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ANTIMICROBIAL ACTIVITY OF HYDROALCOHOLIC EXTRACTS OBTAINED FROM MACROFUNGI COLLECTED IN AN URBAN FOREST FRAGMENT IN TABATINGA, AMAZONAS

ATIVIDADE ANTIMICROBIANA DE EXTRATOS HIDROALCOÓLICOS OBTIDOS DE MACROFUNGOS COLETADOS EM UM FRAGMENTO FLORESTAL URBANO EM TABATINGA, AMAZONAS

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ABSTRACT

Macrofungi are of considerable ecological and biotechnological relevance, particularly because of their ability to produce secondary metabolites with pharmacological potential. This study evaluated the antimicrobial activity of hydroalcoholic extracts obtained from macrofungi collected in an urban forest fragment in the Novo Progresso neighborhood, municipality of Tabatinga, Amazonas, Brazil. During field sampling, eighteen specimens were recorded, of which five morphotypes compatible with species of the order Polyporales were selected for analysis: *Pycnoporus sanguineus*, *Lentinus crinitus*, *Lentinus berteroi*, *Hexagonia hydnoides*, and *Earliella scabrosa*. The extracts were obtained by maceration in 70% ethanol and subsequently evaluated for extraction yield and antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*. Extraction yields ranged from 0.25% to 4.33%, with the highest values observed for *L. berteroi* and *H. hydnoides*. All extracts showed antimicrobial activity at 3.2 mg/mL, indicating potential activity against Gram-positive bacteria, Gram-negative bacteria, and yeasts. These findings suggest the presence of bioactive metabolites and reinforce the potential of Amazonian macrofungi as promising sources for the prospection of new antimicrobial agents. Further studies involving chemical fractionation, minimum inhibitory concentration determination, and structural characterization of active metabolites are needed to better understand the bioactive potential of these species.

Keywords: Amazonian macrofungi; Antimicrobial activity; Hydroalcoholic extracts; Polyporales; Bioprospecting.

RESUMO

Os macrofungos apresentam elevada relevância ecológica e biotecnológica, especialmente devido à capacidade de produzir metabólitos secundários com potencial farmacológico. Este estudo avaliou a atividade antimicrobiana de extratos hidroalcoólicos obtidos de macrofungos coletados em um fragmento florestal urbano localizado no bairro Novo Progresso, município de Tabatinga, Amazonas, Brasil. Durante as coletas, foram registrados dezoito espécimes, dos quais cinco morfotipos compatíveis com espécies da ordem Polyporales foram selecionados para análise: *Pycnoporus sanguineus*, *Lentinus crinitus*, *Lentinus berteroi*, *Hexagonia hydnoides* e *Earliella scabrosa*. Os extratos foram obtidos por maceração em etanol 70% e posteriormente avaliados quanto ao rendimento de extração e à atividade antimicrobiana frente a *Staphylococcus aureus*, *Escherichia coli* e *Candida albicans*. Os rendimentos variaram entre 0,25% e 4,33%, sendo os maiores valores observados para *L. berteroi* e *H. hydnoides*. Todos os extratos apresentaram atividade antimicrobiana na concentração de 3,2 mg/mL, indicando potencial de ação frente a bactérias Gram-positivas, Gram-negativas e leveduras. Esses resultados sugerem a presença de metabólitos bioativos e reforçam o potencial dos macrofungos amazônicos como fontes promissoras para a prospecção de novos agentes antimicrobianos. Estudos futuros envolvendo fracionamento químico, determinação da concentração inibitória mínima e caracterização estrutural dos metabólitos ativos serão necessários para ampliar a compreensão sobre o potencial bioativo dessas espécies.

Palavras-chave: Macrofungos amazônicos; Atividade antimicrobiana; Extratos hidroalcoólicos; Polyporales; Bioprospecção.

1. INTRODUCTION

Fungi constitute one of the most diverse and functionally important groups in the biosphere, playing essential roles in organic matter decomposition, nutrient cycling, and the maintenance of terrestrial ecosystem dynamics (Deacon, 2013; Tedersoo et al., 2014). Despite their ecological and evolutionary relevance, global fungal diversity remains substantially underestimated, since only a small fraction of existing species has been formally described (Blackwell, 2011).

Among fungi, macrofungi are distinguished by the production of reproductive structures visible to the naked eye, known as basidiomata or fruiting bodies, and by their broad morphological, physiological, and metabolic diversity. These features make macrofungi particularly relevant for taxonomic, ecological, and biotechnological studies, as they connect fungal diversity, ecological function, and the production of bioactive metabolites with pharmacological and industrial potential (Ulloa; Hanlin, 2000; Kirk et al., 2008).

In forest environments, saprobic macrofungi are key agents in the degradation of lignocellulosic substrates, including trunks, branches, leaves, and leaf litter. This capacity is associated with the production of extracellular enzymes able to degrade complex polymers such as lignin, cellulose, and hemicellulose, thereby contributing directly to plant biomass decomposition, soil renewal, and the maintenance of biogeochemical cycles, particularly those of carbon and nitrogen (Webster; Weber, 2007; Niego et al., 2023). Without these organisms, organic matter would accumulate and nutrient availability for other ecosystem components would be reduced.

Beyond their ecological importance, macrofungi are increasingly recognized as promising sources of bioactive compounds. They produce several classes of secondary metabolites, including phenolic compounds, terpenoids, quinones, steroids, alkaloids, and polysaccharides, which are frequently associated with antimicrobial, antioxidant, anti-inflammatory, immunomodulatory, and cytotoxic activities (Wasser, 2002; Sułkowska-Ziaja et al., 2023). Phenolic compounds and terpenoids are especially relevant due to their ability to neutralize free radicals and inhibit pathogenic microorganisms, whereas β-glucans have been widely investigated for their immunomodulatory and antitumor properties (Wasser, 2002; Sułkowska-Ziaja et al., 2023).

The antimicrobial activity of fungal metabolites may involve different mechanisms, including changes in cell membrane permeability, interference with protein synthesis, inhibition of enzymes essential for microbial metabolism, and induction of oxidative stress (Sułkowska-Ziaja et al., 2023). This biological potential makes macrofungi important models for the prospection of new bioactive molecules, particularly in the context of increasing microbial resistance to conventional antimicrobials, which is considered one of the major threats to global public health (World Health Organization, 2023).

In antimicrobial screening studies, *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* are commonly used as experimental models. *S. aureus* is a Gram-positive bacterium associated with skin, respiratory, urinary, and systemic infections, with high capacity to develop resistance, including methicillin-resistant strains. *E. coli* is a Gram-negative bacterium that normally belongs to the intestinal microbiota; however, some strains can cause gastrointestinal, urinary, and systemic infections. *C. albicans* is an opportunistic yeast capable of forming biofilms and exhibiting tolerance to conventional antifungals, making it an important model for evaluating antifungal compounds (Tortora; Funke; Case, 2017; Pohl et al., 2022; Viana et al., 2025).

The Amazon is one of the world's major biodiversity centers and harbors a wide variety of organisms that remain poorly studied, including macrofungi. High humidity, relatively stable temperatures, and abundant organic substrates favor the development of diverse fungal communities. However, knowledge of this diversity remains limited, particularly in urban and peri-urban areas of Western Amazonia, where forest fragments may function as reservoirs of mycobiota and organisms with biotechnological potential.

Among the macrofungal groups of greatest ecological and biotechnological relevance, representatives of the order Polyporales are particularly important because they are frequently associated with wood decomposition and ligninolytic enzyme production. Species in this order are able to degrade lignin and cellulose while also synthesizing secondary metabolites of pharmacological interest, such as natural pigments, phenolic compounds, quinones, and terpenoids (Gafforov et al., 2023; Sułkowska-Ziaja et al., 2023). This combination of ecological function and metabolic diversity makes Polyporales especially relevant for bioprospecting studies.

Among the species of interest, *Pycnoporus sanguineus* is a widely distributed tropical polypore characterized by sessile, semicircular basidiomata ranging from orange to scarlet red. It is a saprobic white-rot fungus capable of producing enzymes such as laccases, manganese peroxidases, and lignin peroxidases, as well as phenolic pigments and compounds with antimicrobial and antioxidant activity (Kirk et al., 2008; Ryvarden; Melo, 2014; Kumar et al., 2023).

Species of the genus *Lentinus*, such as *Lentinus crinitus* and *Lentinus berteroi*, also have ecological and biotechnological relevance. These fungi are frequently found in tropical and subtropical regions, especially on dead or decaying wood, where they participate in lignocellulosic degradation and nutrient cycling. Studies indicate that representatives of this genus produce phenolic compounds, terpenes, and polysaccharides associated with antioxidant and antimicrobial activities, reinforcing their potential for natural product prospection (Santos Filho et al., 2023; Meng et al., 2024).

Other representatives of Polyporales, such as *Hexagonia hydnoides* and *Earliella scabrosa*, are also commonly associated with decaying woody substrates. *H. hydnoides* has a hymenophore with well-defined hexagonal pores and contributes to lignin and cellulose degradation, whereas *E. scabrosa* is a ligninolytic fungus widely distributed in tropical and subtropical regions, occurring on dead wood in both natural and urbanized environments (Hibbett et al., 2007; Ryvarden; Melo, 2014). Studies involving ligninolytic fungi show that these organisms can produce oxidative enzymes and secondary metabolites with applications in bioremediation, pollutant degradation, and antimicrobial activity (Pointing, 2001; Zhang et al., 2021; Gafforov et al., 2023).

Given this context, studies focused on the biological evaluation of Amazonian macrofungi are essential for expanding knowledge of regional biodiversity and identifying organisms with biotechnological potential. Therefore, this study aimed to evaluate the antimicrobial activity of hydroalcoholic extracts obtained from macrofungi collected in an urban forest fragment in Tabatinga, Amazonas, against *S. aureus*, *E. coli*, and *C. albicans*.

2. METHODOLOGY

Samples were collected from an urban forest fragment located in the Novo Progresso neighborhood, municipality of Tabatinga, Amazonas, Brazil, a region situated on the tri-border area between Brazil, Colombia, and Peru. The study area is characterized by a humid equatorial climate, high average temperatures, abundant rainfall, and accumulation of decomposing organic matter, conditions that favor the occurrence and development of macrofungi.

Macrofungi were collected manually and selected according to basidiomata integrity and biomass availability for extract preparation. Preliminary morphological characterization was based on macroscopic features, including pileus shape and color, hymenophore type, texture, presence or absence of stipe, basidioma consistency, and colonized substrate. Taxonomic comparison was performed using specialized literature, particularly *Dictionary of the Fungi* (Kirk et al., 2008) and *Introduction to Fungi* (Webster; Weber, 2007), as well as the Index Fungorum and MycoBank databases for nomenclatural standardization. Since no microscopic or molecular analyses were performed, the identifications were considered preliminary.

After collection, the specimens were cleaned to remove surface impurities, fragmented, and macerated to increase the contact surface with the solvent. Hydroalcoholic extracts were obtained by maceration in 70% ethanol (v/v), at a 1:10 mass-to-volume ratio, for 48 h at room temperature and protected from light. The extracts were then filtered through filter paper, and the solvent was removed by evaporation in a sand bath at approximately 50 °C. The resulting crude extracts were dried in an oven at 40 °C for 48 h to remove residual moisture, transferred to amber bottles, and stored at 4 °C until antimicrobial assays.

Antimicrobial activity was evaluated against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*, kindly provided by Fiocruz Amazônia/ILMD. The extracts were solubilized in 1% DMSO to obtain solutions at 3.2 mg/mL. Aliquots of 100 µL were transferred to 96-well plates, followed by addition of the previously standardized microbial inoculum. The plates were incubated at 37 °C for 24 h for bacteria and 48 h for the yeast. After incubation, resazurin was added as a cell viability indicator. Maintenance of the blue color was interpreted as absence of microbial growth, whereas a color change to pink indicated microbial growth. Thus, antimicrobial activity was qualitatively evaluated at the tested concentration, without determination of the minimum inhibitory concentration.

3. RESULTS AND DISCUSSION

3.1. Ecological Aspects Of The Studied Urban Forest Fragment

The sampling area consisted of an urban forest fragment located in the Novo Progresso neighborhood, municipality of Tabatinga, Amazonas, within the humid equatorial climate zone characteristic of Western Amazonia. The environmental conditions observed in the area, including high humidity, shaded microhabitats, abundant leaf litter, and the presence of decaying woody substrates, create favorable conditions for the establishment of saprobic fungal communities. These factors are particularly relevant for lignocellulolytic macrofungi, as the continuous availability of plant-derived substrates supports growth, basidiomata development, and the production of extracellular enzymes involved in lignin and cellulose degradation (Dix; Webster, 1995; Deacon, 2013).

The occurrence of basidiomata on trunks, branches, and decomposing plant material highlights the ecological importance of these organisms in nutrient cycling and in maintaining the functional dynamics of urban forest ecosystems. Although subjected to anthropogenic influence, forest fragments may act as important reservoirs of fungal diversity, especially in tropical regions where fungal communities remain poorly documented.

3.2. Preliminary Morphological Characterization Of Macrofungi

A total of eighteen macrofungal specimens were recorded during field sampling, of which five morphotypes were selected for antimicrobial evaluation based on abundance, morphological preservation, and biomass availability. Based on the observed macromorphological characteristics, the specimens showed morphological compatibility with *Pycnoporus sanguineus*, *Lentinus crinitus*, *Lentinus berteroi*, *Hexagonia hydnoides*, and *Earliella scabrosa*, all representatives of the order Polyporales.

Species attribution was considered preliminary because it relied exclusively on macroscopic characteristics, including pileus shape and coloration, hymenophore structure, basidioma consistency, presence or absence of stipe, and substrate colonization. Despite these limitations, the observed features were consistent with descriptions available for tropical polyporoid fungi. Species belonging to Polyporales are widely recognized for their ecological association with wood decomposition and for their ability to synthesize ligninolytic enzymes and secondary metabolites of biotechnological interest (Santos Filho et al., 2023; Sum et al., 2023).

The morphotype compatible with *P. sanguineus* was distinguished by the intense orange coloration of the pileus, a characteristic frequently associated with phenolic pigments and bioactive compounds previously reported for the genus *Pycnoporus* (Smânia et al., 2002). Specimens compatible with *L. crinitus* and *L. berteroi* were observed on woody substrates and leaf-litter-covered areas, a distribution pattern consistent with the saprobic lifestyle described for species of *Lentinus* (Santos Filho et al., 2023). Likewise, morphotypes compatible with *H. hydnoides* and *E. scabrosa* were exclusively associated with decaying wood, reinforcing their ecological affinity for humid environments rich in lignified substrates.

3.3. Yield Of Hydroalcoholic Extracts

The extraction yields obtained from the hydroalcoholic extracts are presented in Table 1. Differences in yield were observed among the analyzed macrofungi, indicating variation in extraction efficiency between the selected morphotypes.

These differences may reflect variations in basidioma composition, moisture content, structural density, and the concentration of solvent-extractable metabolites. Since 70% ethanol favors the extraction of compounds with intermediate polarity, including phenolic compounds, terpenoids, quinones, pigments, and soluble polysaccharides, the extraction yield may provide indirect evidence of chemical heterogeneity among the evaluated macrofungi (Bitwell et al., 2023; Sułkowska-Ziaja et al., 2023).

Table 1: Yield of hydroalcoholic extracts of macrofungi.

Scientific name	Initial Fresh Biomass (g)	Extract Mass (g)	Extraction Yield (%)
P. sanguineus	1.30	0.01	1.01
L. crinitus	1.70	0.04	2.39
H. hydnoides	14.40	0.44	3.08
L. berteroi	2.00	0.09	4.33
E. scabrosa	3.40	0.01	0.25

Source: Authors (2026).

The differences in extraction yield may be associated with variations in basidioma structure, moisture content, tissue density, and the amount of solvent-extractable metabolites. Ethanol at 70% is commonly used to extract compounds of intermediate polarity, including phenolic compounds, terpenoids, quinones, pigments, and soluble polysaccharides (Bitwell et al., 2023; Sułkowska-Ziaja et al., 2023).

The relatively higher yield observed for *L. berteroi* may suggest a greater availability of extractable compounds or stronger affinity of its metabolites for the hydroalcoholic solvent. In *H. hydnoides*, the yield may be partly related to the greater initial fresh biomass used, as well as to the occurrence of phenolic and pigment-associated compounds previously reported for representatives of the genus (Rahman; Mahmood; Alam, 2021). Conversely, the lower yield observed for *E. scabrosa* may indicate a smaller proportion of soluble compounds or a predominance of structural constituents strongly associated with the fungal cell wall.

Nevertheless, extraction yield should not be interpreted in isolation as an indicator of antimicrobial potential. Biological activity depends mainly on the chemical nature, concentration, and interactions among the metabolites present in the extract.

3.4. Antimicrobial Activity Of The Extracts

All hydroalcoholic extracts evaluated showed antimicrobial activity against *S. aureus*, *E. coli*, and *C. albicans* at 3.2 mg/mL, as shown in Table 2.

Table 2: Antimicrobial activity of hydroalcoholic extracts against the tested microorganisms.

Species	*S. aureus*	*E. coli*	*C. albicans*
P. sanguineus	+	+	+
L. crinitus	+	+	+
H. hydnoides	+	+	+
L. berteroi	+	+	+
L. crinitus (lineage 2)	+	+	+

Source: Authors (2026).

Legend: (+) absence of microbial growth at the tested concentration.

The positive response against the three microorganisms suggests that the extracts contain compounds capable of inhibiting the growth of Gram-positive bacteria, Gram-negative bacteria, and yeasts. This broad inhibitory profile is relevant because *S. aureus*, *E. coli*, and *C. albicans* represent structurally and physiologically distinct microbial groups, with different resistance and adaptation mechanisms.

Although the assays were performed at a single concentration, the results provide useful preliminary evidence of antimicrobial activity. Qualitative screening assays are widely employed in bioprospecting studies because they allow the selection of promising organisms and extracts for subsequent investigations, including chemical fractionation, determination of the minimum inhibitory concentration, and structural characterization of active compounds (Balouiri; Sadiki; Ibnsouda, 2016).

The activity observed for the extract obtained from the morphotype compatible with *P. sanguineus* is consistent with reports describing this species as a producer of phenolic pigments, quinones, and cinnabarin-related compounds associated with antimicrobial activity (Smânia et al., 1995; Chagas et al., 2022). Similarly, the activity observed for the extracts compatible with *L. crinitus* and *L. berteroi* may be related to phenolic compounds, terpenoids, and bioactive polysaccharides previously reported for species of *Lentinus* (Ferreira et al., 2021; Santos Filho et al., 2023).

Extracts obtained from the morphotypes compatible with *H. hydnoides* and *E. scabrosa* also showed antimicrobial activity, despite their differences in extraction yield. This finding reinforces that biological activity depends not only on the amount of extract obtained, but primarily on the chemical composition and potency of the metabolites present. Studies with ligninolytic fungi indicate that even low-yield species may produce highly active metabolites capable of inhibiting microbial growth at low amounts (Alfaro et al., 2021; Peña; Carrasco, 2023).

The inhibition of *C. albicans* is particularly noteworthy, given the clinical relevance of this opportunistic yeast and its increasing tolerance to conventional antifungal agents. Therefore, the results support the potential of Amazonian macrofungi as sources of antifungal and antimicrobial compounds of pharmacological interest (Perfect, 2017; World Health Organization, 2023).

The antimicrobial response observed across all evaluated extracts may also reflect ecological strategies naturally developed by wood-associated macrofungi. In decomposing substrates, fungal communities coexist with dense populations of bacteria, yeasts, and competing fungi, creating selective pressure for the production of secondary metabolites involved in chemical defense and niche establishment. These compounds may act by reducing microbial competition and preserving access to nutritional resources during substrate colonization. Consequently, antimicrobial activity observed under laboratory conditions may represent an ecological function originally shaped in natural environments (Deacon, 2013; Niego et al., 2023).

The broad inhibitory profile observed against both bacterial and fungal microorganisms is consistent with previous studies demonstrating that ligninolytic fungi frequently synthesize compounds with multiple biological targets. Phenolic metabolites, quinones, and oxidative enzymes produced by macrofungi have been associated with membrane destabilization, oxidative imbalance, interference in enzymatic pathways, and inhibition of microbial growth. This multifunctionality has attracted increasing attention for the development of natural antimicrobial agents and biotechnological applications, especially considering the global increase in antimicrobial resistance (Sułkowska-Ziaja et al., 2023; World Health Organization, 2023).

From an applied perspective, the present findings reinforce the value of exploratory screening approaches in regions with limited mycological characterization, such as Western Amazonia. Even preliminary evaluations may contribute to identifying organisms with potential for subsequent biochemical investigation and support future studies involving metabolomic characterization, purification of active fractions, and assessment of mechanisms of action.

3.5. Inferences About Bioactive Compounds Associated With The Observed Activity

The antimicrobial activity observed in the hydroalcoholic extracts suggests the presence of bioactive secondary metabolites among the evaluated macrofungi. Species of the order Polyporales are known to produce phenolic compounds, quinones, terpenoids, pigments, and polysaccharides, which are frequently associated with antimicrobial, antioxidant, and cytotoxic activities (Lysakova et al., 2024; Sułkowska-Ziaja et al., 2023).

In the morphotype compatible with *P. sanguineus*, the intense orange coloration may be related to the production of phenolic pigments and cinnabarin-derived compounds, which have been described for the genus *Pycnoporus* and associated with antibacterial and antifungal activity (Smânia et al., 1995; Smânia et al., 2002). These metabolites may act through oxidative mechanisms, changes in cell membrane integrity, and interference with microbial metabolic processes.

For the morphotypes compatible with *L. crinitus* and *L. berteroi*, the observed activity may be associated with phenolic compounds, terpenoids, and β-glucans, classes frequently reported in species of *Lentinus*. These metabolites may affect the cell wall, plasma membrane, metabolic enzymes, and oxidative balance, thereby contributing to microbial inhibition (Ferreira et al., 2021; Santos Filho et al., 2023).

In the case of the morphotypes compatible with *H. hydnoides* and *E. scabrosa*, antimicrobial activity may be associated with oxidative aromatic compounds and metabolites derived from ligninolytic pathways, which are common in wood-degrading fungi. These compounds are relevant not only for lignin decomposition but also for ecological competition with other microorganisms present in the substrate (Kumar et al., 2023).

Since this study did not include phytochemical, chromatographic, or spectrometric analyses, the relationship between the chemical classes mentioned and the antimicrobial activity observed should be interpreted as a literature-based inference. Further studies involving chemical fractionation, chromatography, mass spectrometry, and quantitative antimicrobial assays are therefore necessary to identify the metabolites responsible for the observed activity.

4. CONCLUSION

The results indicate that macrofungi collected from an urban forest fragment in Tabatinga, Amazonas, are promising sources of bioactive compounds with antimicrobial potential. Hydroalcoholic extracts obtained from morphotypes compatible with *P. sanguineus*, *L. crinitus*, *L. berteroi*, *H. hydnoides*, and *E. scabrosa* inhibited *S. aureus*, *Escherichia coli*, and *C. albicans* at the tested concentration.

Although chemical characterization and minimum inhibitory concentration assays were not performed, the findings provide preliminary evidence of the biotechnological potential of Amazonian macrofungi and highlight urban forest fragments as relevant reservoirs of fungal resources for future bioprospecting studies.

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