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ECOLOGÍA

Chemical analysis of endophytic fungi isolated from mangrove trees in Playa San Pedro Nature Reserve, Buenaventura, Valle del Cauca, Colombia

Análisis químico de hongos endófitos aislados de árboles de mangle en la Reserva Natural Playa San Pedro, Buenaventura, Valle del Cauca, Colombia

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ABSTRACT

Endophytic fungi are well known for their association with a wide variety of plant species, likewise, mangrove plants are well known for harboring a vast variety of fungi with a valuable diversity of bioactive compounds originating from the secondary metabolism that is synthesized in part as a response to the chemical defense against microorganisms, hostile environments, and antagonistic insects. The objective of the present study was to analyze the chemical composition of endophytic fungi isolated from mangrove trees in Buenaventura, Colombia. Analyses of DNA sequences from the internal transcribed spacer ribosomal nuclear region (ITS) were conducted to determine the fungi's identity. The results revealed 17 isolates, belonging to eight fungal families. All isolates were subjected to thin-layer chromatography analysis, observing different phytochemical nuclei eluted in the system (7: 3 hexane: acetone), of these, 23 compounds were recognized using gas chromatography coupled to mass spectrometry; cytotoxicity tests were carried out in human foreskin fibroblast cell line, which did not show a trend in cell viability. The selected endophytic fungi derived from mangrove trees reveal the presence of different chemical compounds, representing an alternative resource of great interest in bioprospecting and bioremediation.

Keywords: Biological activity, bioactive compounds, phytochemical nuclei, saline environments.

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RESUMEN

Los hongos endófitos son bien conocidos por su asociación con una gran variedad de especies vegetales, igualmente, las plantas de mangle son bien conocidas por albergar una amplia variedad de hongos con una fuente valiosa de compuestos bioactivos originados a partir del metabolismo secundario que se sintetizan en parte por la respuesta a la defensa química contra el ataque de microorganismos, ambientes hostiles, e insectos antagonistas. El objetivo del presente estudio fue analizar la composición química de hongos endófitos aislados de árboles de mangle en Buenaventura, Colombia. Se llevaron a cabo análisis de secuencias de ADN de la región nuclear ribosomal espaciador transcrito interno (ITS) para determinar la identidad de los hongos. Los resultados revelaron 17 aislados, pertenecientes a ocho familias fúngicas. Todos los aislados se sometieron a análisis de cromatografía de capa fina, observándose diferentes núcleos fitoquímicos eluidos en el sistema (7:3 hexano: acetona), de los cuales se reconocieron 23 compuestos por medio de cromatografía de gases acoplada a espectrofotometría de masas. También se llevaron a cabo ensayos de citotoxicidad en células de fibroblastos del prepucio humano, los cuales no presentaron una tendencia en la viabilidad celular. Los hongos endófitos derivados de árboles de mangle revelaron la presencia de compuestos químicos que pueden ser un recurso alternativo de gran interés en bioprospección y biorremediación.

Palabras clave: actividad biológica, ambientes salinos, compuestos bioactivos, núcleos fitoquímicos.

INTRODUCTION

Endophytic fungi are associated with a wide variety of plant species and possess a valuable source of bioactive compounds originating from the secondary metabolism which is synthesized as part of the chemical defense against thermal or light changes, nutritional deficiencies, pathogens, predators, or presence of other organisms (Payyavula *et al.* 2012, Khalil *et al.* 2021). The production of secondary metabolites in endophytes is influenced by the type of host; for example, in plants growing in hostile or adverse environments, they are an important source of bio compound-rich endophytic fungi, even playing an important role in the resistance of plant species (Li *et al.* 1996, Yeshi *et al.* 2022).

When growing in adverse or hostile conditions mangrove trees are potential hosts of endophytic fungi as these may fulfill diverse functions such as: protecting them from high salt concentrations, diseases, constant flooding, sedimentation, and pests (Ananda and Sridhar 2002, Gilbert and Sousa 2002, Osorio *et al.* 2017a, 2017b). Such endophytic fungi have been the focus of many studies, due to their ability to synthesize bioactive products with utility in agriculture, biotechnology, and medicine (Strobel *et al.* 2004). Among the most novel biologically active substances include those of anticancer, antimicrobial, antioxidant, antiviral, and insecticidal type (Strobel *et al.* 2004, Gallo *et al.* 2008, Pimentel *et al.* 2011).

In recent decades, research has focused on the search for new and more effective secondary metabolites that enable the treatment of various diseases from molecules derived from natural products, as important source of bioactive molecules. For approximately 30 years, about 300 molecules derived from endophytic fungi have been described, including, peptides, polypeptides, terpenes and steroids, among others, characterized by a broad range of biological effects such as antibiotics, antifungals, antioxidants, and cytotoxins among others (Bhadury *et al.* 2006, Torres *et al.* 2020).

Examples of fungi that recently have been used for the analysis of bioactive compounds include *Aspergillus luchuensis* (mut. Kawachii), isolated from leaves of *Ceriops tagal* (Perr.) C.B.Rob. in Thailand, which showed antimicrobial activity, likewise, based on chemical analyses of fungal endophytes such as *Xylaria feejeensis* (Berk.) Fr. and *Aspergillus luchuensis* extracts showed the presence of tannins, alkaloids, and coumarins (Sopalun *et al.* 2021) Furthermore, one fungal isolate can produce several compounds, for example, *Cladosporium* sp. isolated from *Excoecaria agallocha* L. and *Aspergillus sojae* Sakag. et K.Yamada ex Murak isolated from *Plectranthus amboinicus* Lour. produced fifteen compounds with antibacterial, insecticidal, anticancer, and antioxidant activities and in general as bio-control agents (Wang *et al.* 2018, Elango *et al.* 2020).

Mangrove forests are rich ecosystems of fundamental significance. Yet despite their importance, these biodiversity hotspots are under constant threat due to human and environmental stressors (World Rainforest Movement c2002). Colombia is the only country in South America that has coasts on the Caribbean Sea and the Pacific Ocean along its 3000 km; however, the number of studies on the diversity of microorganisms associated with mangroves and their biological functionality is still very limited, and the research concerning endophytic fungi associated with mangroves has focused mostly on the diversity and ecology (Osorio *et al.* 2015, 2017a, 2017b, Torres *et al.* 2020). The aim of the present study was to identify and analyze the chemical composition of endophytic fungi associated with mangrove trees at the Playa San Pedro Nature Reserve, in Buenaventura.

MATERIALS AND METHODS

Collection of plant material

The plant material was collected in May 2018, in the Playa San Pedro Natural Reserve, Buenaventura, Colombia, which is located 3°50' North, 77°15' West. For the development of this study, initially the inspection of the mangrove forest area was carried out, and then ten portions of branches 10 cm, in length were collected from four mangrove species: *Laguncularia racemosa* (C.F.Gaertn), (Combretaceae), *Mora oleifera* (Caesalpiniaceae) (Triana ex Hemsl), *Pelliciera rhizophorae* (Planch. & Triana), (Tetrameristaceae), and *Rhizophora racemosa* (G. Mey) (Rhizophoraceae) to obtain a total of 40 branches. Finally, the samples were labeled stored at room temperature, and transported to the laboratories of the Universidad del Quindío for the isolation of endophytic fungi.

Isolation of endophytic fungi

The collected plant material was washed with tap water to remove debris, then each branch was cut into discs measuring 5 to 10 mm, they were subjected to surface sterilization following the protocol described by Osorio *et al.* (2017a). The fragments were then transferred to the commercial culture medium Potato Dextrose Agar (PDA - Difco) with streptomycin to avoid bacterial contamination. Samples were incubated for one month at room temperature (approximately 25°C) and constantly checked for mycelial growth observation (Torres *et al.* 2020).

Fungal identification

The cultures obtained were used for DNA extraction following the protocol of Raeder and Broda (1985). The ribosomal Internal Transcribed Spacer (ITS) region including the 5.8 S rDNA region was amplified by polymerase chain reaction (PCR), using standard ITS1 and ITS4 primers (White *et al.* 1990). A 25 µl reaction mixture was prepared for the PCR, it contained 100 ng of DNA, 2.5 µl of PCR reaction buffer (10 mM Tris-HCL, 1.5 mM MgCl2, 50 mM KCL), 1 µl of each primer, 2 µl dNTP (0.2 mM) and 0.5 µl of Faststart Taq DNA Polymerase (Roche Applied Science, Germany). Sterile Sabax water was added to adjust the final reaction volumes to 25 µl. The reactions were performed using an initial denaturation at 94 ˚C for 4 minutes followed by a step of ten cycles consisting of 94 ºC for 20 seconds, the annealing at 55 ºC and an elongation at 72 ºC for 45 s, followed by a further 25 cycles of 94 ºC for 20 s, with an annealing step using the temperature as previously indicated for 40 s with a time increase of 5 s every cycle and elongation for 45 s at 72 ºC. This was concluded with a final elongation step at 72 ºC for 10 minutes. An aliquot of 5 µl of each of the PCR products was stained with GelRedTM nucleic acid gel stain (Biotium, USA), separated on 1 % agarose gels for 20 minutes at 90 Volts and viewed with a Gel Doc EZ Imager (Bio-Rad Laboratories Inc.) to access the success of the PCR. These products were cleaned using Sephadex G-50 columns following the instructions provided by the manufacturers (Sigma Aldrich, Sweden) and the cleaned filtrate was used in the sequencing reactions.

Molecular characterization of endophytic fungi

The sequences obtained from the ITS genetic region were assembled in Sequencher v.5.1 software, to obtain the corresponding consensus. The sequences were aligned in the MAFFT v7 server (Katoh and Standley 2013) and compared with those in the GenBank database (http://www.ncbi.nlm.nih.gov) using the BLASTn algorithm (Altschul et al. 1990).

Bayesian Inference (BI) was performed in Mr. Bayes V3.1.2 software (Ronquist and Huelsenbeck 2003) with two independent runs for five million generations every 100 generations. Maximum Likelihood (ML) analysis was performed in PhyML v. 3.1 software (Guindon and Gascuel 2003), where confidence levels were estimated with 1000

bootstrap replicates. We searched for the evolutionary model that best fit the data set using jModelTest 2.1.10 (Darriba *et al.* 2012). Maximum Parsimony (MP) analysis was performed in PAUP v. 4.0 software (Swofford and Sullivan 2003) with a heuristic search with 1000 replicates. In addition, the consistency index (CI), homoplasy index (HI), rescaled consistency index (RC), retention index (RI), and tree length (TL) were determined. Trees were visualized using the software FigTree v 1.4.3 (Rambaut 2017).

Screening for fungal extracts

Ten isolates were selected to be sown in liquid cultures using Potato Dextrose (PD- Difco), without agar to avid solidification and incubated for one month at room temperature at approximately 25°C, with constant agitation speed of 600 rpm, until sufficient mycelial growth was obtained. The fungal extracts were obtained using the liquid-liquid extraction method for the culture medium and liquid-solid extraction by maceration for the mycelium, using ethyl acetate as solvent. For the liquid-liquid extraction of the culture medium, successive extractions were carried out, where the organic phase was recovered by means of a separating funnel. The mycelium extract was also recovered using gravity filtration with filter paper, separating the solids from the extract. The organic phases remained at room temperature at 25°C until the total evaporation of the solvent. The dried fractions were labeled and stored. Finally, the percentage of extraction was determined for each of the treatments.

Table 1. Isolates of endophytic fungi obtained from mangrove trees in San Pedro Nature Reserve, Buenaventura, Colombia.

Fungal species	Isolate number	Host	GenBank codes ITS
Bipolaris sp.	COLPR-12	*Pelliciera. rhizophorae*	MW029957
Bipolaris sp.	COLPR-15	*P. rhizophorae*	MW029956
Ciboria aestivalis (Pollock) Whetzel	COLLR-3	*Laguncularia racemosa*	MW029950
Coprinellus radians (Desm.) Vilgalys, Hopple & Jacq. Johnson	COLRR-11	*Rhizophorae racemosa*	MW029960
Cylindrobasidium torrendii (Bres.) Hjortstam	COLLR-1	*L. racemosa*	MW029959
C. torrendii	COLPR-13	*P. rhizophorae*	MW029958
Epicoccum nigrum Link	COLRR-10	*R. racemosa*	MW029955
Fusarium oxysporum Schltdl.	COLLR-2	*L. racemosa*	MW029951
F. oxysporum	COLPR-14	*P. rhizophorae*	MW029952
F.oxysporum	COLMO-16	*Mora oleifera*	MW029953
Lasiodiplodia theobromae (Pat.) Griffon & Maubl.	COLLR-17	*L. racemosa*	MW029949
L. venezuelensis T.I. Burgess, P.A. Barber & Mohali	COLRR-9	*R. racemosa*	MW029948
Neofusicoccum batangarum Begoude, Jol. Roux & Slippers	COLLR-5	*L. racemosa*	MW029944
N. batangarum	COLRR-8	*R. racemosa*	MW029945
Neofusicoccum sp.	COLRR-6	*R. racemosa*	MW029946
Neofusicoccum sp.	COLRR-7	*R. racemosa*	MW029947
Neurospora crassa Shear & B.O. Dodge	COLLR-4	*L. racemosa*	MW029954

Qualitative chemical analysis of the endophyte fungal extracts

Chromatographic analysis of each of the fractions was performed by thin layer chromatography analysis used in Aluminum coatings. The plates were eluted in three systems (hexane-acetone 7:3, hexane-ethyl acetate 8:2, and dichloromethane-methanol 9:1). For the development of the chromatographic plates, two systems were used, the first one was the ultraviolet lamp at 2 wavelengths 254 and 365 nm, and in the second one, ammonium cerium (IV) sulfate (NH4)4Ce(SO4)4 was applied to reveal the stains of interest, indicating the presence of secondary metabolites. In addition, extracts of three isolates (1 mg/mL in ethanol) were analyzed by gas chromatography coupled to mass spectrometry (GC-MS) on a Hewlett Packard Model 5890 Plus Series, using an Rxi-5MS column. The methodology used in the chromatographic analysis involved the use of Helium as carrier gas at a flow rate of 1 mL/min, and it was injected in Split mode, on-column injection system with electronic pressure controlled (EPC) and Flame ionization detection (FID). While the column temperature was 45°C, the injector temperature was maintained at 170 °C. Likewise, the temperature program consisted of 45 °C, for 10 min and decreasing at 3 °C/min up to 220 °C for 30 min, the injection volume was 2 µL, and the ionization chamber and transfer line temperatures were 220 °C and 250 °C respectively. The mass spectrometer used has an electron impact ionization system at 70eV, the analyses were performed in Scan mode at intervals of 35 to 500 in m/Z ratio. The components were characterized by a relative comparison of retention times and mass spectrum from the NIST 2013 library present in the equipment, following the protocol proposed by Acevedo *et al.* (2013).

Preliminary phytochemical tests were also performed on seven extracts of the isolated fungi, each test with two extracts: liquids from the culture media and solids from the mycelia treated with ethyl acetate. For the determination of the presence of phytochemical nuclei of alkaloids, diterpenes, flavonoids, phenols, steroids, and terpenes, different specific developers were used for each nucleus, comparing it with a standard solution following the protocol described by Sanabria (1983).

Table 2. Phytochemical analysis for the detection of secondary metabolites present in extracts of endophytic fungi isolated from mangrove tree branches from the San Pedro Buenaventura Natural Reserve, Valle del Cauca, Colombia. - = Absence of metabolite; + = Presence of metabolite.

			Secondary metabolites				
	Endophytic fungi	Host	Alkaloids	Diterpenes - steroids	Phenols	Flavonoids	Terpenes
Culture media	*Bipolaris* sp.	*Pelliciera rhizophorae*	+	+	+	+	+
	C. aestivalis	*Laguncularia racemosa*	-	+	+	+	+
	C. radians	*Rhizophora racemosa*	-	+	-	+	+
	F. oxysporum	*P. rhizophorae*	-	-	-	+	-
	L. venezuelensis	*R. racemosa*	+	+	-	+	+
	Neofusicoccum sp.	*R. racemosa*	-	+	-	+	+
	N. crassa	*L. racemosa*	-	-	+	-	+
Mycelium	*Bipolaris* sp.	*P. rhizophorae*	+	+	+	+	+
	C. radians	*R. racemosa*	-	+	-	+	+
	F. oxysporum	*P. rhizophorae*	+	+	-	+	+
	L. venezuelensis	*R. racemosa*	-	+	-	-	+
	N. crassa	*L. racemosa*	-	+	+	+	+

Cytotoxicity Bioassays

Considering the highest percentage yields, cytotoxicity tests were performed for two extracts of two endophyte species, these tests were determined by the methyl thiazole tetrazolium (MTT) tetrazolium salt technique (Studzinski 1999), where the human foreskin fibroblast (HFF) cell line was used, the cells were cultured in 96-well plates with culture media containing D-MEN supplemented with 3% horse serum, 1 % non-essential amino acids and 1% streptomycin. The plates were incubated at 37°C (Torres *et al.*

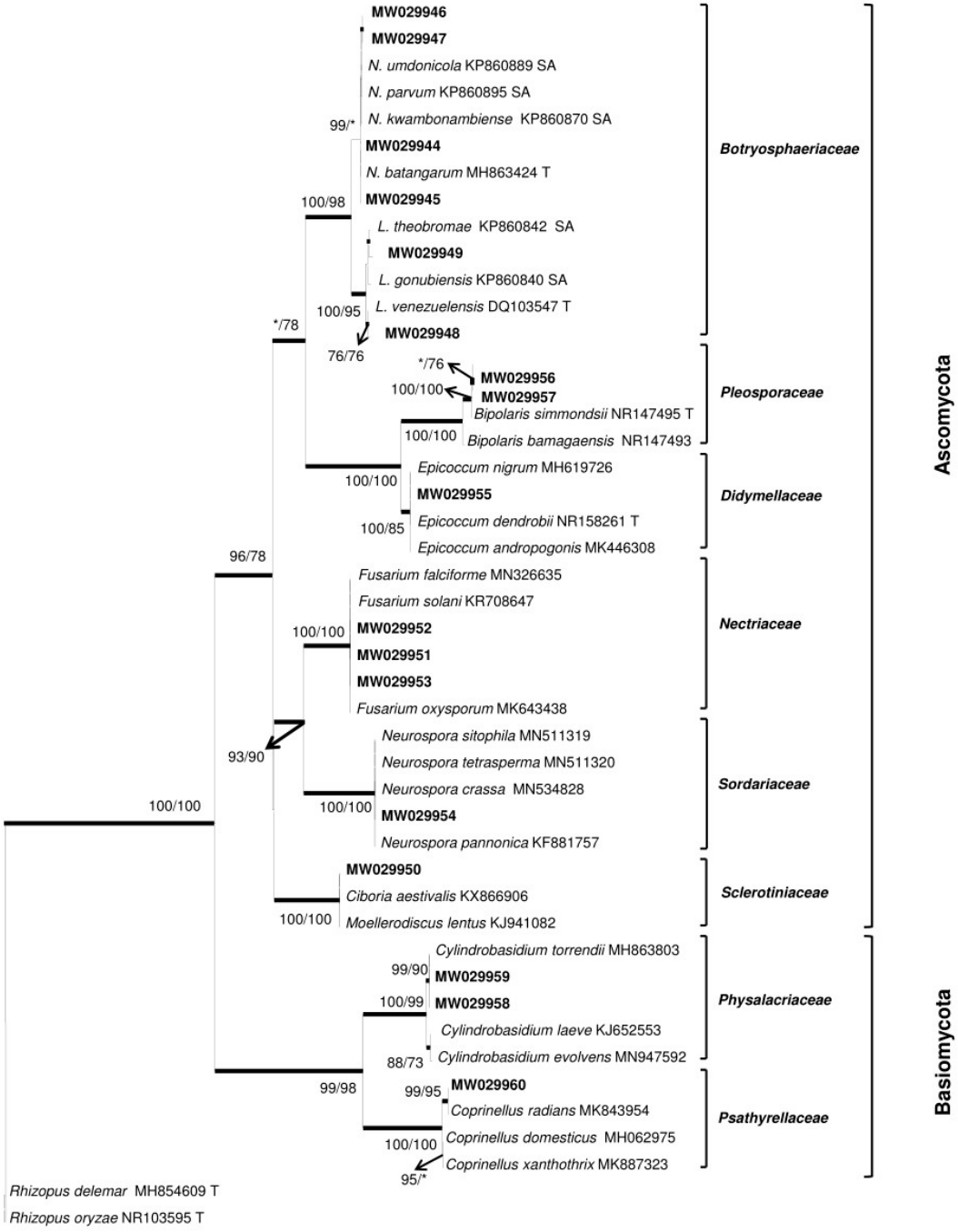

Figure 1. Phylogram analyses of the ITS data set including the Posterior probabilities ≥95% of the Bayesian inference and represented by thick branches, maximum likelihood and Bootstrap support values >70%, are indicated near nodes as maximum parsimony and maximum likelihood, with bootstrap support values <70% indicated with *. The isolates of endophytic fungi from mangroves distributed in the San Pedro Natural Reserve **in bold**, grouped within the Botryosphaeriaceae, Didymellaceae, Nectriaceae, Physalacriaceae, Pleosporaceae, Psathyrellaceae, Sclerotiniaceae and Sordariaceae; the remaining isolates, not in bold, were obtained from the GenBank database.

2020). Subsequently, extracts dissolved in 1 % dimethyl sulfoxide (DMSO) were added at four concentrations 100, 200, 500, and 1000 µg/mL. Finally, cell viability was evaluated with respect to control (untreated) cells using the formula: % viability = (Average optical density of treated cultures/ Optical density of negative control) x 100.

RESULTS

Isolation and identification of endophytic fungi

Out of the 40 branches collected, 17 pure isolates were obtained. Of these, five were isolated from *Laguncularia racemosa*, six from *Rhizophora racemosa*, five from *P. rhizophorae,* and one from *M. oleifera*. DNA was extracted and the ITS region was successfully amplified from the 17 isolates obtained from the four mangrove species. The fragments obtained were approximately 460-610 bp. in size, and compared with the NCBI Genbank, allowing preliminary identification of the study isolates with identity rates of 97 and 99 %. Sequences of all species were deposited in GenBank (Table 1).

A total of eleven fungal groups were identified, these include *Bipolaris* sp. (Pleosporaceae) (MW029956, MW029957), *Ciboria aestivalis* (Pollock) (Sclerotiniaceae) (MW029950), *Coprinellus radians* (Vilgalys, Hopple and Jacq. Johnson) (Psathyrellaceae) (MW029960), *Cylindrobasidium torrendii* (Bres) (Physalacriaceae) (MW029958, MW029959), *Epicoccum nigrum* (Link) (Didymellaceae) (MW029955), *Fusarium oxysporum* (Schlechtendal) (Nectriaceae) (MW029951, MW029952, MW029953), *Lasiodiplodia theobromae* (Griffon and Maubl) (Botryosphaeriaceae) (MW029949), *Lasiodiplodia venezuelensis* (T.I. Burgess, P.A. Barber and Mohali) (Botryosphaeriaceae) (MW029948), *Neofusicoccum batangarum* (Begoude, Jol. Roux and Slippers) (Botryosphaeriaceae) (MW029944, MW029945), *Neofusicoccum* sp. (Botryosphaeriaceae) (MW029946, MW029947) and *Neurospora crassa* (Shear and B. O. Dodge) (Sordariaceae) (MW029954).

Bayesian inference, maximum likelihood, and maximum parsimony phylogenetic analyses of the ITS data set for 17 fungal isolates, showed a phylogram with eight families,

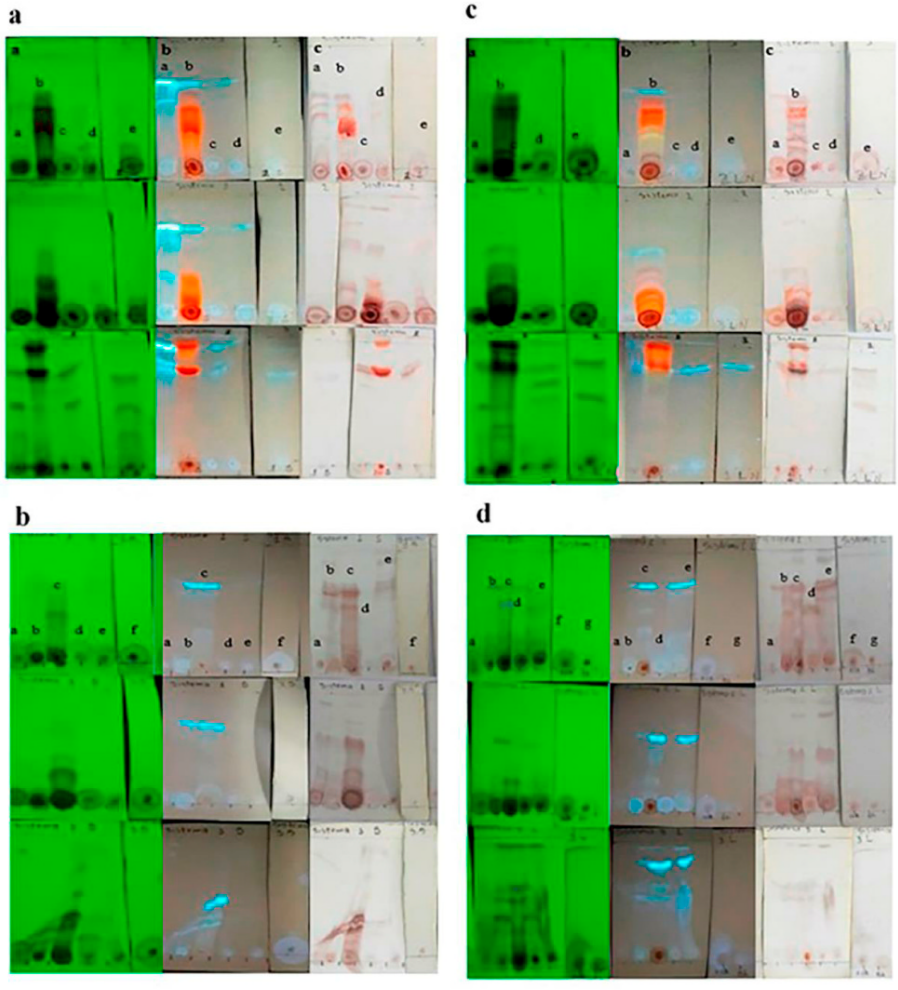

Figure 2. Thin layer chromatography of extracts. **a**. Solid samples of the extracts - a. *L. theobromae*, b. *C. radians*, c. *C. aestivalis*, d. *N. crassa*, e. *Neofusicoccum* sp. **b**. Solid samples of the extracts - a. *F. oxysporum*, b. *Bipolaris* sp. c. *C. torrendii*, d. *F. oxysporum*, e. *N. batangarum*, f. *F. oxysporum*. **c**. Corresponds to liquid samples of the extracts - a. *L. theobromae*, b. *C. radians*, c. *C. aestivalis*, d. *N. crassa*, e. *L. venezuelensis*. **d**. Liquid samples of the extracts -a. *F. oxysporum*, b. *Bipolaris* sp., c. *F. oxysporum*, d. *N. batangarum*, e. *F. oxysporum*, f. *Neofusicoccum* sp., g. *C. torrendii*.

represented by the genera *Bipolaris, Ciboria, Coprinellus, Cylindrobasidium, Epicoccum, Fusarium, Lasiodiplodia, Neofusicoccum,* and *Neurospora*. Such analyses provided evidence of a congruence between the obtained phylogenic trees which are supported by the bootstrap values >70 % and posterior probability ≥95 % (Fig. 1).

Screening for fungal extracts

Once the extracts were obtained from ten of the isolated endophytic fungi: *Bipolaris* sp., *C. aestivalis*, C. *radians, C. torrendii, F. oxysporum, L. theobromae, L. venezuelensis, N. batangarum, Neofusicoccum* sp. and *N. crassa,* the liquid phase (liquid culture media) was separated from the solid phase (mycelia) and a total of twelve extracts were obtained by the solid-liquid extraction method (S-L) and twelve extracts by the liquid-liquid extraction method (L-L). The masses and percent yield for mycelium in *C. radians* was (1.82 %), *C. torrendii* (3.166 %), *F. oxysporum* (1.36 %), *L. theobromae* (1.66 %), *L. venezuelensis* of (6 %), *Neofusicoccum* sp. (1.5 %) and *N. crassa* (9.23 %), and in the yield of liquid medium *C. aestivalis* (0.91 %), *F. oxysporum* (0.33 %), *Neofusicoccum* sp. (0.70 %), *N. batangarum* (0.25 %) and *N. crassa* (0.57 %).

Qualitative chemical analysis of the endophytic fungal extracts

In the thin layer chromatography, the phytochemical nuclei identified in the fractions presented a better separation, evidencing the presence of different nuclei eluted in the 7:3 hexane: acetone system (Fig. 2). According to this result, the chromatographic plates were eluted in the 7:3 hexane: acetone system and then sprayed with the phytochemical test developers, where the presence of phytochemical nuclei of the alkaloids, diterpenes, flavonoids, phenols, steroids, and terpenes were identified for most of the species analyzed (Table 2). Furthermore, 23 compounds from three isolates of endophytic fungi: *Bipolaris* sp., *C. radians,* and *F. oxysporum* were identified by using gas chromatography coupled to mass spectrometry, of these, twelve compounds were present in the three fungal species (Table 3).

Cytotoxicity bioassays

Cytotoxicity tests for *Bipolaris* sp. extracts were found to be toxic at concentrations of 100 and 200 µg/mL, while at 500 and 1000 µg/mL they are viable for the entire cell line. The results for *N. crassa* indicate that all concentrations are toxic to HFF cells but to a greater degree the 500 µg/mL concentration, which is below 35% (Fig. 3).

Table 3. Presence of 23 compounds from *Bipolaris* sp., *Coprinellus radians* and *Fusarium oxysporum* species identified through gas chromatography coupled to mass spectrometry.

Compounds	Retention time (min)	Fungal genera		
		Bipolaris sp.	*Coprinellus radians*	*Fusarium oxysporum*
2-aminopyridine	11.25	X		X
Bornyl acetate	29.5	X	X	X
Carvone	35.46	X	X	X
Caryophyllene	59.31	X		
Citronelal	28.32	X	X	X
Decanal	32.41		X	
Dodecanol	40.67	X	X	X
Ethyl isovalerate	5.2	X	X	X
Eucalyptol	27.05	X		
Ferulic acid	37.55		X	
g-decalactone	39.26	X		
Heptadecane	54.34	X	X	X
Hexanoic acid	5.09	X	X	X
Hydnocarpic acid	32.37	X	X	X
Lauric Acid	49.2	X	X	X
Macrophomic acid	39.79		X	
Menthyl acetate	32.57	X	X	X
o -phenylphenol	38.46	X	X	
Pinosilvina	44.85		X	
Piperonal	38.85			X
Stearic Acid	57.86	X	X	X
Sulcatone	25.7	X	X	X
Unknown Diterpene	55.19	X		

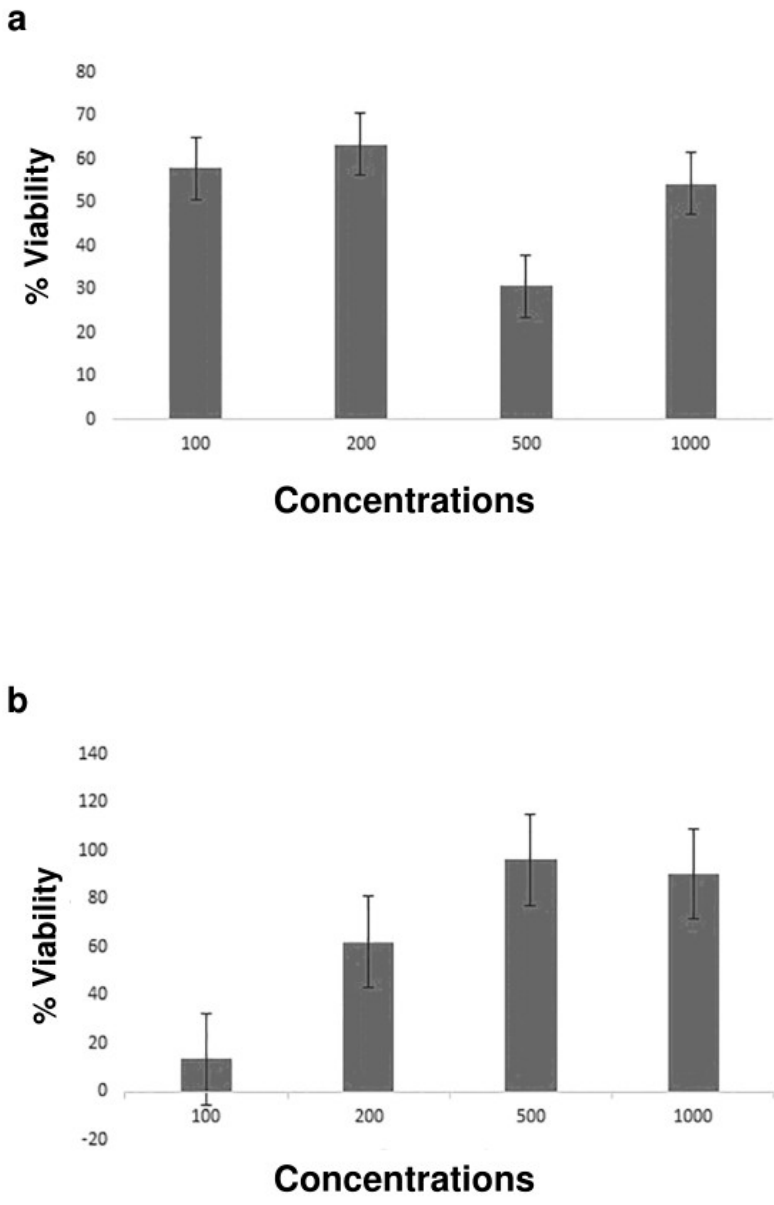

Figure 3. Cytotoxic effect on human foreskin fibroblast cells. **a**. *Bipolaris* sp. **b**. *Neurospora crassa*. Extracts evaluated at concentrations of 100, 200, 500 and 1000 µg/mL.

DISCUSSION

Despite the importance of mangrove trees in Colombia, the information about the associated microorganisms and their metabolism is still very limited. In this study, we identified 17 fungal isolates, belonging to nine genera based on the DNA sequence data sets. Of these, *Bipolaris* sp., *C. aestivalis*, *C. torrendii*, *F. oxysporum*, and *L. venezuelensis* have been reported in a wide variety of mangrove species and other plants as phytopathogenic and as a prolific source of secondary metabolites (Slippers & Wingfield 2007, Tan *et al.* 2016, Mohali *et al.* 2017, Kouipou-Toghueo 2020, Maehara *et al.* 2020, Harwoko *et al.* 2021), ratifying that these fungi play a key role in a vast array of potential issues (Rodriguez *et al.* 2009, Hyde *et al.* 2019, Vilarino-Godinho *et al.* 2019, Becarelli *et al.* 2021, El-Sayed *et al.* 2022, Wen *et al.* 2022).

Results in this study have led to the determination of phytochemical nuclei, including alkaloids, flavonoids, phenols, steroids, and terpenes using a liquid medium and mycelium. Interestingly, previous reports have highlighted the ability of endophytic fungi to protect their hosts against harsh conditions through the production of diverse bio-

compounds (Gao *et al.* 2010, O'Hanlon *et al.* 2012, Grabka *et al.* 2022), moreover, it is also considered that such compounds are produced by the plants in response to different biotic and abiotic pressures, conferring avoidance and tolerance (Li *et al.* 1996, Yeshi *et al.* 2022).

Furthermore, the activity of the HFF cell line in *Bipolaris* sp. was also tested, showing higher cell viability (96 %), as the concentrations increased in 100, 200, and 500 µg/mL, however, in concentrations of 1000 µg/mL such viability decreased (90 %). *Neurospora crassa*, presented a positive effect on HFF when concentrations increased in 100 and 200 µg/mL, with a viability of 57 % and 63 %, nevertheless, when these were exposed to concentrations of 500 µg/mL such viability decreased to 33 %, interestingly, the viability increased again with a considerable intensification of concentration at 1000 µg/mL (54 %), indicating not evidenced of an orderly trend (Torres *et al.* 2020).

Irregular effects observed during cytotoxicity activity assays, are consistent with previous studies, where cell viability resulted inversely proportional to concentrations due to errors when adding the MTT solutions into the wells, causing a decrease in the tested cells and changes in the concentrations (Mestizo 2016). Likewise, Veciana *et al.* 2014 established that toxic levels for cells are due to cell viability below 75 %, being viable at percentages above 75 %. Therefore, further studies in other cell lines are suggested to elucidate the cytotoxic effects and to determine their potential on cell viability.

Mangrove trees are important reservoirs of endophytic fungi, which represent a potential source of compounds to be used under different approaches. However, to harness the Colombian biodiversity and its chemical constituents, further studies with more sophisticated analytical techniques will be also required.

PARTICIPATION OF AUTHORS

SVVC: data acquisition, analysis, writing, editing. AMRS: data acquisition, analysis, writing, review and editing. RL: data acquisition, design, and commenting on the manuscript, JAO: conception, design, data acquisition, analysis and writing, review, and editing.

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CONFLICT OF INTEREST

The authors declares that is no conflict of interest.

LITERATURE CITED

Acevedo D, Navarro M, Monroy L. 2013. Composición química del aceite esencial de hojas de orégano (*Origanum vulgare*). Inf. Tecnol. 24(4):43-48. doi: https://doi.org/10.4067/S0718-07642013000400005

Altschul F, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. J. Mol. Biol. 215(3):403-410. doi: https://doi.org/10.1016/S0022-2836(05)80360-2

Ananda K, Sridhar KR. 2002. Diversity of endophytic fungi in the roots of mangrove species on the west coast of India. Can. J. Microbiol. 48(10):871-878. doi: https://doi.org/10.1139/w02-080

Becarelli S, Chicca I, La China S, Siracusa G, Bardi A, Gullo M, Petroni G, Bernard-Levin D, Di Gregorio S. 2021. A New *Ciboria* sp. for Soil Mycoremediation and the Bacterial Contribution to the Depletion of Total Petroleum Hydrocarbons. Front. Microbiol. 8(12). doi: https://doi.org/10.3389/fmicb.2021.647373

Bhadury P, Mohammad B, Wright P. 2006. The current status of natural products from marine fungi and their potential as anti-infective agents. J. Ind. Microbiol. Biotechnol. 33(5):325. doi: https://doi.org/10.1007/s10295-005-0070-3

Darriba D, Taboada GL, Doallo R, Posada D. 2012. jModelTest2: more models, new heuristics and parallel computing. Nat. Methods. 9(772). doi: https://doi.org/10.1038/nmeth.2109

Elango D, Manikandan V, Jayanthi P, Velmurugan P, Balamuralikrishnan B, Ravi AV, Shivakumar MS. 2020. Selection and characterization of extracellular enzyme production by an endophytic fungi *Aspergillus sojae* and its bio-efficacy analysis against cotton leafworm, *Spodoptera litura*. Curr. Plant Biol. 23. doi: https://doi.org/10.1016/j.cpb.2020.100153

El-Sayed, ESR, Hazaa MA, Shebl MM, Amer MM, Mahmoud SR, Khattab AA. 2022. Bioprospecting endophytic fungi for bioactive metabolites and use of irradiation to improve their bioactivities. AMB Expr. 12(46). doi: https://doi.org/10.1186/s13568-022-01386-x

Gallo M, Guimaraes D, Momesso L, Pupa M. 2008. Natural products from endophytic fungi. En: Saikai R, editores. Microbial Biotechnology. Pitam Pura: New India Publishing Agency. p. 139-158.

Gao F, Dai C, Liu X. 2010. Mechanisms of fungal endophytes in plant protection against pathogens. Afr. J. Microbiol. Res. 4:1346-1351. doi: https://doi.org/10.5897/AJMR.9000480

Gilbert GS, Sousa WP. 2002. Host Specialization among Wood□Decay Polypore Fungi in a Caribbean Mangrove Forest1. Biotropica 34(3):396-404. doi: https://doi.org/10.1111/j.1744-7429.2002.tb00553.x

Grabka R, d'Entremont TW, Adams SJ, Walker AK, Tanney JB, Abbasi PA, Ali S. 2022. Fungal endophytes and their role in agricultural plant protection against pests and pathogens. Plants. 11(3):384. doi: https://doi.org/10.3390/plants11030384

Guindon S, Gascuel O. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst. Bio. 52(5):696-704. doi: https://doi.org/10.1080/10635150390235520

Harwoko H, Daletos G, Stuhldreier F, Lee J, Wesselborg S, Feldbrügge M, Müller WEG, Kalscheuer R, Ancheeva E, Proksch P. 2021. Dithiodiketopiperazine derivatives from endophytic fungi *Trichoderma harzianum* and *Epicoccum nigrum*. Nat. Prod. Res. 35(2):257-265. doi: https://doi.org/10.1080/14786419.2019.1627348

Hyde KD, Xu J, Rapior S, Jeewon R, Lumyong S, Niego AG, Abeywickrama PD, Aluthmuhandiram JVS, Brahamanage RS, Brooks S, Chaiyasen A, Thilini KW, Chomnunti P, Chepkirui C, Chuankid B, de Silva NI, Doilom M, Faulds C, Gentekaki E, Gopalan V, Kakumyan P, Harishchandra D, Hemachandran H, Hongsanan S, Karunarathna A, Karunarathna SC, Khan S, Kumla J, Jayawardena RS, Liu JK, Liu N, Luangharn T, Macabeo APG, Marasinghe DS, Meeks D, Mortimer PE, Mueller P, Nadir S, Nataraja KN, Nontachaiyapoom S, O'Brien M, Penkhrue W, Phukhamsakda C, Ramanan US, Rathnayaka AR, Sadaba RB, Sandargo B, Samarakoon BC, Tennakoon DS, Siva R, Sriprom W, Suryanarayanan TS, Sujarit K, Suwannarach N, Suwunwong T, Thongbai B, Thongklang N, Wei D, Wijesinghe SN, Winiski J, Yan J, Yasanthika E, Stadler M. 2019. The amazing potential of fungi: 50 ways we can exploit fungi industrially. Fungal Divers. 97:1-136. doi: https://doi.org/10.1007/s13225-019-00430-9

Khalil AMA, Abdelaziz AM, Khaleil MM, Hashem AH. 2021. Fungal endophytes from leaves of *Avicennia marina* growing in semi-arid environment as a promising source for bioactive compounds. Lett. Appl. Microbiol. 72(3):263-274. doi: https://doi.org/10.1111/lam.13414

Kouipou-Toghueo RM. 2020. Bioprospecting endophytic fungi from *Fusarium* genus as sources of bioactive metabolites. Mycology. 11(1):1-21. doi: https://doi.org/10.1080/21501203.2019.1645053

Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol. Bio. Evol. 30(4):772-780. doi: https://doi.org/10.1093/molbev/mst010

Li JY, Strobel G, Sidhu R, Hess WM, Ford EJ. 1996. Endophytic taxol producing fungi from bald cypress, *Taxodium distichum*. Microbiol. 142(8):2223-2226. doi: https://doi.org/10.1099/13500872-142-8-2223

Maehara S, Yamane C, Kitamura C, Hinokuma M, Hata T. 2020. High ophiobolin A production in endophytic fungus *Bipolaris* sp. associated with *Datura metel*. Nat. Prod. Res. 34(20):2990-2992. doi: https://doi.org/10.1080/14786419.2019.1597352

Mestizo M. 2016. Síntesis, caracterización y estudio citotóxico de nuevos complejos de Co (II), Cu (II) y Ni (II) con ligandos de cumarina tipo salen con potencial actividad anticancerígena. [Tesis]. Bogotá. Universidad de los Andes.

Mohali SR, Castro-Medina F, Úrbez-Torres JR, Gubler WD. 2017. First report of *Lasiodiplodia theobromae* and *L. venezuelensis* associated with blue stain on *Ficus insipida* wood from the Natural Forest of Venezuela. For. Pathol. 47(5):1-5. doi: https://doi.org/10.1111/efp.12355

O'Hanlon KA, Knorr K, Jorgensen LN, Nicolaisen M, Boelt B. 2012. Exploring the potential of symbiotic fungal endophytes in cereal disease suppression. Biol. Control. 63(2):69-78. doi: https://doi.org/10.1016/j.biocontrol.2012.08.007

Osorio JA, Wingfield MJ, De Beer ZW, Roux J. 2015. *Pseudocercospora mapelanensis* sp. nov, associated with a fruit and leaf disease of *Barringtonia racemosa* in South Africa. Australasian Plant Pathol. 44:349-359. doi: https://doi.org/10.1007/s13313-015-0357-4

Osorio JA, Crous CJ, Wingfield MJ, De Beer ZB, Roux J. 2017a. An assessment of mangrove diseases and pests in South Africa. For Inter. J. For. Res. 90(3):343-358. doi: https://doi.org/10.1093/forestry/cpw063

Osorio JA, Crous CJ, De Beer ZW, Wingfield MJ, Roux J. 2017b. Endophytic *Botryosphaeriaceae*, including five new species, associated with mangrove trees in South Africa. Fungal. Biol. 121(4):361-393. doi: https://doi.org/10.1016/j.funbio.2016.09.004

Payyavula RS, Navarre DA, Kuhl JC, Pantoja A, Pillai SS. 2012. Differential effects of environment on potato phenylpropanoid and carotenoid expression. BMC Plant. Bio. 12:39. doi: https://doi.org/10.1186/1471-2229-12-39

Pimentel MR, Molina G, Dionísio AP, Maróstica MR, Pastore GM. 2011. The use of endophytes to obtain bioactive compounds and

their application in biotransformation process. Biotechnol. Res. Int. 2011:1-11. doi: https://doi.org/10.4061/2011/576286

Raeder U, Broda P. 1985. Rapid preparation of DNA from filamentous fungi. Letr. Appl. Microbiol. 1(1):17-20. doi: https://doi.org/10.1111/j.1472-765X.1985.tb01479.x

Rambaut A. 2017. FigTree-version 1.4. 3, a graphical viewer of phylogenetic trees. Computer program distributed by the author. Web site. [Last accessed: 06 jan 2020].

Rodriguez RJ, White JF, Arnold AE, Redman RS. 2009. Fungal endophytes: diversity and functional roles. New Phytol. 182(2):314-330. doi: https://doi.org/10.1111/j.1469-8137.2009.02773.x

Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinform. 19(12):1572-1574. doi: https://doi.org/10.1093/bioinformatics/btg180

Sanabria A. 1983. Análisis fitoquímico preliminar: metodología y su aplicación en la evaluación de 40 plantas de la familia Compositae. Universidad Nacional de Colombia, Facultad de Ciencias, Departamento de Farmacia.

Slippers B, Wingfield MJ. 2007. Botryosphaeriaceae as endophytes and latent pathogens of woody plants: diversity, ecology and impact. Fungal Biol. Rev. 21(2-3):90–106. doi: https://doi.org/10.1016/j.fbr.2007.06.002

Sopalun K, Laosripaiboon W, Wachirachaikarn AW, Iamtham S. 2021. Biological potential and chemical composition of bioactive compounds from endophytic fungi associated with Thai mangrove plants. S. Afr. J. Bot. (141):66-76. doi: https://doi.org/10.1016/j.sajb.2021.04.031

Strobel G, Daisy B, Castillo U, Harper J. 2004. Natural products from endophytic microorganisms. J. Nat. Prod. 67(2):257-268. doi: https://doi.org/10.1021/np030397v

Studzinski G. 1999. Cell growth, differentiation and senescence: a practical approach. Department of Pathology and Laboratory Medicine. New Jersey: Oxford University Press.

Swofford D, Sullivan J. 2003. Phylogeny inference based on parsimony and other methods using PAUP*. In: Salemi M, Vandamme AM, editors. The Phylogenetic Handbook: A Practical Approach to DNA and Protein Phylogeny. Inglaterra: Cambridge University Press. p. 160-206.

Tan YP, Crous PW, Shivas RG. 2016. Eight novel *Bipolaris* species identified from John L. Alcorn's collections at the Queensland Plant Pathology Herbarium (BRIP). Mycol. Prog. 15:1203-1214. doi: https://doi.org/10.1007/s11557-016-1240-6

Torres D, Vallejo SV, Linnakoski R, Rojas AM, Osorio JA. 2020. Caracterización de compuestos bioactivos presentes en hongos endófitos asociados a manglares de la Reserva Natural San Pedro, Buenaventura, Valle del Cauca. [Tesis]. [Quindío]: Universidad del Quindío.

Veciana G, Cortés C, Torro M, Sirvent S, Rizo B, Gil G. 2014. Evaluación de la citotoxicidad y bioseguridad de un extracto de polifenoles de huesos de aceitunas. Nutr. Hosp. 29:1388-1393. doi: https://dx.doi.org/10.3305/nh.2014.29.6.7141

Vilarino-Godinho BT, Férrer-Melo ÍA, Aparecida-Gomes E, Hilsdorf-Piccoli R, Gomes-Cardoso P. 2019. Endophytic fungi community in *eremanthus erythropappus* tree from anthropogenic and natural areas of minas gerais. SciELO journals. Dataset. 25(3). doi: https://doi.org/10.1590/01047760201925032642

Wang L, Han X, Zhu G, Wang Y, Chairoungdua A, Piyachaturawat P, Zhu W. 2018. Polyketides from the endophytic fungus *Cladosporium* sp. isolated from the mangrove plant *Excoecaria agallocha*. Front. Chem. 6:344. doi: https://doi.org/10.3389/fchem.2018.00344

Wen J, Okyere SK, Wang S, Wang J, Xie L, Ran Y, Hu Y. 2022. Endophytic Fungi: An Effective Alternative Source of Plant-Derived Bioactive Compounds for Pharmacological Studies. J Fungi. (Basel) 8(2):205. https://doi.org/10.3390/jof8020205

White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, editors. PCR Protocols: a guide to methods and applications. San Diego, California, USA: Academic Press. p. 315-322. doi: https://doi.org/10.1016/B978-0-12-372180-8.50042-1

World Rainforest Movement (c2002) Mangroves Local Livelihoods vs. Corporate Profits. 9974-7719-1-9

Yeshi K, Crayn D, Ritmejerytė E, Wangchuk P. 2022. Plant secondary metabolites produced in response to abiotic stresses has potential application in pharmaceutical product development. Molecules. 27(1):313. doi: https://doi.org/10.3390/molecules27010313