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Original Article

Tapping into Tapajos: antibacterial potential of fungal strains isolated from decaying wood in the Brazilian Amazon

Explorando o Tapajós: potencial antibacteriano de cepas fúngicas isoladas de madeira em decomposição na Amazônia brasileira

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Abstract

The emergence of bacterial resistance to antimicrobials poses a significant health threat. To address this issue, exploring the fungal diversity in freshwater environments in the Amazon Forest has potential in the search for new antimicrobials. This study aimed to investigate the production of antibacterial metabolites by aquatic fungi from Amazon lakes, specifically Lake Juá and Lake Maicá (Brazil-PA). The fungal isolates were obtained from wood fragments submerged in these lakes, and the ethyl acetate extracts were evaluated for antibacterial activity against *Staphylococcus aureus* ATCC 25923, *S. aureus* (MRSA), ATCC 43300, *Escherichia coli* ATCC 25922, and *E. coli* (ESBL) NCTC 13353. Additionally, toxicity of the extracts (EtOAc with antimicrobial activity) against human fibroblasts MRC-5 was investigated. The study identified 40 fungal strains with antimicrobial screening, and the ethyl acetate extracts of *Fluviatispora* C34, *Helicascus* C18, *Monodictys* C15, and *Fusarium solani* LM6281 exhibited antibacterial activity. *F. solani* LM6281 showed the lowest minimum inhibitory concentration (MIC) of 50 µg/mL against *S. aureus* strains and MIC of 100 µg/mL against *E. coli* strains including ESBL. The cytotoxicity (IC_{50}) of the extract (EtOAc) of *F. solani* LM6281 was 34.5 µg/mL. Preliminary studies of the TLC culture and RNM-H from the extract (EtOAc) of *F. solani* suggested the presence of substances from the class of terpenes, quinones, phenolics, and flavonoids. This study highlights the potential of submerged wood fungi in the Amazon region to produce antibacterial substances, thus identifying them as sources of novel bioactive compounds with potential use in the pharmaceutical industry and regional bioeconomy.

Keywords: antibacterial metabolites, aquatic fungi, Amazon lakes, antimicrobial resistance, bioactive compounds.

Resumo

O surgimento de resistência bacteriana a antimicrobianos representa uma ameaça significativa à saúde. Para abordar esta questão, explorar a diversidade fúngica em ambientes de água doce na Floresta Amazônica tem potencial na busca de novos antimicrobianos. Este estudo teve como objetivo investigar a produção de metabólitos antibacterianos por fungos aquáticos de lagos amazônicos, especificamente os lagos Juá e Maicá (Brasil-PA). Os isolados fúngicos foram obtidos de fragmentos de madeira submersos nesses lagos, e os extratos de acetato de etila foram avaliados quanto à atividade antibacteriana contra *Staphylococcus aureus* ATCC 25923, *S. aureus* (MRSA), ATCC 43300, *Escherichia coli* ATCC 25922 e *E. coli* (ESBL) NCTC 13353. Além disso, a toxicidade dos extratos (EtOAc com atividade antimicrobiana) contra fibroblastos humanos MRC-5 foi investigada. O estudo identificou 40 cepas de fungos com triagem antimicrobiana, e os extratos de acetato de etila de *Fluviatispora* C34, *Helicascus* C18, *Monodictys* C15 e *Fusarium solani* LM6281 exibiram atividade antibacteriana. *F. solani* LM6281 apresentou a menor concentração inibitória mínima (CIM) de 50 µg/mL contra cepas de *S. aureus* e CIM de 100 µg/mL contra cepas de *E. coli* incluindo ESBL. A citotoxicidade (IC50) do extrato (EtOAc) de *F. solani* LM6281 foi de 34,5 µg/mL. Estudos preliminares da cultura TLC e RNM-H do extrato (EtOAc) de *F. solani* sugeriram a presença de substâncias da classe dos terpenos, quinonas, fenólicos e flavonoides. Este estudo destaca o potencial de fungos de madeira submersos na região amazônica para produzir substâncias antibacterianas, identificando-os como fontes de novos compostos bioativos com potencial de uso na indústria farmacêutica e na bioeconomia regional.

Palavras-chave: metabólitos antibacterianos, fungos aquáticos, lagos amazônicos, resistência antimicrobiana, compostos bioativos.

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1. Introduction

In recent times, there has been a surge in global efforts to combat antimicrobial resistance on a global scale (Tejpar et al., 2022). The indiscriminate use of antimicrobials has led to the emergence of pathogenic strains (Sutherland et al., 2019). Researchers studying antimicrobials have identified certain bacterial strains as a major public health concern, including methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus* (VRE), penicillin-resistant *Streptococcus pneumoniae*, and Enterobacteriaceae that produce beta-lactamases (Shamsrizi et al., 2020). In response to antimicrobial resistance, numerous studies are being conducted to search for new antibacterial substances (Hyde et al., 2019; Kim et al., 2019). Historically, actinobacteria and fungi have been the primary sources of antibacterial substances, accounting for about 70% (De La Hoz-Romo et al., 2022; Charousová et al., 2019; Du et al., 2020). Fungi, specifically species such as *Penicillium* Link, *Cephalosporium* Corda, *Acremonium* Link, and *Fusarium* Link, have enabled the discovery of antimicrobial substances like penicillins, cephalosporins, and fusidic acid (Ajah et al., 2018; Newaz et al., 2023; Farhat et al., 2019; Nelsonjoseph et al., 2022). To discover new fungal metabolites for use as antimicrobials, researchers have been motivated to isolate fungal strains from environments such as the bottom of the sea, animal intestines, and extreme environments (Segundo et al., 2022; Adebayo et al., 2021). Previous research has shown that freshwater fungi have the potential to produce metabolites that possess antibacterial properties. Some examples of these metabolites include Quinaphthin, which is produced by *Helicoon richonis* (Boud.) Linder (El-Elimat et al., 2021), *Anguillosporal* from *Anguillospora longissimi* (Sacc. & P.Syd.) Ingold (Singh and Sati, 2020), and various bioactive substances from *Kirschsteiniothelia* D.Hawksw. (Ho et al., 2003), as well as Massarilactones A and B, which are found in *Massarina tunicate* (*Kirschsteiniothelia* D.Hawksw.) (Oh et al., 2003; Abdel-Wahab et al., 2007). The Amazon rainforest is known for its high levels of biodiversity, with between 10-20% of all known plant and animal species found there (Díaz and Malhi, 2022). In addition to its well-known flora and fauna, the Amazon rainforest also contains a vast and largely unexplored microbial diversity. The biotechnological potential of fungi found in the Amazon region has yet to be fully explored, particularly regarding the production of antimicrobial compounds by freshwater fungi. To address this knowledge gap, our research aimed to investigate the antibacterial activity of fungi that decompose submerged wood in lakes within the Brazilian Amazon.

2. Materials and Methods

2.1. Bacterial strains

For our antibacterial assays, we utilized several reference gram-positive strains, including *S. aureus* ATCC 43300 (MRSA) and *S. aureus* ATCC 25923, as well as two gram-negative strains, *E. coli* ATCC 25922 and *E. coli* (ESBL) NCTC 13353. These standard strains were maintained in the Laboratory of Medical Mycology at the National Institute of Amazonian Research – INPA. To prepare for the experiments, we subcultured the strains onto sheep blood agar and MacConkey agar media (Biomerieux®, France) and incubated them at 35 °C for up to 24 hours.

2.2. Fungal strains

We conducted a study to explore the antibacterial properties of 40 fungal strains isolated from submerged wood samples gathered from two locations - Lake Juá (2°25′57″S, 54° 46′39″W) and Lake Maicá (2°27′29.0″S, 54° 40′10.8″W). The wood samples were collected from the embayment of the lower Tapajós and Amazon Rivers, respectively. Collection of wood samples was done once at each location during October and November 2017. We collected 30 submerged wood fragments from each site that exhibited signs of active decomposition. For details on the isolates and their identification, please refer to our published article (Tejpar et al., 2022).

2.3. Molecular identification of isolates

The fungi used in this study were identified based on their morphology and through sequence analysis of two genetic markers: the internal transcribed spacer (ITS) and the 28S ribosomal RNA (rRNA) gene. To isolate genomic DNA, we followed a previously described protocol. For amplification of the ITS sequence, we used the forward primer ITS1 and the reverse primer ITS4 (White et al., 1990). To amplify the D1/D2 domain of the 28S rRNA gene, we used the primer pair NL1 and NL4 (Fliegerova et al., 2006). We compared the sequences of the ITS and rRNA gene regions with those in the NCBI database and used the software MUSCLE to perform multiple alignments of the ITS gene region, which were implemented in MEGA X version 10.2.4. To construct the phylogenetic tree, we used neighbor-joining (NJ) with bootstrap analysis, including 1000 replicates.

2.4. Screening of antibacterial producers

Fungal isolates were cultivated on Petri dishes containing potato dextrose agar (PDA) at room temperature for 7-10 days. To obtain secondary metabolites, the fungi were cultured in a broth using a method similar to that described by Lima et al. (2011), with some modifications. The bioprocesses were conducted in triplicate. A 150 mL Erlenmeyer flask containing 50 mL of potato dextrose broth (made up of 120 g/L potato infusion and 10 g/L dextrose) was used. The medium was inoculated with the fungi at a concentration of 1 x 10^4 spores/mL and incubated under static conditions at room temperature in the dark for 14 days. After this period, the broth was filtered using a qualitative filter (Whatman® Grade 4, cellulose type) and stored at -4 °C. The filtrates were thawed and extracted with ethyl acetate (EtOAc; SYNTH®) at a 1:1 solvent ratio. The solvent was then subjected to a rotary evaporator (Fisatom, series 550, Brazil) at 60 rpm and 30 °C, and the resulting mass was diluted in 10% DMSO at a concentration of 3.2 mg/mL. The EtOAc fractions were examined for antibacterial activity using an agar diffusion assay, and the fractions that displayed antibacterial activity were further analyzed to determine their minimum inhibitory concentration.

2.5. Agar diffusion assay

In order to assess the antimicrobial properties of the fungal extracts using the Clinical and Laboratory Standards Institute (CLSI, 2012) standard M02-A11 with some modifications. To prepare a bacterial inoculum, a DensiCHECKTM-plus spectrophotometer was used to achieve a turbidity of 0.5 on the MacFarland scale, resulting in a standardized suspension containing about 1-2 × 10^8 CFU/mL. The bacterial suspension was then spread in three different directions on the surface of Mueller Hinton agar using a sterile swab. Circular wells were made in the agar plates to accommodate the fungal extracts. A volume of 100 µL of fungal extracts was added to each well, and the test was performed in triplicate. Positive controls of oxacillin (50 µg/mL) and amoxicillin (50 µg/mL) as well as a negative control of 1% DMSO were used for each analysis. The plates were incubated under aerobiosis at 36 °C for 16-18 hours, and the inhibition zones were evaluated.

2.6. Minimum inhibitory concentration determination

For the isolates that displayed activity against at least one of the tested bacteria, the minimum inhibitory concentration (MIC) assay was conducted in triplicate according to the modified guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2012) in the USA. The sensitivity tests to antimicrobial agents by dilution for aerobic growth bacteria were performed using the standard M7 - A9 methodology. Stock solutions of ethyl acetate extracts from fungi were initially prepared at a concentration of 3.2 mg/mL and dissolved in 10% DMSO. Positive controls for *Staphylococcus* and *Escherichia* strains were oxacillin and amoxicillin, respectively, used at concentrations ranging from 50 to 0.048 µ/mL (CLSI, 2012). Fungal extracts were used in decreasing concentrations ranging from 1600 to 1.56 µL/mL. A bacterial inoculum of 10 µL was added at a concentration of 1x10^7 CFU /mL, and incubation was conducted for 16-20 hours at 37°C. The assays were performed in duplicate, and readings were recorded after incubation by visual comparison against positive controls and further visualization with the Alamar BlueTM (Thermo Fisher Scientific, USA) reagent. The contents of the wells that exhibited the inhibition of bacterial growth were then inoculated in Muller Hinton agar to determine bactericidal or bacteriostatic activity, and the plates were incubated at 35 °C for 24 hours.

2.7. Cytotoxicity test

The cytotoxicity of the extracts was evaluated using the Alamar BlueTM assay (Merck, Darmstadt, Germany). MRC-5 (human fibroblast) cells were cultured in DMEM (Dulbecco's Modified Eagle's Medium) supplemented with 10% fetal bovine serum and kept in a CO_2 incubator at 37°C with 5% CO_2. The cells were seeded in 96-well plates at a density of 0.5 × 104 cells per well. After 24 hours, the extracts were dissolved in DMSO, added to each well (10 µg/mL), and incubated for 48 hours. Doxorubicin (5 µg/mL) was used as a positive control. Negative controls (blanks) received the same amount of DMSO and had the same final DMSO concentrations as the samples (0.1%). Two hours before the end of the incubation, 10 µL of Alamar BlueTM was added to each well. The fluorescent signal was measured using a multiplate reader with an excitation wavelength range of 530-560 nm and an emission wavelength of 590 nm. The signals obtained were proportional to the number of viable cells in the sample.

2.8. Thin-Layer Chromatography (TLC)

To identify the chemical classes within the crude EtOAc extract, we employed Thin-Layer Chromatography (TLC). The extract was first dissolved in 1 mL of EtOAc and then applied onto a G60 F254 silica chromatographic plate (measuring 5 cm x 5 cm) with an aluminum reverse phase using glass capillaries in 10 µL aliquots. The chromatographic plates were then eluted in a chromatographic vat, which had been previously saturated with various organic solvent systems in binary combinations, serving as the mobile phase. Specifically, we utilized a 5 mL hexane (HX) / dichloromethane (DCM) system with a ratio of 40:60 (v/v). Once elution was complete, the plates were visualized under ultraviolet light (UV) at both 254 and 365 nm wavelengths. In addition, we employed various chemical reagents, such as ceric sulfate ($Ce(SO4)_2$), to detect terpenes, universal revealer sulfuric anisaldehyde, ferric chloride ($FeCl_3$) to detect phenolic substances, aluminum chloride ($AlCl_3$) and NP/PEG (an ethanolic solution with 1% 2-aminoethyl diphenylborinate/ethanolic solution with 5% polyethylene glycol) to confirm the presence of phenolics with specificity for flavonoids and phenolic acids, KOH to confirm the presence of quinones, and Dragendorff's reagent to reveal alkaloids.

2.9. Hydrogen nuclear magnetic resonance

We acquired the ^{1}H Nuclear Magnetic Resonance (NMR) spectra for the crude EtOAc extract of the *Fusarium solani* LM6281 fungus using a Brucker BioSpin AG spectrometer, model Fourier 300 Ultrashield, 300 MHz. The extract was solubilized in 500 µL of deuterated chloroform (CDCl3), with TMS added as an internal reference (signal at 0.00 ppm) for the chemical shift of the resonance signals in the spectra. We used 15 mg of the extract to obtain the hydrogen spectra at a frequency of 300 MHz, which we then processed and exported using the TopSpinTM program, version 4.0.6.

2.10. Statistical analysis

We reported the results as mean ± standard deviation (SD) from three independent experiments, each performed in triplicate. Statistical differences ($p < 0.05$) in cytotoxicity tests were determined using analysis of variance (ANOVA), followed by Tukey's or Bonferroni's post-tests in GraphPad Prism 6.0 for Windows (Graphpad, San Diego, CA).

3. Results

Forty fungal isolates were evaluated for their ability to produce antimicrobials from submerged wood (Table 1). Supplementary taxonomical information for each isolate can be found in Table S1 Supplementary Material.

The culture extracts (EtOAc) of *Fluviatispora* K.D.Hyde C34 and *Monodicty* S.Hughes C15 exhibited antibacterial activity against *E. coli* ATCC 25922 and *E. coli* (ESBL) NCTC 13353, while *Helicascus* Kohlmeyer C18 and *Fusarium* LM6281 extracts exhibited activity against all four bacterial strains (Table 1).

Fusarium LM6281, a notable antimicrobial producer in this study, was sequenced for the ITS region (ITS1-5.8S-ITS2) and showed 99% similarity to access NR163531.1 *Fusarium solani* (Mart.) Sacc.. TYPE material in the NCBI GenBank database. The obtained nucleotide sequence was submitted to NCBI GenBank with the accession number MT017524, and the strain's identification was confirmed through morphological analysis.

The ethyl acetate (EtOAc) extracts of all fungi that showed inhibitory activity were analyzed to determine their minimal inhibitory concentration (MICs) using the microdilution method (as shown in Table 2).

Among the tested fungi, *F. solani* LM6281 had the lowest MIC values, with a concentration of 50 µg/mL against *S. aureus* ATCC 29923 and *S. aureus* ATCC 43300 (MRSA), and a concentration of 100 µg/mL against *E. coli* ATCC 25922 and *E. coli* (ESBL) NCTC 13353.

To study the toxicity of the culture extracts (EtOAc), a dose-response assay was conducted to investigate their effect on MRC-5 cells. The culture extract of *F. solani* LM6281 and *Helicascus* C18 exhibited IC50 values of 34.5 µg/mL and >50 µg/mL, respectively.

An exploratory thin-layer chromatography (TLC) was conducted to characterize the culture extract (EtOAc) from *F. solani* LM6281 using several chemical revelators to investigate the chemical complexity of the extract and identify the chemical classes present in it. Preliminary results of the TLC showed positive reactions to ceric sulfate, ferric chloride, and 2-aminoethyl diphenylborinate (as illustrated in Figure 1). The ethyl acetate extract from the fungus *Fusarium solani* LM6281 was analyzed using ^{1}H NMR, and the resulting spectrum is presented in Figure 2. The extract displayed signals between 11.3-13.6 ppm, which are indicative of hydrogen atoms from hydroxyl groups chelated to carbonyl oxygens. Additionally, signals characteristic of methyl groups (sharp singlets) were observed in the range of 3.5-4 ppm. The range of 6-7.5 ppm exhibited signals from hydrogens that are attached to aromatic rings. Furthermore, in the range of 0.5-3 ppm, signals characteristic of CH, CH_2, and CH_3 were observed without distinguishing the formats, while long chains of CH_2 were observed at 1.26 ppm.

Table 1. Antibacterial activity of ethyl acetate (EtOAc) extracts of fungi isolated from decaying submerged wood in Juá and Maicá lakes, Pará, Brazil.

Taxon	Antibiotic Inhibition halo (mm)			
				E. coli
	***S. aureus* ATCC 25923**	***S. aureus* ATCC 43300 (MRSA)**	***E. coli* ATCC 25922**	**ESBLNCTC 13353**
				Clinical strain
Fluviatispora C34	**-**	**-**	**9**	**8**
Fusarium solani LM6281	**36**	**32**	**21**	**20**
Monodictys C15	**-**	**-**	**12**	**13**
Helicascus C18	**18**	**14**	**12**	**13**
Positive control	**23 (Oxa)**	**18 (Oxa)**	**32 (Amo)**	**30 (Amo)**
Negative control (DMSO 10%)	-	-	-	-

Oxa: oxacillin; Amo: amoxicillin.

Table 2. Minimum Inhibitory Concentration (MIC in µg mL^{-1}) of the ethyl acetate (EtOAc) fungal extract from decaying submerged wood in Juá and Maicá lakes, Pará, Brazil, presenting antibacterial activity. Tests using the broth microdilution method. Positive controls with commercial antibacterials.

Test extracts	Bacterial strains and MIC test extracts (µg/ml)			
	***S. aureus* ATCC 25923**	***S. aureus* ATCC 43300 (MRSA)**	***E. coli* ATCC 25922**	***E. coli* (ESBL) NCTC 13353**
Fusarium solani LM6281	50 ^{c}	50 ^{t}	100 ^{c}	100 ^{t}
Helicascus C18	200 ^{t}	100 ^{t}	-	200 ^{t}
Fluviatispora C34	-	-	200 ^{c}	200 ^{t}
Monodictys C15	200 ^{c}	200 ^{t}	200 ^{t}	200 ^{t}
Positive control OXA	0.1	0.1	-	-
Positive control AMO	-	-	6.25	12.5
Negative control DMSO 10%	-	-	-	-

OXA: oxacillin; AMO: amoxicillin; ^{t}bacteriostatic; ^{c}bactericidal.

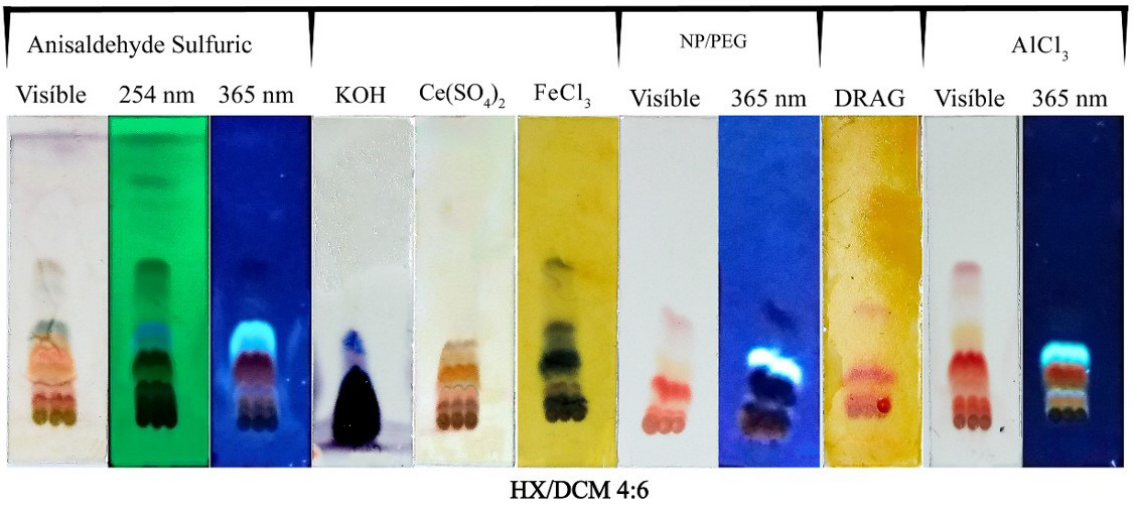

Figure 1. Comparative TLC plates of crude EtOAc extract of *Fusarium solani* LM6281 with different chemical and physical developers. The chromatoplates were eluted in a chromatographic vat previously saturated with different organic solvent systems in binary combinations used as mobile phase (5 mL of the hexane (HX) / dichloromethane (DCM) system (40:60 – v / v).

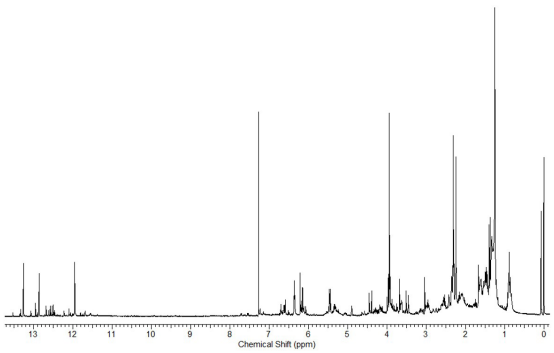

Figure 2. ^{1}H NMR spectra of the crude EtOAc extract of the fungus *Fusarium solani* LM6281.

4. Discussion

The present study investigated the ability of fungal isolates to produce antimicrobial compounds from submerged wood. Among the 40 isolates tested, four fungal isolates (*Fluviatispora* C34, *Monodicty* C15, *Helicascus* C18, and *Fusarium* LM6281) exhibited significant antimicrobial activity against different bacterial strains. The culture extracts (EtOAc) of *Fluviatispora* C34 and *Monodicty* C15 exhibited antibacterial activity against *E. coli* ATCC 25922 and *E. coli* (ESBL) NCTC 13353, while *Helicascus* C18 and *Fusarium* LM6281 extracts exhibited activity against all four bacterial strains tested.

Isolates from genera *Fluviatispora, Monodicty,* and *Helicascus* were investigated for their ability to produce antimicrobial substances.

The genus *Fluviatispora* belongs to the family Halosphaeriaceae, and there have been no reports of this genus producing compounds with antimicrobial activities. However, the family Halosphaeriaceae has been reported as producers of the substance Halosmysin A, which exhibits activity against leukemias. (Yamada et al., 2020).

Investigations of the marine-derived fungus *Monodictys putredinis* (Wallr.) S.Hughes led to the isolation of two novel dimeric chromanones that consist of two uniquely modified xanthone-derived units the a Monodictyochromone A e B (Pontius et al., 2008). The compounds were examined for their cancer chemopreventive potential. *Monodictys castaneae* (Wallr.) S.Hughes SVJM139 have been shown to exhibit antimicrobial activity against various bacteria (Visalakchi and Muthumary, 2009). The fungi *Helicascus*, on the other hand a study investigated the biological activities of this group by obtaining isolates from liquid cultures, and reported the presence of new lactones produced by a marine fungus belonging to the species *Helicascus kanaloanus* Kohlmeyer (ATCC 18591) (Poch and Gloer, 1989).

Sequence analysis of *Fusarium* LM6281 showed that it had 99% similarity to access NR163531.1 *Fusarium solani* TYPE material in the NCBI GenBank database. The obtained nucleotide sequence was submitted to NCBI GenBank, and the strain's identification was confirmed through morphological analysis. *Fusarium solani* LM6281 also had the lowest MIC values, indicating that it is a promising source of antimicrobial compounds against bacterial strains. *Fusarium solani* is a well-known fungal species that is known to produce a wide range of secondary metabolites with antimicrobial properties (Somwanshi and Bodhankar, 2015; Khan et al., 2018).

The dose-response assay conducted to study the toxicity of the culture extracts (EtOAc) showed that *F. solani* LM6281 and *Helicascus* C18 exhibited IC50 values of 34.5 µg/mL and >50 µg/mL, respectively. The IC50 value of *F. solani* LM6281 extract indicates moderated toxicity to MRC-5 cells. Research studies have investigated the effects of certain metabolites produced by *Fusarium solani* on MRC-5 cells. Studies have shown that fumonisin B1 has cytotoxic effects on different types of cells. For example, in a study conducted by Liu (Liu et al., 2019), human liver cells (hepatocytes) were treated with varying concentrations of fumonisin B1.

The results demonstrated that fumonisin B1 caused cellular damage, including morphological changes, increased oxidative stress, and induction of cell death through apoptosis.

Equisetin is a secondary metabolite produced by *Fusarium equiseti* (Corda) Sacc., and the study aimed to assess its potential as an anticancer agent (Wang et al., 2014; Khan et al., 2018). Langseth et al. (1998) reported that the *Fusarium* species produce different trichothecenes and other toxins and exhibit variations in cytotoxicity, both among different species of this genus and within each species.

The exploratory TLC conducted to characterize the culture extract (EtOAc) from *F. solani* LM6281 showed positive reactions to ceric sulfate, ferric chloride, and 2-aminoethyl diphenylborinate, indicating the presence of certain chemical classes in the extract. The ^{1}H NMR analysis of the ethyl acetate extract from *F. solani* LM6281 showed the presence of signals from hydroxyl groups chelated to carbonyl oxygens, methyl groups, hydrogens attached to aromatic rings, CH, CH_2, and CH_3 groups without distinguishing the formats, and long chains of CH_2. These chemical findings suggest the potential presence of terpenes, quinones, phenolics, and flavonoids.

The most important secondary metabolites produced by *Fusarium* that have been extensively studied for their antimicrobial activity are: beauvericin, fusaric acid, equisetin, bikaverin, and fusarin C, anthranilates, fumonisins, alkaloids and quinone (Sondergaard et al., 2016; Hilario et al., 2016; Kyekyeku et al., 2017; Ibrahim et al., 2021) Beauvericin and fusaric acid have been reported to exhibit antifungal activity against various plant pathogens, while equisetin has shown potent antibacterial activity against gram-positive bacteria (Sood et al., 2017). Bikaverin has been reported to possess broad-spectrum antimicrobial activity against bacteria and fungi, while fusarin C has been shown to exhibit potent antifungal activity against various plant pathogens (Sondergaard et al., 2016). These secondary metabolites hold great promise as potential candidates for the development of novel antimicrobial agents (Mendonça et al., 2021).

Overall, the results of this study demonstrate that submerged wood could serve as a source of antimicrobial-producing fungi, with *Fusarium solani* LM6281 showing particular promise. Further studies can be conducted to investigate the chemical composition of the extract and its mechanism of action, as well as the potential for the development of new antimicrobial agents.

5. Conclusions

In conclusion, this study provides valuable insights into the potential of submerged wood as a source of antimicrobial-producing fungi. The findings highlight the ability of four fungal isolates, including *Fusarium solani* LM6281, to produce compounds with significant antibacterial activity against various bacterial strains. The chemical analysis of the extract from *F. solani* LM6281 revealed the presence of various chemical classes, which may contribute to its antimicrobial properties. These results suggest that further investigation of the extract's chemical composition and mechanism of action is warranted. The secondary metabolites produced by *F. solani* hold promise as potential candidates for the development of novel antimicrobial agents. Overall, this study provides a foundation for future research aimed at harnessing the potential of fungal isolates from submerged wood for the development of new antimicrobial agents.

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Supplementary Material

Supplementary material accompanies this paper.

Table S1. Strain code and similarity (ITS Barcode) of 14 taxa isolated from samples of submerged wood collected in Lakes Juá and Maicá, Santarém, Pará, Brazil

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