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OCCURRENCE AND ANTIMICROBIAL ACTIVITY OF AGARICOMYCETES OF THE STATE OF ACRE, BRAZIL

OCORRÊNCIA E ATIVIDADE ANTIMICROBIANA DE AGARICOMICETES DO ESTADO DO ACRE, BRASIL

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RESUMO

Agaricomicetos são fungos macroscópicos que produzem uma grande variedade de compostos bioativos de interesse para a saúde humana, porém, estudos de ocorrência e atividades biológicas realizados com Agaricomicetes amazônicos são raros. Assim, o objetivo deste trabalho foi descrever a ocorrência e avaliar a atividade antimicrobiana de extratos de basidioma de Agaricomicetos coletados em dois fragmentos de floresta amazônica no Estado do Acre. As coletas foram realizadas no Parque Zoobotânico e na Fazenda Experimental Catuaba. Extratos etanólicos foram preparados a partir de 31 basidiomas de Agaricomicetos e avaliados pelo teste *cup plate*. Os extratos etanólicos foram testados contra a bactéria *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae* e o fungo *Candida albicans*. Foram coletados 69 Agaricomicetos, classificados nas ordens Agaricales (53,6%), Polyporales (40,6%) e Auriculariales (5,8%). Foram identificados 53,6% dos basidiomicetos coletados, distribuídos em 8 famílias, 12 gêneros e 7 espécies. Dos 31 extratos etanólicos testados para atividade antimicrobiana, o extrato de Agaricomycetes Polyporales 5.221 e *Oudemansiella cubensis* apresentou atividade antibacteriana contra *S. aureus*. Este estudo contribuiu para o primeiro relato de atividade antibacteriana de *Oudemansiella cubensis*.

Palavras-chave: Cogumelo medicinal. Atividade antibacteriana. *Oudemansiella cubensis*.

ABSTRACT

Agaricomycetes are macroscopic fungi that produce a wide variety of bioactive compounds of interest to human health, however, occurrence studies and biological activities done with Amazonian Agaricomycetes are rare. Thus, the objective of this work was to describe the occurrence and evaluate the antimicrobial activity of basidioma extracts from Agaricomycetes collected in two fragments of Amazonian forest in the State of Acre. The collections were made in the Zoobotanical Park and the Experimental Farm Catuaba. Ethanol extracts were prepared from 31 basidiomas of Agaricomycetes and evaluated by the *cup plate* test. The ethanol extracts were tested against the bacteria *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae* and the yeast fungus *Candida albicans*. A total of 69 Agaricomycetes were collected, classified in the orders Agaricales (53.6%), Polyporales (40.6%) and Auriculariales (5.8%). 53.6% of the collected basidiomycetes were identified, distributed in 8 families, 12 genera and 7 species. Of the 31 ethanol extracts tested for antimicrobial activity, the extract from Agaricomycetes Polyporales 5.221 and *Oudemansiella cubensis* had antibacterial activity against *S. aureus*. This study contributed to the first report of antibacterial activity of *Oudemansiella cubensis*.

Key words: Medicinal Mushroom. Antibacterial activity. *Oudemansiella cubensis*.

1. INTRODUCTION

Agaricomycetes are macroscopic fungi of the Phylum Basidiomycota, popularly known as mushrooms and stick-ears, found in substrates such as litter and trunks. This group of fungi includes many species with medicinal properties, the best known in the world are *Ganoderma lucidum*, *Grifola frondosa* and *Trametes versicolor* [1].

Mushrooms can have different biological activities as antitumor [2], antiviral [3], antimicrobial [4], anti-inflammatory [5], antioxidant [6] and immunomodulatory [7]. They present these activities due the presence of secondary metabolites, such as terpenes, steroids, anthraquinones, derivatives of benzoic acid and quinolones, but also of primary metabolites, such as oxalic acid, peptides and proteins [8].

Among the biological activities presented by mushrooms, antimicrobial activity has become essential due to increased resistance of microorganisms to antimicrobials. Many studies have researched antimicrobial substances in Agaricomycetes, with the objective of discovering bioactive compounds able to be a promising antibiotic against resistant microorganisms and of relevant interest to public health [9].

In Brazil, some studies have evaluated the antimicrobial activity of different species of Agaricomycetes [10-19]. Studies done in the Brazilian Amazon, it was found that there is a small number, and the evaluation of the antimicrobial activity of *Pycnoporus sanguineus* can be reported [20], another that evaluated eight species of Agaricomycetes [21] and recently, the antibacterial activity of mushrooms collected in southwestern Amazonia was evaluated, where 14 species had activity [22].

Due to the few studies done with Agaricomycetes, mainly in the Brazilian Amazon, the objective of this work was to describe the occurrence and evaluate the antimicrobial activity of basidioma extracts from Agaricomycetes collected in two fragments of Amazon rainforest in the State of Acre, Brazil.

2. MATERIALS AND METHODS

Study area

Collections were made at the Zoobotanical Park (ZP) (9°57'8 "S - 67 ° 52'25" W), at the Federal University of Acre (UFAC) in the city of Rio Branco, Acre and at the Catuaba Experimental Farm (CEF) (10º04'S and 67º37'W), located near the confluence of BR-364 and BR-317, city of Senador Guiomard, Acre (Figure 1).

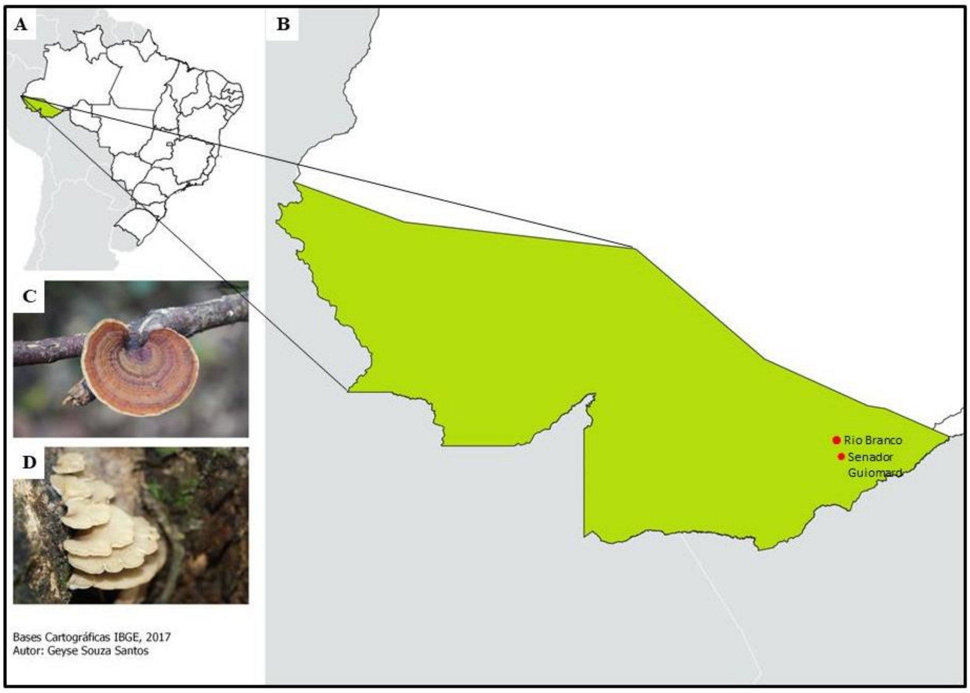

Fig.1. Location of Agaricomycetes collection areas in the State of Acre, Brazil. **A**: Brazil; **B**: State of Acre; **C-D**: Agaricomycetes collected in the two fragments of the Amazon rainforest.

Collection and identification

Agaricomycetes were collected from pre-existing trails, the fungi basidioma was removed from the substrate with a pocket knife and stored in paper bags. At the time of collection, all specimens were photographed, numbered and the substrate was described. Basidiomas were taken to the Microbiology Laboratory at UFAC, observation and analysis of macroscopic and microscopic characteristics were made. After all the observations, the material was dehydrated at 45 °C for 24-48 h for the production of desiccans [23].

The macroscopic characteristics observed in individuals of the order Polyporales were color, shape, consistency, position in the substrate, basidioma size and number of pores per mm [24]. For the order Agaricales, the capillary, stipple and lamellae were observed while fresh [25], the color description followed a color chart [26].

For observation of microscopic structures, freehand cuts were made with steel blade on the basidiomas, the cuts were placed between blade and cover slip with 3% potassium hydroxide and Congo red [27,28]. Identification keys were used according to specific literature [29-37] and databases Index Fungorum (http://www.indexfungorum.org) and Mycobank (http://www.mycobank.org/).

Production of basidioma extracts

For extraction, 1 g of dry basidioma of 31 Agaricomycetes were weighed, crushed and macerated with ethanol for 24 h for 3 times [20].

After maceration, the samples were filtered and dried at 37 °C, until the solvent was completely evaporated. The yields of the extracts were calculated and dissolved in dimethyl sulfoxide (DMSO) at a concentration of 20 mg mL^{-1}.

Antimicrobial activity

Antimicrobial activity was made using a cup-plate test [38]. Gram-positive bacteria *Staphylococcus aureus* (ATCC 12598) and *Streptococcus pneumoniae* (ATCC 11733), Gram-negative bacteria *Escherichia coli* (ATCC 10536) and *Klebsiella pneumoniae* (ATCC 700603), and the yeast fungus *Candida albicans* (ATCC 90028) were used as test microorganisms. These species are microorganisms that normally cause infections in humans.

Three to five bacterial colonies, isolated, of the same morphological type were selected on Petri dish with Müller-Hinton agar medium (MH), and the same process made with the yeast colonies grown on Sabouroud-dextrose-agar (SDA).

The colonies of microorganisms were transferred to tubes with 5 mL of sterile 0.9% saline and standardized to obtain an optical turbidity comparable to that of the standard solution of 0.5 for bacteria and 1.0 for fungus, according to the scale of McFarland. With a sterile swab, the standardized microbial suspension was inoculated in Petri dishes with the culture medium MH agar for bacteria and SDA for fungus, in three different directions.

Circular holes (cup plate technique) with a diameter of 5 mm were made over the medium, 20 µL of the fungal extract were added and the plates were kept at 4 °C for 24 h to diffuse the extracts. The plates were then incubated at 37 °C for 18h and the inhibition halos were read with antibiogram rule measured in millimeters. The results were recorded considering the average values of the three repetitions.

3. RESULTS

A total of 69 Agaricomycetes were collected at Zoobotanical Park and Catuaba Experimental Farm. These were classified in the orders Agaricales (53.6%), Polyporales (40.6%) and Auriculariales (5.8%).

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Of the total collected Agaricomycetes, 36 individuals (53.6%) were identified, and 46.4% remained at the taxonomic level of order. Agaricomycetes identified were distributed in 8 families, 12 genera and 7 species (Table 1, Figure 2).

Fig. 2. Basidiomata of Agaricomycetes occurring in the Zoobotanical Park (ZP) and Catuaba Experimental Farm (CEF). **A-B**: *Coriolopsis caperata* (Berk.) Murrill, **C-D**: *Favolus tenuiculus* P. Beauv, **E-F**: *Gloeporus thelephoroides* (Hook.) G. Cunn, **G-H**: *Hexagonia papyracea* Berk., **I-J**: *Oudemansiella cubensis* (Berk. & M. A. Curtis), **K-L**: *Trametes modesta* (Kunze ex Fr.) Ryvarden, **M-N**: *Trogia cantharelloides* (Mont.) Pat.

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Table 1. Agaricomycetes identified by order, family, genus and species and substrate collected. Tr: trunk, L: litter, S: soil, Tw: Twig.

Order	Family	Genus/Species	Substrate
Agaricales	**Hygrophoraceae**	*Hygrocybe* sp.	L
	Marasmiaceae	*Marasmius* sp. 1	Tw
		Marasmius sp. 2	L
		Marasmius sp. 3	L
		Marasmius sp. 4	Tr
		Marasmius sp. 5	L
		Trogia cantharelloides (Mont.) Pat	L
	Physalacriaceae	*Oudemansiella* sp.	L
		Oudemansiella cubensis (Berk. & M. A. Curtis)	Tr
	Tricholomataceae	*Leucopaxillus* sp.	L
Auriculariales	**Auriculariaceae**	*Auricularia* sp. 1	Tr
		Auricularia sp. 2	Tr
		Auricularia sp. 3	Tr
		Auricularia sp. 4	Tr
Polyporales	**Ganodermataceae**	*Amauroderma* sp. 1	S
		Amauroderma sp. 2	S
		Amauroderma sp. 3	S
		Amauroderma sp. 4	S
		Amauroderma sp. 5	S
		Amauroderma sp. 6	S

	Amauroderma sp. 7	S
	Amauroderma sp. 8	S
	Amauroderma sp. 9	S
	Amauroderma sp. 10	S
Meruliaceae	*Podoscypha* sp. 1	L
	Podoscypha sp. 2	Tw
	Podoscypha sp. 3	S
	Podoscypha sp. 4	S
	Gloeporus thelephoroides (Hook.) G. Cunn	Tw
Polyporaceae	*Coriolopsis caperata* (Berk.) Murrill	Tr
	Favolus sp. (Fr.) Fr.	Tr
	Favolus tenuiculus P. Beauv.	Tr
	Hexagonia papyracea Berk.	Tr
	Trametes modesta (Kunze ex Fr.) Ryvarden	Tr

Agaricomycetes were found in five types of substrates, trunk (34.9%), litter (30.4%), soil (21.7%), twig (11.6%), and bamboo (1.4%). Only Agaricomycetes of the order Polyporales were found in all types of substrates (Figure 3).

Of the 31 ethanolic extracts analyzed for antimicrobial activity, the extract of Agaricomycetes Polyporales 5.221 and Oudemansiella cubensis (Figure 4) had antibacterial activity against S. aureus with halos of 5-10 mm in diameter (Table 2).

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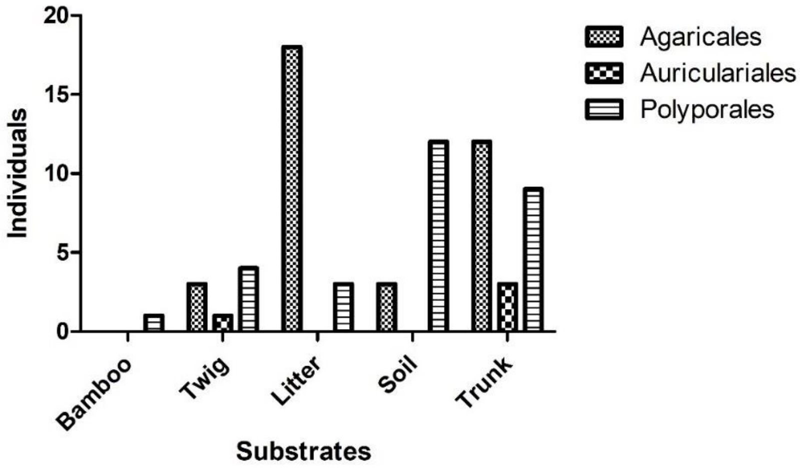

Fig. 3. Agaricomycetes collected at Zoobotanical Park and Catuaba Experimental Farm distributed in taxonomic order and types of substrates.

Table 2. Registration number, taxonomic identification and antibacterial activity of ethanolic extracts of Amazonian Agaricomycetes basidiomas.

Registration number	Taxonomic Identification	Microorganism				
		Sta	***Spn***	***Eco***	***Kpn***	***Cal***
5.183	*Oudemansiella cubensis*	2.0 ± 0.0	-	-	-	-
5.221	Polyporales	8.0 ± 0.0	-	-	-	-

Sta: *Staphylococcus aureus*; **Spn**: *Streptococcus pneumoniae*; **Eco**: *Escherichia coli*; **Kpn**: *Klebsiella pneumoniae*; **Cal:** *Candida albicans*.

Values are represented as mean ± dp (= 3).

Fig. 4. Agaricomycetes with antibacterial activity against *S. aureus*. A-C: *O. cubensis*, D-E: Polyporales 5.221.

4. DISCUSSION

Of the total of 69 Agaricomycetes collected at Zoobotanical Park and Catuaba Experimental Farm, the order Agaricales had the largest number of individuals (53.6%), followed by the order Polyporales (40.6%). Currently, the order Agaricales is the group with the highest number of species identified in Brazil, when compared to the other orders of Agaricomycetes, with 924 species registered in the country [39].

Comparing the genera identified in this work with one of the first works done in this region, it was possible to observe that the genera *Amauroderma*, *Favolus*, *Hexagonia*, *Marasmius*, *Podoscypha* and *Trametes* were also registered [40]. In another recent research done at Zoobotanical Park, the genera in common with this work were *Amauroderma*, *Auricularia*, *Favolus*, *Ganoderma*, *Gloeporus*, *Marasmius*, *Phellinus*, *Trametes* and *Trogia* [41].

In a research with the objective of reviewing the species deposited in the Herbariums of the Amazon, 33 species of the Herbarium of UFAC were identified, among them, the species *Ganoderma australe*, *Gloeporus thelephoroides* and *Agaricomycetes* of the genera *Amauroderma*, *Phellinus* and *Trametes* coincide with the species found in this study [42].

Studies done with the identification and registration of Agaricomycetes in the State of Acre are limited to a few studies. The most recent study showed 15 species of Agaricomycetes that have not yet reported this for that region [43]. One of the factors for this low number of studies is the deficiency of fungal taxonomists for this region, with consequent few species registered for Acre.

Agaricomycetes collected were found more frequently in the trunk (34.9%) and litter (30.4%) substrates. One of the ecological roles played by fungi is recycling. Due to fungal decomposition, substrates such as lignocellulose in wood are degraded, helping to release essential nutrients back into the environment [44].

From the ethanol extracts analyzed for antimicrobial activity, the extracts of Polyporales 5.221 and *Oudemansiella cubensis* showed antibacterial activity against *S. aureus* (Tabela 2). The ability of mushrooms to have antimicrobial activity is due the presence of molecules in their basidiomas with different molecular weights, in addition, these organisms need antibacterial and antifungal compounds to survive in their natural environment [45,46].

Extracts of the species *Irpex lacteus* and *Laetiporus sulphureus*, of the order Polyporales, also had activity against *S. aureus* [13,47].

In a study testing the antibacterial activity of extracts of Amazonian Agaricomycetes, extract from 13 species of the order polyporales had activity, and the species *Cymatoderma* sp., *Ganoderma* cf. *australe*, *Favolus tenuiculus* and *Gloeoporus thelephoroides* had against *S. aureus* [22].

Studies that evaluated the antimicrobial activity of extracts of the species *O. canarii* (possibly belonging to *O. cubensis*, based on the current taxonomic concept), had antimicrobial activity against *C. albicans*, *C. glabrata*, *C. krusei* and *C. tropicalis* [13,21]. Another species analyzed was *O. mucida*, also able to inhibit the growth of *C. albicans* [48].

Oudemansiella species are known to produce the bioactive compounds strobilurins and oudemansins, able to inhibit the growth other fungi, even at low concentrations [21]. Mucidin, an antimicrobial substance isolated from mycelial cultures of *O. mucida*, has high antimicrobial activity against a wide variety of bacteria and yeasts [49]. There were no reports of antimicrobial activity of *O. cubensis* for bacterial species, so this work contributes to the first report.

5. CONCLUSIONS

The fragments of the Amazon rainforest from State of Acre have a great wealth of Agaricomycetes, with higher frequency of individuals of the order Agaricales and Polyporales. This study contributes to the identification of species of Agaricomycetes, and was also the first report of antibacterial activity of *Oudemansiella cubensis*.

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