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Chemistry

Prospection and antibacterial screening of metabolic extracts of endophytic fungi isolated from *Tibouchina granulosa* (Desr.) Cogn. (Melastomataceae)

Prospecção e *screening* antibacteriano de extratos metabólicos de fungos endofíticos isolados de *Tibouchina granulosa* (Desr.) Cogn. (Melastomataceae)

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ABSTRACT

The multidrug resistance of pathogenic microorganisms against widely used antimicrobials has grown in recent years. Among the different sources of bioactive compounds, endophytic fungi stand out for their ability to produce important classes of bioactive substances. The present study investigated the chromatographic profiles and antimicrobial activity against 10 pathogenic strains (four included in critical priority by WHO) of the extracts of 12 endophytic fungi isolated from *Tibouchina granulosa* (Melastomataceae). The activity of the metabolites was evaluated using broth microdilution to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Screening of partial chemical profiles was obtained using HPLC-DAD. Extracts of all fungi inhibited the proliferation of 4–10 pathogenic bacterial strains tested. At concentrations between 500 and 1,000 µg mL-1, *Xylaria berteroi* extract inhibited the growth of all strains tested, while *Diaporthe oxe* inhibited eight strains. Chemical analysis demonstrated diverse chromatographic profiles with the possibility of different classes of specialized metabolites, including polyketides, alkaloids, furanones, and terpenoids. Overall, endophytic fungi isolated from *Tibouchina granulosa* were found to synthesize different natural bioactive compounds, highlighting their potential for use in chemical prospecting and characterization.

Keywords: Bioactive molecules; Endophytes; HPLC; Human pathogens; MIC

RESUMO

A multirresistência de microrganismos patogênicos contra antimicrobianos amplamente utilizados tem se fortalecido nos últimos anos; dentre as diferentes fontes de compostos bioativos, os fungos endofíticos se destacam por sua capacidade de produzir importantes classes de substâncias bioativas. O presente estudo investigou os perfis cromatográficos e a atividade antimicrobiana contra 10 cepas patogênicas (4 incluídas em prioridade crítica pela OMS) dos extratos de 12 fungos endofíticos isolados de Tibouchina granulosa (Melastomataceae). A atividade dos metabólitos foi avaliada usando microdiluição em caldo para determinar a MIC e MBC. A triagem de perfis químicos parciais foi obtida usando HPLC-DAD. Extratos de todos os fungos podem inibir a proliferação de 4 a 10 cepas bacterianas patogênicas testadas. Em concentrações entre 500 e 1.000 µg mL-1, o extrato de Xylaria berteroi inibiu o crescimento de todas as cepas testadas e Diaporthe oxe inibiu oito cepas. A análise química demonstrou diversos perfis cromatográficos com a possibilidade de diferentes classes de metabólitos especializados, incluindo policetídeos, alcalóides, furanonas e terpenóides. No geral, fungos endofíticos isolados de Tibouchina granulosa sintetizam diferentes compostos bioativos naturais, indicando sua promessa para prospecção e caracterização química.

Palavras-chave: Moléculas bioativas; Endófitos; HPLC; Patógenos humanos; MIC

1 INTRODUCTION

Microorganisms are characterized by their ability to evolve constantly, both biologically and genetically, in response to bioactive substances in their environment, resulting in the development of resistance to these substances. It is in this context that the indiscriminate use of antimicrobials has promoted the emergence of microorganisms that exhibit resistance to many widely used drugs (Junior et al., 2018).

In 2017, the World Health Organization (Who) published for the first time a list of 12 “priority pathogens” for research on novel antimicrobials, which they classified as “critical priority” (*Pseudomonas aeruginosa* and members of the family *Enterobacteriaceae*), “high priority” (*Staphylococcus aureus* and *Salmonella* spp.), or “medium priority” (*Streptococcus pneumoniae* and *Shigella* spp.) (Who, 2017). However, the 2020 annual report, in which candidate antimicrobial substances were presented, the WHO revealed that this line of research has advanced slowly since 2017. Among the antibiotics approved by regulatory agencies, 82% belong to classes with reported resistance (WHO, 2021).

Brazil is home to 20% of the world's plant biodiversity and boasts the richest environmental diversity on the planet (UNEP, 2019). The *Tibouchina granulosa* (Melastomataceae) is a native plant of the Brazilian Atlantic Forest biome, and is widely used for ornamentation. Studies on plant extracts of the melastomataceous species have documented several bioactivities, such as antifungal (Kuster *et al*., 2009), antibacterial, and antiparasitic (Tracanna et al., 2015), antinociceptive (Dias et al., 2016), anti-inflammatory (Ramírez-Atehortúa et al., 2018), and antioxidant (Bomfim et al., 2021) properties. However, the bioactivities of the endophytic fungi associated with these plant species are relatively understudied.

In the search for new bioactive substances, new pharmaceutical products have been derived from compounds found in plant extracts, as well as from the interaction between plants and their associated microorganisms, such as endophytic filamentous fungi (El Sayed, 2021; El Sayed, 2022). Endophytes are known to synthesize diverse chemical metabolites, such as steroids (Su et al., 2022), alkaloids (Ma et al., 2022), phenols (Liu et al., 2020) isocoumarins (Li et al., 2022), xanthones (Sritharan et al., 2019), quinones (Kamel et al., 2020), terpenoids (Lua et al., 2020), cytochalasins (Medina et al., 2019), peptides (Saikia et al., 2022), lipids (Bekiesch et al., 2019), glycosides (Wang et al., 2022) and other substances, representing promising sources of new bioactive compounds (Rai et al., 2021).

Many of these secondary or specialized metabolites produced by endophytic fungi have already been reported to exhibit biological activity, such as antiprotozoal (Golias et al., 2020), antiphytopathogenic (Rajani et al., 2021), antidiabetic (Agrawal et al., 2022), antimicrobial (Demeni et al., 2021), antifungal (Sishuba et al., 2021), phytopromotatory (Khan et al., 2021), antioxidant (Palupi et al., 2021), anticancer (Lim et al., 2021, El-Sayed, 2022), and biofilm-degrading (Matias et al., 2021) properties. The antibacterial activity of these microorganisms is also noteworthy. Studies have previously reported on the action of endophytes with action against all pathogens listed as "priority pathogens" by the WHO, including *Pseudomonas aeruginosa* (Bodele

et al, 2022), *Proteus mirabilis* (De Oliveira Chagas et al., 2017), *Klebsiella pneumoniae* (Pelo et al., 2020), *Staphylococcus aureus* (Wu et al., 2018), and *Salmonella enteritidis* (Gond et al., 2012).

To this end, in an exploratory study, Golias et al. (2020) isolated and identified endophytic fungi from the healthy leaves of *T. granulosa*. The fungi identified belonged to the genera *Cercospora*, *Colletotrichum*, *Diaporthe*, *Fusarium*, *Hypoxylon*, *Nigrospora*, *Phyllosticta*, and *Xylaria*. Metabolites found in the ethyl acetate fraction of the crude extract of *Phyllosticta capitalensis* exhibited inhibitory activity against promastigotes of *Leishmania amazonensis* and *Leishmania infantum*.

Based on the previous findings mentioned above, the objective of the present study was to evaluate the antimicrobial activity of metabolites derived from the previously isolated endophytic fungi against pathogenic microorganisms of great relevance to human health.

2 MATERIALS AND METHODS

2.1 Endophytic fungi culture and crude extract preparation

The endophytic fungi were isolated and identified by Golias *et al*. (2020), and form part of the Collection of Endophytic and Environmental Microorganisms of the Microbial Biotechnology Laboratory of the State University of Maringá (CMEA-UEM). The following isolates were used: *Cercospora* sp. Tg154, *Colletotrichum jiangxiense* Tg29, *Colletotrichum karstii* Tg13, *Colletotrichum siamense* Tg55, *Diaporthe* cf. *hevea* 1 Tg12, *Diaporthe endophytica* Tg57, *Diaporthe oxe* Tg32, *Diaporthe paranaenses* Tg73, *Fusarium circinatum* Tg134, *Phyllosticta capitalensis* Tg06, *Xylaria berteroi* Tg168, and *Xylaria grammica* Tg79.

The endophytes were activated in potato dextrose agar (PDA; Reatec®) medium supplemented with 50 µl mL^{-1} tetracycline and incubated for 7 days at 28°C. Subsequently, six 5-mm diameter discs of each fungal colony were transferred to an

Erlenmeyer flask containing 100 mL of potato dextrose broth (PDB; Acumedia®) and incubated for 21 days at 28°C.

For metabolite extraction, the fermented broth was first filtered using a glass funnel and cotton and then centrifuged in 50 mL conical tubes at 1,400 × *g* for 15 min. The supernatant was transferred to a separation funnel, and the solvent was added at a 1:5 ratio (ethyl acetate:fermented broth). This step was repeated three times. The solvent was collected and subjected to rotary evaporation at 37°C and 600 mmHg pressure (TE-210; Tecnal). The ethyl acetate fractions were stored at 80°C in an ultra-freezer until use.

2.2 Bacterial activation

The gram-negative pathogenic bacteria *Pseudomonas aeruginosa* (ATCC 15442), *Pseudomonas hydrophila* (ATCC 7966), *Salmonella enteritidis* (ATCC 13076), *Shigella flexneri* (ATCC12022), *Proteus mirabilis* (ATCC 25933), and *Klebsiella pneumoniae* (ATCC 700603), as well as the gram-positive pathogenic bacteria *Enterococcus faecalis* (ATCC 19433), *Staphylococcus epidermidis* (ATCC 12228), *Staphylococcus aureus* (ATCC 25923), and *Bacillus subtilis* (ATCC 6633) were activated in Mueller–Hinton broth (MH; Becton Dickinson®) at 37°C for 24 h. Subsequently, the bacterial inoculum were suspended in 0.9% saline solution until a turbidity equivalent to 0.5 on the McFarland scale was reached, followed by dilution at 1:10 (inoculum: 0.9% saline solution) to obtain an inoculum of 1×10^5 colony forming units per milliliter ($CFU \cdot mL^{-1}$). The bacteria *P. aeruginosa*, *P. hydrophila*, *P. mirabilis*, and *K. pneumoniae* are classified as “priority 1” or “critical” by the WHO, while the species *S. enteritidis* and *S. aureus* are classified as “priority 2” or “high” and *S. flexneri* is classified as “priority 3” or “medium” (WHO 2020).

2.3 Antimicrobial activity

The activity of crude extracts from fungal cultures was evaluated using broth microdilution to determine the minimum inhibitory concentration (MIC) (i.e. the lowest concentration capable of inhibiting bacterial growth visible to the naked eye) and the

minimum bactericidal concentration (MBC) (i.e. the lowest concentration at which no growth is observed in the agar medium) using protocol M7-A of the National Committee for Clinical Laboratory Standards (NCCLS) (CLSI, 2018).

The crude extract was diluted in 5% DMSO, resuspended in MH broth until the final concentration was 2,000 μg mL^{-1}, and sterilized by passing through a 0.45-μm membrane filter (Filtrilo®). In each well of a sterile 96-well plate, 100 μL of MH broth was dispensed. Subsequently, 100 μL of the sterile crude extract were added to each well of the first column, and the mixtures were serially diluted (1:2) to obtain concentrations of 0.98–1,000 μg mL^{-1}. Another 100 μL of MH broth was added to the microbial growth control wells. Finally, 5 μL of bacterial inoculum was distributed in all wells, and the plates were incubated at 37°C for 24 h.

After incubation, the MIC was determined from the well corresponding to MIC, using 10-μL aliquots seeded in a Petri dish containing MH agar to determine MIC. After seeding on agar to the determined MIC, 10 μL of aqueous triphenyl tetrazolium chloride solution (1% TTC; VETEC®) was added to each well, and the plate was re-incubated for another hour at 35°C. The presence of red coloration in the wells was interpreted as negative evidence of the inhibitory effect of the test crude extract, while the absence of coloration was considered positive evidence (Ayres *et al*., 2008). The tests were performed in triplicate. Wells without bacterial growth were used to determine the MBC. To this end, aliquots of 10 μL of each well were grown in Mueller-Hinton agar (MHA) for 24 h to 37°C. The MBC was considered as the lowest concentration that inhibited bacterial growth.

2.4 Screening of partial chemical profiles of fungal extracts using high-performance liquid chromatography with a diode array detector (HPLC-DAD)

Chromatograms were obtained using HPLC (Prominence; Shimadzu) with two LC-20AR pumps, the DGU-20A5R degasser, the SIL-10AF automatic injector, the SPD-M20A DAD, and the MBC-20ª controller (Shimadzu).

Approximately 4.0 mg of fraction obtained from each endophytic fungus was resuspended in 2 mL of methanol (HPLC grade, Merck®). The suspension was filtered through a PTFE hydrophilic filter and analyzed using HPLC-DAD under the following chromatographic conditions: injection volume, 10 µL; flow rate, 0.8 mL min-1; mobile phase, HPLC-grade methanol (Merck®) and Milli-Q water (Millipore®); stationary phase, Supelcosil LC-18 column (25 cm × 4.6 mm, 5 µm i.d.). An exploratory elution gradient of 5–100% methanol over 30 min and 100% methanol for 10 min was set. At the end of the run, a return gradient of 100–5% methanol for 5 min, with a 15-min waiting time for column reconditioning, was set. Spectra in the UV–Vis region were recorded using the DAD in a 190–800 nm scan range, with 254 nm selected as the wavelength to obtain the chromatograms and analyze the data.

3 RESULTS AND DISCUSSON

In the present study, the amount of crude extract produced from 12 endophytic fungi isolated in the healthy leaves of T. granulosa was evaluated (GOLIAS et al., 2020). The T. granulosa leaves were surface-sterilized and cut into small fragments of about 5 mm × 2 mm. Subsequently, five leaf fragments were arranged per petri dish containing BDA medium plus tetracycline (Sigma, St. Louis, MO) and incubated at 28°C for 7 days. The crude extract of endophytes was tested for antimicrobial activity against gram-positive and gram-negative pathogenic bacteria (Table 1).

All endophytic fungi of T. granulosa evaluated in this study produced specialized metabolites that could inhibit the proliferation of 4–10 bacterial strains at concentrations of 500 or 1,000 µg mL-1 depending on the fungal extract (Table 1). In the decreasing order of antibacterial activity of their extracts, the tested fungi were arranged as follows: Xylaria berteroi (inhibited the growth of all 10 strains tested), Diaporthe oxe (8), Diaporthe endophytica (6), Diaporthe cf. hevea 1 (6), Colletotrichum siamense (6), Phyllosticta capitalensis (6), Cercospora sp. (4), Colletotrichum karstii (4),

Colletotrichum jiangxiense (4), Diaporthe paranaenses (4), Fusarium circinatum (4), and Xylaria grammica (4).

Table 1 – Antimicrobial activity of the ethyl acetate fraction of endophytic fungi isolated from *Tibouchina granulosa* (Desr.) Cogn. (Melastomataceae) against human pathogenic microorganisms

(Continued)

Endophytic fungi		**Pathogenic microorganisms**			
		Gram-positive			
		B. subtilis	***S. aureus***	***S. pidermidis***	***E. faecalis***
Cercospora sp	MBC	-	-	-	-
	MIC	1000*	-	-	-
Colletotrichum jiangxiense	MBC	-	-	-	-
	MIC	1000	1000	-	-
Colletotrichum karstii	MBC	-	-	-	-
	MIC	1000	1000	-	-
Colletotrichum siamense	MBC	-	1000	-	-
	MIC	1000	500	1000	-
Diaporthe cf. hevea 1	MBC	-	-	-	-
	MIC	1000	1000	-	-
Diaporthe endophytica	MBC	1000	1000	-	-
	MIC	500	500	1000	1000
Diaporthe oxe	MBC	-	-	-	-
	MIC	1000	1000	1000	-
Diaporthe paranaenses	MBC	-	-	-	-
	MIC	1000	1000	-	-
Fusarium circinatum	MBC	-	-	-	-
	MIC	1000	1000	-	-
Phyllosticta capitalensis	MBC	-	-	-	-
	MIC	1000*	1000	-	-
Xylaria berteroi	MBC	-	1000	-	-
	MIC	1000	500	1000	1000
Xylaria grammica	MBC	-	-	-	-
	MIC	1000	1000	-	-

Table 1 – Antimicrobial activity of the ethyl acetate fraction of endophytic fungi isolated from *Tibouchina granulosa* (Desr.) Cogn. (Melastomataceae) against human pathogenic microorganisms (Conclusion)

Endophytic fungi		**Pathogenic microorganisms**					
		Gram-negative					
		P. hydrophila	***K. pneumoniae***	***S. enteritidis***	***S. flexneri***	***P. aeruginosa***	***P. mirabilis***
Cercospora sp	MBC	-	-	-	-	-	-
	MIC	1000	1000	-	1000	-	-
Colletotrichum jiangxiense	MBC	-	-	-	-	-	-
	MIC	-	-	1000	1000	-	-
Colletotrichum karstii	MBC	-	-	-	-	-	-
	MIC	-	1000	1000	-	-	-
Colletotrichum siamense	MBC	-	-	1000	1000	-	-
	MIC	-	-	500	500	1000	-
Diaporthe cf. hevea 1	MBC	-	-	-	-	-	-
	MIC	1000	1000	1000	1000	-	-
Diaporthe endophytica	MBC	-	-	-	1000	1000	-
	MIC	-	-	-	500	500	-
Diaporthe oxe	MBC	-	-	-	-	1000	-
	MIC	1000	1000	1000	1000	500	-
Diaporthe paranaenses	MBC	-	-	-	-	-	-
	MIC	-	-	1000	1000	-	-
Fusarium circinatum	MBC	-	-	-	-	-	-
	MIC	-	-	1000	1000	-	-
Phyllosticta capitalensis	MBC	-	-	-	-	-	-
	MIC	1000	1000	1000	1000	-	-
Xylaria berteroi	MBC	-	-	1000	-	1000	-
	MIC	1000	1000	500	1000	500	1000
Xylaria grammica	MBC	-	-	-	-	-	-
	MIC	-	1000	1000	-	-	-

*Concentration in $\mu g.mL^{-1}$; "-": no activity at the concentrations evaluated. MBC: minimum bactericidal concentration; MIC: minimum inhibitory concentration. Strains: *Bacillus subtilis, Enterococcus faecalis, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, Pseudomonas hydrophila, Salmonella enteritidis, Shigella flexneri, Staphylococcus aureus, and Staphylococcus epidermidis.* Source: Authors (2023)

In terms of the susceptibility of microorganisms to fungal metabolites, 60.4% (n = 29) of the tested strains that were sensitive to these extracts were gram-positive bacteria, compared to 51.4% (n = 37) of sensitive gram-negative bacteria (Table 1).

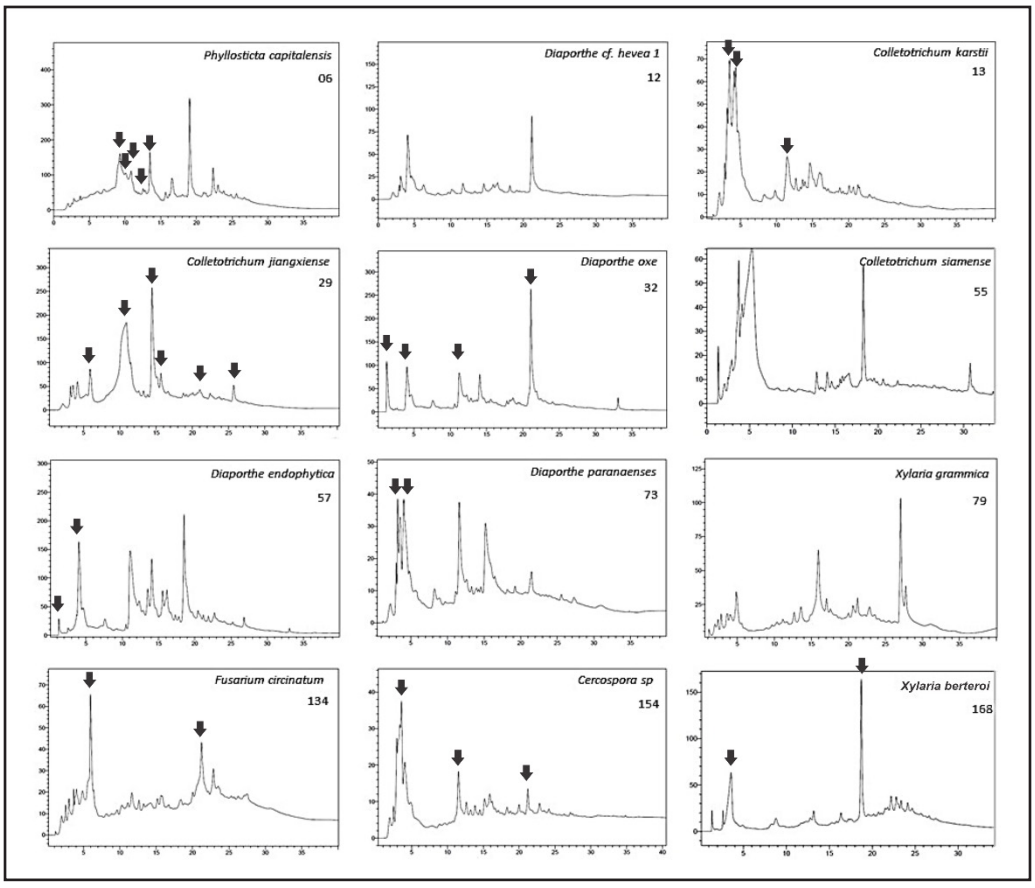
Bacillus subtilis and *Staphylococcus aureus* were the most susceptible microorganisms to the crude extract produced by the endophytic fungi tested, being inhibited by metabolites of all species, with the exception of *Cercospora* sp., which did not inhibit its growth. *S. flexneri* and *Salmonella enteritidis* were inhibited by metabolites produced by 10 of the 12 fungi tested: *K. pneumoniae* (7), *P. hydrophila* (5), *P. aeruginosa, S. epidermidis* (4), *E. faecalis* (2), and *P. mirabilis* (1) (*Xylaria berteroi*).

Cercospora species are economically and ecologically important fungi, representing a major group of parasites that cause immense damage due to their high phytopathogenicity, justifying the extensive biochemical and molecular research conducted to elucidate the underlying mechanisms (Świderska-Burek et al., 2020). Felisbino et al. (2021) evaluated the antibacterial activity of the crude ethyl acetate extract of *Cercospora brachiata* against the bacteria *Actinomyces naeslundii* and *Streptococcus sanguinis*, reporting a MIC of 100 μg mL^{-1} and 200 μg mL^{-1}, respectively. The authors highlight some of the identified compounds, such as fatty acids, esters, and steroids. Mookherjee et al. (2020) evaluated the antimicrobial activity of the endophyte *Cercospora* sp. PM018 against various bacterial strains. Among 20 test microbes, the highest antibacterial activity of PM018 was observed against *S. aureus*, *Ralstonia solanacearum*, and *E. coli*. Mannitol, palmitic acid, and stearic acid are among some of the critical antibacterial constituents isolated from this endophyte.

The chromatogram obtained for the ethyl acetate fraction of the fungal extract of *Cercospora* sp. showed six major peaks (Figure 1). Peak 4 showed a retention time (RT) of 4 min and maximum absorbance bands in the UV region between 200–220 and 258 nm. Peaks 5 (RT = 11.5 min) and 6 (RT = 21.2 min) showed bands in the region of 200 and 260 and 200–220 nm, respectively. According to Domzalski et al. (2021) and Wu et al. (2019), these bands correspond to compounds belonging to the class of alkaloids.

In addition, compounds belonging to the furanone (Kim et al., 2018) and terpenoid (Bass and Niemann, 1978) classes have also been reported with absorption bands in this region. Therefore, further chemical analyses are warranted to characterize these fractions, including the classes of specialized metabolites that constitute them. Our HPLC-DAD analysis provided information on the complexity of samples and indicated possible classes of compounds, generating valuable data for future experiments aimed at the isolation, characterization, and prospection of these compounds.

Figure 1 – Chromatograms of fungal extracts obtained using HPLC-DAD (254 nm) of endophytic fungi isolated from *Tibouchina granulosa*



The x-axis corresponds to the retention time and the y-axis is the intensity of absorbance signal of HPLC-DAD analysis. Source: Authors (2023)

Although alkaloids are known to possess antimicrobial activity (Youssef et al., 2021), few studies to date have reported on alkaloids produced by the endophytic

Cercospora sp. (Weltmeier et al., 2011). To the best of our knowledge, this study is novel in its reporting of bioactivity potentially conferred by alkaloids in this genus, which warrants further exploration.

Furthermore, fungi of the genus *Colletotrichum* are known to cause anthracnose and post-harvest rot in fruits (Phoulivong et al., 2010), as well as keratitis in humans (Shivaprakas et al., 2011). Moreover, as endophytes, they are known as the major producers of specialized metabolites (Moraga et al., 2019). In the present study, species of the genus *Colletotrichum* (*C. jiangxiense*, *C. karstii*, and *C. siamense*) produced metabolites capable of inhibiting the growth of 46.7% of the microorganisms tested. Specifically, *B. subtilis*, *S. aureus*, and *S. enteritidis* were inhibited by metabolites from these three fungi. Notably, *C. siamense* also showed inhibitory activity against *S. epidermidis*, *S. flexneri*, and *P. aeruginosa*, and *C. jiangxiense* also showed inhibitory activity against *S. flexneri*.

The chromatogram obtained from the ethyl acetate fraction of the fungal extract of *Colletotrichum jiangxiense* showed six major peaks, with RTs of ~5.9–25.7 min (Figure 1). Spectra in the UV region of substances corresponding to peaks 2, 4, and 6 showed the maximum absorbance bands in the region between 215 and 280 nm. Compounds belonging to the polyketide (Lai et al., 2020) and alkaloid (Sangster & Stuart, 1965; Wu et al., 2019) classes, which are widely detected in fungi, have been reported to present absorption bands close to those obtained for the *Colletotrichum jiangxiense* fraction in this study (Lai et al., 2020; Sangster et al., 1965; Wu et al., 2019).

Contrary to the evaluated species of the genus, *Colletotrichum karstii* showed three peaks (peak 3, 4 and 5 with RTs of 4.6–14.6 min) with bands in the region of 200 and 260 nm; for peak 4 (RT = 4.3 min), the maximum absorbance band was recorded between 200 and 220 nm (Figure 1). In a previous study, Padmathilake et al. (2017) identified substances belonging to the furanone group with bands at 200 and 260 nm. Luo et al. (2019) identified three new polyketides produced by endophytic *Colletotrichum gloeosporioides*: (2S)-2,3-dihydro-5,6-dihydroxy-2-methyl-4H-1-benzopyran-4-one, (2'R)-

2-(2'-hydroxypropyl)-4-methoxyl-1,3-benzene-diol, and 4-ethyl-3-hydroxy-6-propenyl-2H-pyran-2-one. The first polyketide showed antimicrobial activity against *Bacillus cereus* (MIC = 12.5 $\mu g \cdot mL^{-1}$), while the third showed activity against *Staphylococcus albus*, *Bacillus subtilis*, and *Staphylococcus aureus* with the same MIC value. Overall, our data corroborate previous reports on the antimicrobial activity of this genus.

Zou et al. (2000) have reported another notable example of the action of polyketides isolated from the *Colletotrichum* sp. Specifically, the authors tested collectotric acid isolated from the endophyte *C. gloeosporioides* and recorded MICs of 25 and 50 $\mu g \cdot mL^{-1}$ against *B. subtilis* and *S. aureus*, respectively. Consistently, in the present study, *B. subtilis* and *S. aureus* were inhibited by the metabolites of *C. siamense*, *C. jiangxiense*, and *C. karstii* (Zou et al., 2000), confirming the sensitivity of these pathogenic strains to metabolites produced by endophytes of the genus *Colletotrichum* and the immense potential of these substances.

Furthermore, alkaloids and terpenes extracted from *Colletotrichum* species have been widely reported (MORAGA et al., 2019). Five *Colletotrichum* species (*C. karstii*, *C. arxii*, *C. aegnigma*, *C. cordylinicola*, and *Colletotrichum* sp.) isolated from different parts of *Cinchona calisaya* Wedd have been reported to exhibit antibacterial activity against *S. aureus* and *E. coli* (Radiastuti et al., 2017).

Fractions of endophytic fungal extracts of *Diaporthe* spp. showed inhibitory activity against all pathogens tested, except *Proteus mirabilis*. The fraction of *Diaporthe endophytica* extract showed the strongest bactericidal effect within the genus, exhibiting inhibitory activity against *B. subtilis*, *S. aureus*, *S. flexneri*, and *P. aeruginosa*, followed by *Diaporthe oxe*, which exhibited the same activity/concentration against *P. aeruginosa.*

In Brazil, 272 endophytic fungi were isolated from *Schinus terebinthifolius* (Dos Santos et al., 2021), of which only 26 have been tested for their antimicrobial activity. *Diaporthe terebinthifolii* CMRP1430 and *D. terebinthifolii* CMRP1436 have been reported to exhibit antimicrobial activity against *E. coli*, *P. aeruginosa*, and *S. aureus*. These reports indicate that fungi of this group show great potential for their antimicrobial activity.

The chromatograms of *Diaporthe endophytica*, *Diaporthe oxe*, and *Diaporthe paranaenses* presented the same maximum absorbance bands (200, 220, and 258 nm, respectively) for peaks 1 and 2, with RTs of 4.0–11.6 min (Figure 1), which correspond to substances in the classes of alkaloids (Sangster & Stuart, 1965; Wu et al., 2019). The production of alkaloids by endophytic fungi of the genus *Diaporthe* has been reported previously (Maehara et al., 2012; Cui et al., 2017a; Cui et al., 2017b), compounds that have been attributed to the inhibitory activity of these fungi against *Mycobacterium tuberculosis* (Cui et al., 2017a).

The chromatogram of the ethyl acetate fraction of *Diaporthe oxe* extract showed four major peaks (Figure 1). In a previous study, Wang et al. (2018) identified polyketide group metabolites, such as azafilones, corresponding to the observed absorption band of peak 4 (RT = 21 min and UV_{max} = 223 and 332 nm) reported in the present study.

Fusarium circinatum showed inhibitory activity against *B. subtilis*, *S. aureus*, *S. enteritidis*, and *S. flexneri*. Moreover, the chromatogram of the ethyl acetate fraction of this fungal extract showed three major peaks: peaks 1 and 3 showed RTs of 6 and 22.8 min and absorption spectra in regions between 200–220 and 253 nm and 200 and 258 nm, respectively (Figure 1).

The antimicrobial activity of metabolites produced by an unidentified species of the genus *Fusarium* (Du et al., 2020) isolated from *Securinega suffruticosa* was linked to the high content of alkaloids (0.170%) that inhibited the growth of *Staphylococcus aureus* at 1,000 $\mu g \cdot mL^{-1}$.

Phyllosticta capitalensis showed inhibitory activity against *B. subtilis*, *S. aureus*, *P. hydrophila*, *K. pneumoniae*, *S. enteritidis*, and *S. flexneri*. Among the five major peaks present in its chromatogram, those observed at RTs of 10.7 and 13.5 min with maximum absorbance bands at 200 and 317 nm were peculiar. Xiao et al. (2016) isolated isocoumarins from *Aspergillus* sp., which showed absorption bands close to those detected for compounds in the *P. capitalensis* extract.

P. capitalensis isolated from the hypocotyls of *Bruguiera sexangulado* in a Chinese mangrove was characterized and eight specialized metabolites, including four

meroterpenes and four policetides, were detected. Meroterpenes, guignardone A, and guignardone J have been reported to exhibit antimicrobial activity against *S. aureus* (Xu et al., 2021), with MICs of 25 and 50 $\mu g \cdot mL^{-1}$.

Xylaria berteroi was the only species among the endophytic fungi tested whose specialized metabolites presented inhibitory activity against all microorganisms evaluated at concentrations between 500 and 1,000 $\mu g \cdot mL^{-1}$. In addition, it presented bactericidal activity against *S. aureus*, *S. enteritidis*, and *P. aeruginosa*. Thus, this strain likely represents an important source of bioactive metabolites. In contrast, *Xylaria grammica* showed inhibitory activity against *B. subtilis*, *S. aureus*, *K. pneumoniae*, and *S. enteritidis*.

Although no study has reported on the antimicrobial activity of the endophytes *Xylaria berteroi* and *Xylaria grammica*, other properties have already been described, since *Xylaria* species present a wide chemical diversity of specialized bioactive metabolites (Song et al., 2014).

Among of the specialized metabolites of *Xylaria* species exhibiting antimicrobial activities, 4-cyanomethoxybenzoic (Rakshith et al., 2013), 5-carboxylmellein and cytochalasin D (Pongcharoen et al., 2007), 1β, 4β, 7α-trihydroxyeudesmane (Wang et al., 2007), haloroselinic acid (Chinworrungsee et al., 2001), and piliform acid (Rukachaisirikul et al., 2013) have been reported. Overall, the rich diversity of isolated substances and their activities within the genus *Xylaria*, in addition to the antimicrobial activity of *Xylaria berteroi* and *Xylaria grammica* documented in the present study, highlights the importance of this data for future research on the prospection of new biomolecules from these endophytic fungi.

4 CONCLUSIONS

To summarize, the findings of this study highlight *Tibouchina granulosa* as a promising source of endophytes for the production of antimicrobial compounds. Our analyses demonstrated that the ethyl acetate fraction of endophytic fungal

extracts contains compounds with potential antimicrobial activities. Based on data obtained through the HPLC-DAD analysis of extracts of endophytic fungi isolated from *Tibouchina granulosa*, as well as comparisons with the existing literature, the detected absorbance bands in the UV–Vis region were found to primarily correspond to compounds belonging to the classes of polyketides, alkaloids, furanones, and terpenoids, as possible constituents of the analyzed samples. However, these chemical characterization data are preliminary, and further analyses are warranted for the isolation and characterization of specialized metabolites produced by the tested endophytes. Nonetheless, our findings can guide the future exploration and prospection of metabolites produced by *Tibouchina granulosa* endophytes. Overall, the present study demonstrates the exploration potential of the collection of endophytes isolated from *Tibouchina granulosa* for the prospection and identification of natural antibacterial compounds of fungal origin.

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