species Msh-1 against *Aspergillus fumigatus* and *Candida albicans*. Jundishapur Journal of Microbiology, 8(9), 1–4.

Shen HS, Shao S, Chen JC, Zhou T. 2017 – Antimicrobials from Mushrooms for Assuring Food Safety. Comprehensive Reviews in Food Science and Food Safety, 16(2), 316–329.

Suay I, Arenal F, Asensio FJ, Basilio A et al. 2000 – Screening of basidiomycetes for antimicrobial activities. Antonie Van Leeuwenhoek, 78(2), 129–140.

Valgas C, Souza SM, Smânia EFA, Smânia A. 2007 – Screening methods to determine antibacterial activity of natural products. Brazilian Journal of Microbiology, 38(2), 369–380.

Yamaç M, Bilgili F. 2006 – Antimicrobial activities of fruit bodies and/or mycelial cultures of some mushroom isolates. Pharmaceutical biology, 44(9), 660–667.

Younes I, Sellimi S, Rinaudo M, Jellouli K, Nasri M. 2014 – Influence of acetylation degree and molecular weight of homogeneous chitosans on antibacterial and antifungal activities. International journal of food microbiology, 185, 57–63.