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Full Length Research Paper

Antibacterial activity of endophytic fungi isolated from *Croton lechleri* (Euphorbiaceae)

Sebastião Luiz Pires Vargas[1*], Erlan Kessyo Braga de Albuquerque[2]; Sandra Albuquerque Lima Ribeiro[3]; Atilon Vasconcelos de Araujo[1], Renildo Moura da Cunha[3] and Clarice Maia Carvalho[3]

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***Croton lechleri* is a native species from the Amazon and used with relative frequency in folk medicine in Brazil and other countries. Diversity and antibacterial activity of endophytic fungi associated with this plant were studied here. Samples of leaves and stems were used and 575 endophytic fungi were isolated (307 from leaves and 268 from stems), comprising 284 morphotypes distributed in 13 genera and unknown. The most frequently isolated genera were *Phomopsis* (30.78%), *Penicillium* (21.57%) and *Pestalotiopsis* (16.70%). Diversity and richness of species were higher in leaf tissues. Fifty-five fungi presented antibacterial activity. The fungi with the highest activity were *Phomopsis* (6.34%), *Penicillium* (3.17%), and those unknown (5.28%). *Penicillium* sp. 9 showed the highest antibacterial activity against *Enterococcus faecalis* and *Phomopsis* sp. 8 and *Phomopsis* sp. 9 against *Streptococcus pneumoniae* and *Staphylococcus aureus*. *Curvularia* sp. 1 and a fungus that could not be identified (Unknown sp. 9), showed the highest antibacterial activity against *Klebsiella pneumoniae* and *Escherichia coli*, respectively. Only two fungi (*Penicillium* sp. 9 and *Curvularia* sp. 1) inhibited the five tested bacteria. Endophytic fungi of *C. lechleri* harbor a great diversity of endophytic fungi, which have the potential for producing antibacterial compounds.**

Key words: Dragon's blood, antibacterial agent, endophytic fungi, microbial interaction.

INTRODUCTION

Croton lechleri, a tree that grows in Mexico, Venezuela, Ecuador, Peru, and Brazil, popularly known as dragon's blood (Gupta et al., 2008), is used by local communities to cure respiratory infections, diarrhea, gastric ulcers,

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herpes, and skin infections (Carlson, 2002).

C. lechleri latex has curative effects (Pieters et al., 1995), being antitumor (Rossi et al., 2003; Alonso et al., 2012; Montopoli et al., 2012), antioxidant (Lopes et al., 2004; Marino et al., 2008; Rossi et al., 2011), antibacterial (Bussmann et al., 2010; Rossi et al., 2011), antidiarrheal (Cottreau et al., 2012), and antimutagenic (Lopes et al., 2004; Fão et al., 2012; Rossi et al., 2013). The traditional use of plants with medicinal properties has promoted studies on the diversity and bioprospection of endophytic fungi (Strobel et al., 2004).

The presence of endophytic microorganisms in medicinal plants has been observed in many species (Hilarino et al., 2011; Premalatha and Kalra, 2013; Bezerra et al., 2015). These organisms are often involved in complex relationships between the synthesis, degradation, and accumulation of secondary metabolites of biotechnological interest (Müller et al., 2016). In many cases, there is an important symbiotic interaction with host plant, involving the production of compounds that can reduce herbivory in plant tissues, confer resistance to plant pathogens, and produce growth regulators to increase plant development (Kumar and Verma, 2017).

Endophytic microorganisms inhabit the interior of plants for at least one period of its life cycle and may colonize inter- and intracellular spaces of plants (Azevedo et al., 2000). These organisms do not harm plants but exhibit an asymptomatic relationship (Hardoim et al., 2015).

The use of endophytic microorganisms as a source of bioactive compounds or secondary metabolites is an interesting strategy since these microorganisms inhabit the interior of plants without causing any apparent symptom of the disease and growing in this environment involves continuous metabolic interaction between endophyte and host (Finkel et al., 2017).

Although several studies on biological activities and chemical composition of *C. lechleri* can be found in the literature, none of them is related to endophytic community and biological activity of its metabolites. Thus, endophytic fungi from leaves and stems of *C. lechleri* and their in vitro biotechnological potential for controlling pathogenic bacteria were assessed in this study.

MATERIALS AND METHODS

Collection of plants and isolation of endophytic fungi

Samples of leaves and stems from three individuals of *C. lechleri* were collected at the Federal University of Acre (9°57′26.2″ S and 67°52′29.1″ W) between September 2014 and February 2015.

The collected botanical material was processed and samples with no disease signs were selected and washed to eliminate the excess epiphytic. Samples were separated for preparing culture media containing plant tissue, stem, or leaf extracts, and samples for isolating endophytic fungi.

Samples were disinfected with 70 % alcohol for 1 min, 2.5% sodium hypochlorite for 2 min, 70% alcohol for 30 s, and washed in sterile distilled water twice (Pereira et al., 1993).

Tissues were fragmented into 5 mm diameter samples and inoculated in potato dextrose agar (PDA) and Sabouraud dextrose agar (SDA) culture media, with and without 10% plant tissue extract. Plant extract was produced by using 100 g of fresh leaves or stems in 500 mL distilled water and filtered on filter paper. An infusion of 200 g of potato was added to prepare PDA+extract medium or 500 mL distilled water to prepare SDA+extract medium, being solubilized the reagents (Lima et al., 2011). All media received chloramphenicol (100 µg mL^{-1}) in order to inhibit bacterial growth. The inoculated samples were incubated at 18 and 28°C and observed daily for 30 days (Azevedo et al., 2010).

Fungi were purified in PDA culture medium, classified into morphotypes according to their macromorphological characteristics, and stored using mineral oil and distilled water techniques (Azevedo et al., 2010). For identification, macro and micromorphological analyses were performed and compared with specific literature (Barnett and Hunter, 1998).

Fungus cultivation and metabolite extraction

Endophytic fungi were cultured in potato dextrose broth (PD) by inoculating ten blocks of pure culture agar (5 mm^2) under active growth and with 14 days in 125 mL Erlenmeyer flasks containing 20 mL of medium. Flasks were incubated for 14 days at 28°C without shaking. Subsequently, 2 mL of broth was extracted with an equal volume of ethyl acetate by liquid-liquid partition and the extract in ethyl acetate was collected and evaporated. The crude extract was dissolved in 300 µL dimethylsulfoxide (DMSO) for antibacterial bioassay (NCCLS, 2003).

Antibacterial activity determination

The antibacterial activity of fungal extracts was determined by the agar diffusion method against the pathogenic bacteria *Staphylococcus aureus* (ATCC 12598), *Streptococcus pneumoniae* (ATCC 11733), *Enterococcus faecalis* (ATCC 4083), *Escherichia coli* (ATCC 10536), and *Klebsiella pneumoniae* (ATCC 700603) (NCCLS, 2003). Pathogenic bacteria were cultured at 37°C for 4 to 6 h in Luria-Bertani medium and their turbidity was adjusted to 0.5 McFarland scale. Bacteria were then inoculated into Petri dishes containing Muller-Hinton agar (MH), deposited on these paper disks (5 mm diameter) and then 20 µL of endophytic extract, and incubated at 37°C for 24 h. The endophytic extract that did not allow bacterial growth around the disc was considered as having antibacterial activity, and the inhibition halos produced were measured in millimeters. All determinations were performed in triplicate (NCCLS, 2003).

Statistical analysis of data

The infection index (FI) was calculated from the relationship between the number of fragments from which the endophytic fungi emerged and the total number of fragments used in the experiment (Azevedo et al., 2010).

The relative frequency of isolation (RF) was calculated as the number of isolates of a species divided by the total number of isolates, being expressed as a percentage (Bezerra et al., 2015). For the diversity analysis of the endophytic community of *C. lechleri*, the Simpson and Shannon indices were used to calculate the number of dominant species (Bezerra et al., 2015).

The formula for calculating the Simpson diversity index is $1 - \Sigma (pi)^2$. Shannon-Wiener diversity $(H') = -\Sigma\ pi \ln pi$, where pi is the

Table 1. Number and relative frequency percentages of endophytic fungi isolated from *C. lechleri* according to plant tissue, culture medium and temperature.

Genus	Plant tissue		Culture medium						Temperature (°C)		T[a]	RF (%)
	Leaf	Stem	PDA	PDA+ leaf	PDA+stem	SDA	SDA+leaf	SDA+stem	18	28		
Phomopsis	63	114	59	25	43	36	4	10	82	95	177	30.78
Penicillium	82	42	27	-	18	17	45	17	52	72	124	21.57
Pestalotiopsis	69	27	32	20	-	24	20	-	52	44	96	16.70
Colletotrichum	16	7	-	1	6	15	-	1	8	15	23	4.00
Aspergillus	12	8	10	-	8	-	2	-	18	2	20	3.48
Fusarium	14	5	6	6	-	7	-	-	8	11	19	3.30
Xylaria	3	14	-	-	9	8	-	-	4	13	17	2.96
Guignardia	2	12	4	2	-	8	-	-	6	8	14	2.43
Curvularia	4	7	-	-	1	4	-	6	3	8	11	1.91
Nigrospora	2	4	-	-	4	2	-	-	-	6	6	1.04
Chaetomium	1	-	-	1	-	-	-	-	1	-	1	0.17
Paecilomyces	-	1	-	-	-	-	-	1	-	1	1	0.17
Rhizopus	1	-	1	-	-	-	-	-	-	1	1	0.17
Unknown	38	27	11	13	15	16	5	5	33	32	65	11.30
T[a]	307	268	150	68	104	137	76	40	267	308	**575**	
RF (%)	53.39	46.61	26.09	11.83	18.09	23.83	13.22	6.96	46.43	53.57		

a= total identified in the sample.

proportion of species colonization frequency in a sample. Species equivalence (E) was calculated by using the following formula: E = H / ln S, where S is the number of species in the sample (Bezerra et al., 2015).

RESULTS

A total of 575 fungi (307 from leaves and 268 from stems) were isolated from 160 fragments of *C. lechleri* and grouped into 284 fungal morphotypes, distributed in 13 genera and unknown. The infection index (FI) for *C. lechleri* was 93%, being 96% for leaves and 90% for stems. Colonization and frequency of endophytic fungi were higher in leaves (53.39%) than in stems (46.61%) of *C. lechleri* (Table 1).

The most frequent genera were *Phomopsis* (30.78%), *Penicillium* (21.57%), and *Pestalotiopsis* (16.70%) isolated from leaf and stem. Three endophytic genera (*Rhizopus*, *Paecilomyces*, and *Chaetomium*) had a lower frequency and were isolated only once (0.17 %) (Table 1). Two genera were isolated only from leaf (*Chaetomium* and *Rhizopus*) and one exclusively from the stem (*Paecilomyces*).

Endophytic fungi richness was higher in *C. lechleri* leaves. Among the 575 analyzed fungi, only 65 (11.30%) were not identified. The medium with the highest amount of isolated endophytic fungi was PDA+plant tissue extract (29.91%), followed by PDA (26.09%) (Figure 1).

The temperature was also a factor that influenced the isolation of endophytic fungi from *C. lechleri*. In this sense, 308 fungi (53.57%) were isolated at 28°C and only 267 (46.43%) at 18°C, having as a specialist at 18°C the genus *Chaetomium* and at 28°C the genus *Paecilomyces* and *Rhizopus*.

Endophytic community diversity isolated from different tissues of *C. lechleri* was compared using the α-diversity indices (Table 2). Simpson and Shannon-Wiener endophytic fungi diversity were higher in leaves. Species richness was also higher in leaves. Little difference was observed regarding species uniformity among the studied tissues.

The antibacterial activity of ethyl acetate extract from each of the 284 fungal morphotypes was analyzed (Table 3). Only two samples inhibited all the tested bacteria, that is, the extract of *Penicillium* sp. 9 and *Curvularia* sp. 1. The extracts of *Phomopsis* sp. 3, 4, and 10 and *Pestalotiopsis* sp. 2 had antibacterial action only against gram-positive bacteria, *S. aureus*, and *S. pneumoniae*. Only the extract of *Penicillium* sp. 6 showed activity for the gram-negative bacteria *E. coli* and *K. pneumoniae*.

Among the tested extracts, 55 (19.37%) presented antibacterial activity against at least one of the five tested bacteria. *S. aureus* presented lower resistance to the tested extracts, presenting a sensitivity to 33 samples (11.62%), while *E. faecalis* presented the highest resistance, with sensitivity only to six samples (2.11%). Nineteen (6.70%), 16 (5.63%), and 11 (3.87%) endophytic extracts were active against *E. coli*, *S. pneumoniae*, and *K. pneumoniae*, respectively. Only extracts from *Curvularia* sp. 1 (2.1152) and *Penicillium*

Table 2. Diversity indices of endophytic fungi from C. lechleri according to plant tissue, culture medium, and temperature.

Diversity index	Abundance	Species richness	Shannon-Wiener diversity	Simpson diversity	Species evenness
Tissue type					
Leaf	307	157	4.64	0.99	0.81
Stem	268	128	4.40	0.98	0.79
Culture medium					
PDA	150	77	3.86	0.97	0.77
PDA+Leaf	68	37	3.13	0.93	0.74
PDA+Stem	104	57	3.69	0.96	0.80
SDA	137	63	3.75	0.97	0.76
SDA+Leaf	76	20	2.60	0.91	0.60
SDA+Stem	40	30	3.24	0.95	0.75
Temperature (°C)					
18	308	131	4.40	0.98	0.77
28	267	155	4.67	0.99	0.84
Total sample	575	284	5.21	0.99	0.82

Table 3. Antimicrobial activity of endophytic fungi isolated from *C. lechleri* against pathogenic bacteria species.

Endophytic fungi	Isolate	Antagonistic activity against*				
		Efa	Spn	Sau	Eco	Kpn
Phomopsis sp. 1	2.1157	-	-	-	10.5±0.5	-
Phomopsis sp. 2	2.1183	-	-	8.7±1.1	-	-
Phomopsis sp. 3	2.1187	-	11.5±0.9	15.4±1.1	-	-
Phomopsis sp. 4	2.1188	-	11.2±0.6	10.9±0.9	-	-
Phomopsis sp. 5	2.1198	-	-	-	8.6±0.7	-
Phomopsis sp. 6	2.1231	-	-	-	8.7±1.3	-
Phomopsis sp. 7	2.1269	-	-	-	6.4±0.5	-
Phomopsis sp. 8	2.1264	-	18.4±0.5	-	-	-
Phomopsis sp. 9	2.1367	-	10.9±1.1	16.3±0.6	7.0±0.8	-
Phomopsis sp. 10	2.1430	-	7.5±0.8	11.2±0.8	-	-
Phomopsis sp. 11	2.1473	8.3±0.4	-	-	-	-
Phomopsis sp. 12	2.2507	-	-	8.3±0.2	-	-
Phomopsis sp. 13	2.2610	-	-	8.1±0.8	-	-
Phomopsis sp. 14	2.2653	-	6.9±0.4	-	-	-
Phomopsis sp. 15	2.2717	-	9.7±0.6	-	-	-
Phomopsis sp. 16	2.2719	-	8.9±0.3	-	-	-
Phomopsis sp. 17	2.2697	-	8.4±0.5	-	-	-
Phomopsis sp. 18	2.3021	-	-	10.7±0.0	-	-
Penicillium sp. 1	2.1339	-	-	7.8±0.5	-	-
Penicillium sp. 2	2.1351	-	-	12.2±0.1	11.9±0.2	12.1±0.4
Penicillium sp. 3	2.1391	-	8.4±0.3	12.9±0.6	-	12.4±0.5
Penicillium sp. 4	2.1429	-	-	9.0±0.3	-	12.8±0.6
Penicillium sp. 5	2.1478	-	-	6.5±0.8	-	16.2±0.6
Penicillium sp. 6	2.1636	-	-	-	11.3±0.2	7.6±0.5
Penicillium sp. 7	2.2635	-	-	13.9±0.9	-	-

Table 3. Cont'd.

Penicillium sp. 8	2.2698	11.3±0.5	-	13.1±0.2	-	-
Penicillium sp. 9	2.2710	10.5±0.4	11.4±0.4	12.7±0.5	11.2±0.3	9.2±0.4
Xylaria sp. 1	2.2609	-	-	9.3±0.4	-	-
Xylaria sp. 2	2.2630	-	-	-	6.8±0.0	-
Xylaria sp. 3	2.2976	-	-	-	-	8.4±0.2
Fusarium sp. 1	2.1431	8.6±0.3	11.4±0.2	-	-	-
Fusarium sp. 2	2.1388	-	-	-	-	13.2±0.3
Aspergillus sp. 1	2.1477	-	-	6.2±0.4	19.3±0.1	-
Aspergillus sp. 2	2.2875	9.9±0.2	-	7.9±0.3	11.9±0.3	12.7±0.6
Pestalotiopsis sp. 1	2.1114	-	-	8.0±0.3	-	-
Pestalotiopsis sp. 2	2.2705	-	11.2±0.2	7.2±0.6	-	-
Curvularia sp. 1	2.1152	10.1±0.0	8.5±0.4	10.8±0.7	9.7±0.2	19.0±0.1
Colletotrichum sp. 1	2.2707	-	-	7.9±0.3	-	-
Rhizopus sp. 1	2.2685	-	-	9.2±0.3	-	-
Paecilomyces sp. 1	2.1611	-	-	-	8.0±0.2	-
Unknown sp. 1	2.1132	-	-	12.5±0.2	-	-
Unknown sp. 2	2.1150	-	-	7.3±0.3	-	-
Unknown sp. 3	2.1207	-	13.1±0.2	-	10.2±0.4	-
Unknown sp. 4	2.1243	-	-	-	13.2±0.3	-
Unknown sp.5	2.1254	-	-	7.1±0.2	-	-
Unknown sp.6	2.1260	-	-	8.9±0.3	-	15.4±0.4
Unknown sp.7	2.1267	-	-	-	12.4±0.5	-
Unknown sp.8	2.1281	-	-	8.4±0.3	-	-
Unknown sp.9	2.1294	-	-	7.3±0.1	17.1±0.2	-
Unknown sp.10	2.1375	-	-	-	10.3±0.2	-
Unknown sp.11	2.1377	-	-	13.0±0.3	-	-
Unknown sp.12	2.2631	-	-	-	6.7±0.7	-
Unknown sp.13	2.2729	-	-	18.0±0.7	-	-
Unknown sp.14	2.2885	-	6.5±0.7	-	-	-
Unknown sp.15	2.3468	-	-	7.8±0.5	-	-
Chloramphenicol		13.3±0.4	19.0±0.8	19.3±0.5	26.0±0.0	13.7±0.5
Total		6	16	33	19	11

*Efa = *Enterococcus faecalis*; Spn = *Streptococcus pneumoniae*; Sau = *Staphylococcus aureus*; Eco = *Escherichia coli*; Kpn = *Klebsiella pneumoniae.*

sp. 9 (2.2710) presented antagonism for all the tested bacteria. *Penicillium* sp. 8 (2.2698) showed the highest activity against *E. faecalis*, while *Phomopsis* sp. 8 (2.1264) and *Phomopsis* sp. 9 (2.1367) presented the highest activity against *S. pneumoniae* and *S. aureus*, respectively. The fungi with the highest activity against gram-negative were *Curvularia* sp. 1 (2.1152) against *K. pneumoniae* and a fungus that could not be identified (Unknown sp. 9 - 2.1294) against *E. coli* (Figure 2).

DISCUSSION

A total of 575 endophytic fungi of *C. lechleri* were isolated, with colonization and frequency of endophytic fungi higher in leaves (53.39%) than in stems (46.61%) (Table 1). Studies with endophytic fungi have isolated most frequently fungi from stem than from leaves, unlike that observed in *C. lechleri* (Banhos et al., 2014; Bezerra et al., 2015). Other studies showed leaves with the highest colonization of endophytic fungi (Souza et al., 2004).

Similar to the results obtained in this study, *Phomopsis* and *Pestalotiopsis* were also one of the most frequent genera isolated as endophytic (Hilarino et al., 2011; Ferreira et al., 2015). Some fungal genera exhibited specificity in relation to the culture medium of isolation. *Rhizopus* was isolated only in PDA medium, *Chaetomium*

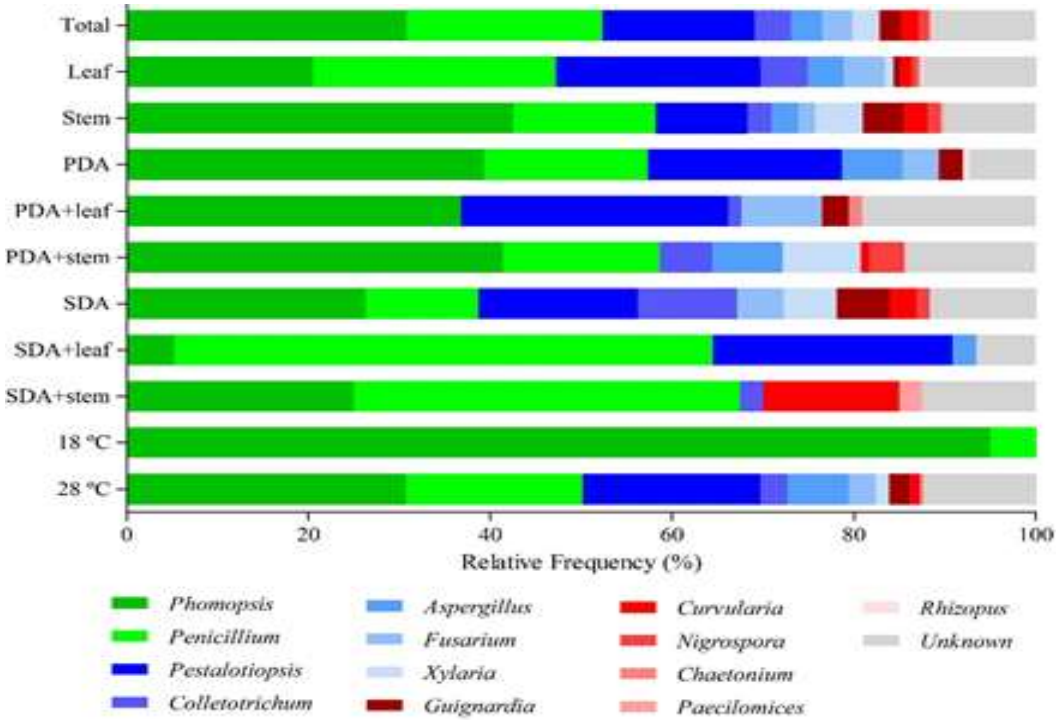

Figure 1. Fungal endophytic communities isolated from *C. lechleri* according to plant tissue, culture medium, and temperature.

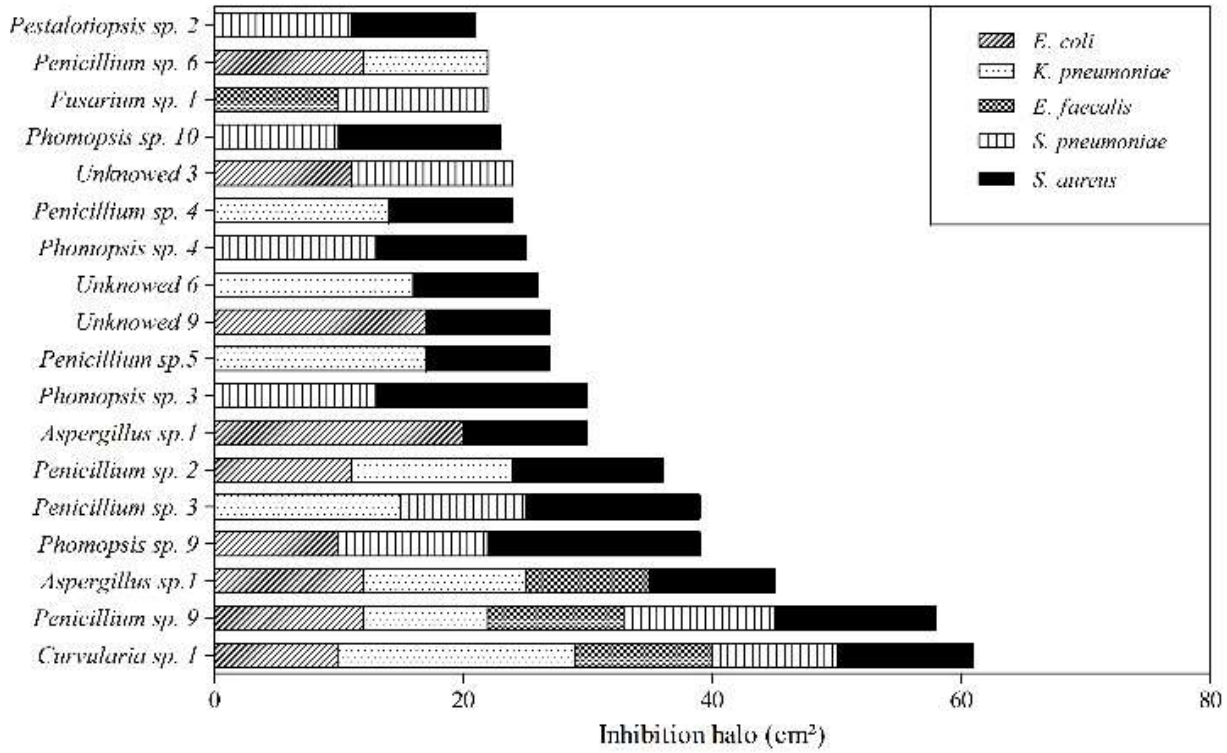

Figure 2. Antimicrobial activity (inhibition halo, in cm^2) against gram-positive and gram-negative bacteria presented by endophytic fungi isolated from *Croton lechleri*. Each value is expressed as the average of three independent experiments performed in triplicate.

only in PDA+extract, and *Paecilomyces* only in SDA+extract. Although PDA medium is the most used for isolating endophytic fungi, other culture media should be used to provide different nutritional sources. It is not common to use various culture media for isolating endophytic fungi (Pimentel et al., 2006), as well as the use of culture media with plant extracts is rarer still (Lima et al., 2011).

Two isolation temperatures were used to increase the number and diversity of endophytic fungi since it is possible to isolate fungi with slower growth at 18°C. However, few studies can be found in the literature using an isolation temperature of 18°C (Souza et al., 2004). In general, temperatures between 25 and 30°C are commonly used (Lima et al., 2011; Banhos et al., 2014; Bongiorno et al., 2016) or an ambient temperature of 28°C (Costa et al., 2012; Tayung et al., 2012), showing a large variation in this environmental factor (Table 1).

Endophytes have been reported as prolific producers of antimicrobial compounds. *Phomopsis*, *Penicillium* and *Xylaria* were the fungal genera that presented the highest antibacterial activity. Fungi of these genera are well known in the literature for their biological activities and several studies prove their potential as producers of biologically active secondary metabolites (Kobayashi et al., 2003; Prachya et al., 2007; Rukachaisirikul et al., 2008).

Fungi of the genus *Phomopsis* have often been isolated as endophytic and have demonstrated antibacterial activity (Kamei, 2008; Siqueira, 2008; Garcia et al., 2012; Deshmukh et al., 2015). Similar to that observed for the endophytic fungi *C. lechleri*, *Penicillium*, *Aspergillus*, and *Xylaria* stood out in secondary metabolite production, being among the genera frequently isolated as endophytic from tissues of several plants and the most frequently selected in bioprospecting studies (Elias, 2015).

In addition, other studies with endophytes have observed fungi of the genus *Penicillium* with antibacterial activity (Pastre et al., 2007; Borges, 2014; Bezerra et al., 2015). Fungi of the genus *Xylaria* are often isolated as endophytic from tropical plants and their metabolites have antibacterial activity, as observed in prospecting studies (Souza et al., 2004; Campos et al., 2015).

Endophytic fungi of the genus *Curvularia* have been observed in prospecting studies with antibacterial activity (Furtado et al., 2007; Bezerra et al., 2013; Nascimento et al., 2015).

Conclusion

C. lechleri hosts a rich community of endophytic fungi with antibacterial potential against bacteria pathogenic to human, especially against Gram positive. This study intends to contribute to the understanding of endophytic/plant interactions and open new perspectives on the biotechnological potential of endophytic microorganisms from Amazonian plants, which are practically unexplored in this field but have a great potential.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

Alonso AJC, Ortiz ES, Domínguez F, López GT, Chávez M, Jesús Ortiz AT, García AC (2012). Antitumor effect of *Croton lechleri* Mull. Arg.(Euphorbiaceae). Journal of Ethnopharmacology, 140(2):438-442.

Azevedo JL, Araújo WL, Lacava PT, Marcon J, Lima AOS, Sobral JK, Pizzirani-Kleiner AA (2010). Meios de cultura utilizados para o estudo de microrganismos. In: Pizzirani-Kleiner AA et al. (eds) Guia prático: isolamento e caracterização de microrganismos endofíticos. CALO, Piracicaba. P 167.

Azevedo JL, Maccheroni Jr W, Pereira JO, Araújo WL (2000). Endophytic microorganisms: a review on insect control and recent advances on tropical plants. Electronic Journal of Biotechnology, 3(1):15-16.

Banhos EF, Souza AQL, Andrade JC, Souza ADL, Koolen HHF, Albuquerque PM (2014). Endophytic fungi from *Myrcia guianensis* at the Brazilian Amazon: distribution and bioactivity. Brazilian Journal of Microbiology, 45(1):153-161.

Barnett HL, Hunter BB (1998). Illustrated genera of imperfect fungi (No. Ed. 4). American Phytopathological Society (APS Press).

Bezerra J, Nascimento C, Barbosa R, Silva D, Svedese V, Silva-Nogueira E, Gomes B, Paiva L, Souza-Motta C (2015). Endophytic fungi from medicinal plant *Bauhinia forficata*: Diversity and biotechnological potential. Brazilian Journal of Microbiology, 46(1):49-57.

Bezerra JDP, Santos MGS, Barbosa RN, Svedese VM, Lima DMM, Fernandes MJS, Gomes BS, Paiva LM, Almeida JSC, Souza CMM (2013). Fungal endophytes from cactus *Cereus jamacaru* in Brazilian tropical dry forest: a first study. Symbiosis, 60(2):53-63.

Bongiorno VA, Rhoden SA, Garcia A, Polonio JC, Azevedo JL, Pereira JO, Pamphile JA (2016). Genetic diversity of endophytic fungi from Coffea arabica cv. IAPAR-59 in organic crops. Annals of Microbiology, 66(2):855-865.

Borges E (2014). Fungos endofíticos de Arbutus unedo L.: diversidade, propriedades antimicrobianas e composição volátil (Doctoral

dissertation).
Bussmann R, Malca GG, Glenn A, Sharon D, Chait G, Díaz D, Pourmand K, Jonat B, Somogy S, Guardado G (2010). Minimum inhibitory concentrations of medicinal plants used in Northern Peru as antibacterial remedies. Journal of Ethnopharmacology, 132(1):101-108.
Campos FF, Sales PA, Romanha AJ, Araujo MSS, Siqueira EP, Resende JM, Alves TMA, Martins OA, dos Santos VL, Rosa CA, Zani CL, Cota BB (2015). Bioactive endophytic fungi isolated from *Caesalpinia echinata* Lam. (Brazilwood) and identification of beauvericin as a trypanocidal metabolite from *Fusarium* sp. Memórias do Instituto Oswaldo Cruz, 110(1):65-74.
Carlson TJ (2002). Medical ethnobotanical research as a method to identify bioactive plants to treat infectious diseases. Advances in Phytomedicine, 1:45-53.
Costa IP, Maia LC, Cavalcanti MA (2012). Diversity of leaf endophytic fungi in mangrove plants of Northeast Brazil. Brazilian Journal of Microbiology, 43(3):1165-1173.
Cottreau J, Tucker A, Crutchley R, Garey KW (2012). Crofelemer for the treatment of secretory diarrhea. Expert Review of Gastroenterology and Hepatology, 6(1):17-23.
Deshmukh SK, Verekar SA, Bhave SV (2015). Endophytic fungi: A reservoir of antibacterials. Frontiers in microbiology, 5:715.
Elias LM (2015). Bioprospecção de fungos endofíticos isolados de guaranazeiros da Amazônia. Phd thesis, Escola Superior de Agricultura "Luiz de Queiroz".
Fão F, Zan RA, Brondani FMM, Ramos LJ, Oliveira DUM (2012). Análise do potencial mutagênico da seiva da casca de *Croton lechleri* (müll. arg), no estado de Rondônia, Amazônia Ocidental. SaBios - Revista de Saúde e Biologia, 7:91-98.
Ferreira MC, Vieira MDA, Zani CL, Alves TMD, Sales PA, Murta SMF, Romanha AJ, Gil L, Amanda GDC, Zilli JE, Vital MJS, Rosa CA, Rosa LH (2015). Molecular phylogeny, diversity, symbiosis and discover of bioactive compounds of endophytic fungi associated with the medicinal Amazonian plant *Carapa guianensis* Aublet (Meliaceae). Biochemical Systematics and Ecology, 59:36-44.
Finkel OM, Castrillo G, Paredes SH, González IS, Dangl JL (2017). Understanding and exploiting plant beneficial microbes. Current Opinion in Plant Biology, 38:155-163.
Furtado DCM, Amorim EPR, Galvão ALB, Carnaúba JP, Oliveira MN (2007). Ocorrência de *Curvularia lunata* e *Curvularia eragrostidis* em *Tapeinochilus ananassae* no estado de Alagoas. Summa Phytopathologica, 33(2):201-201.
Garcia A, Rhoden S, Bernardi JW, Orlandelli R, Azevedo J, Pamphile J (2012). Antimicrobial Activity of Crude Extracts of Endophytic Fungi Isolated from Medicinal Plant *Sapindus saponaria* L. Journal of Applied Pharmaceutical Science, 2(10):35-40.
Gupta D, Bleakley B, Gupta RK (2008). Dragon's blood: botany, chemistry and therapeutic uses. Journal of Ethnopharmacology, 115(3):361-380.
Hardoim PR, Overbeek LSV, Berg G, Pirttila AM, Compant S, Campisano A, Doring M, Sessitsch A (2015). The hidden world within plants: Ecological and evolutionary considerations for defining functioning of microbial endophytes. Microbiology and Molecular Biology Reviews, 79(3):293-320.
Hilarino MPA, Oki Y, Rodrigues L, Santos JC, Corrêa Junior A, Fernandes GW, Rosa CA (2011). Distribution of the endophytic fungi community in leaves of *Bauhinia brevipes* (Fabaceae). Acta Botanica Brasilica, 25(4):815-821.
Kamei SH (2008). *Nectria* sp., *Phomopsis* sp. e *Xylaria* sp. fungos endofíticos isolados de *Borreria verticillata* (Rubiaceae) L. Paper presented at the Congresso Brasileiro de Genética, Salvador, P 34.
Kobayashi H, Meguro S, Yoshimoto T, Namikoshi M (2003). Absolute structure, biosynthesis, and anti-microtubule activity of phomopsidin, isolated from a marine-derived fungus *Phomopsis* sp. Tetrahedron, 59:455-459.
Kumar A, Verma JP (2017). Does plant—Microbe interaction confer stress tolerance in plants: a review? Microbiological Research, 207:41-52.
Lima AM, Salem JI, Souza JVB, Cortez ACA, Carvalho CM, Chaves FCM, Veiga VF (2011). Effects of culture filtrates of endophytic fungi obtained from *Piper aduncum* L. on the growth of *Mycobacterium tuberculosis*. Electronic Journal of Biotechnology, 14:1-6.
Lopes M, Saffi J, Echeverrigaray S, Henriques JAN, Salvador M (2004). Mutagenic and antioxidant activities of *Croton lechleri* sap in biological systems. Journal of Ethnopharmacology, 95(2-3):437-445.
Marino S, Gala F, Zollo F, Vitalini S, Fico G, Visioli F, Iorizzi M (2008). Identification of minor secondary metabolites from the latex of *Croton lechleri* (Muell-Arg) and evaluation of their antioxidant activity. Molecules, 13(6):1219-1229.
Montopoli M, Bertin R, Chen Z, Bolcato J, Caparrotta L, Froldi G (2012). *Croton lechleri* sap and isolated alkaloid taspine exhibit inhibition against human melanoma SK23 and colon cancer HT29 cell lines. Journal of Ethnopharmacology, 144(3):747-753.
Müller CA, Obermeier MM, Berg G (2016). Bioprospecting plant-associated microbiomes. Journal of Biotechnology, 235:171-180.
Nascimento TL, Oki Y, Lima DMM, Almeida-Cortez JS, Fernandes GW, Souza-Motta CM (2015). Biodiversity of endophytic fungi in different leaf ages of *Calotropis procera* and their antimicrobial activity. Fungal Ecology, 14:79-86.
Nattinal Committee for Clinical Laboratory Standards (NCCLS) (2003). Performance standards for antimicrobial disk susceptibility tests. Approved standard M2-A8. Wayne, Pennsylvania, P 36.
Pastre R, Marinho AM, Rodrigues-Filho E, Souza AQ, Pereira JO (2007). Diversidade de policetídeos produzidos por espécies de *Penicillium* isoladas de *Melia azedarach* e *Murraya paniculata*. Química Nova, 30:1867-1871.
Pereira JO, Azevedo JL, Petrini O (1993). Endophytic fungi of *Stylosanthes*: a first report. Mycologia, 85(3):362-364.
Pieters L, De Bruyne T, Van Poel B, Vingerhoets R, Totté J, Berghe DV, Vlietinck A (1995). In vivo wound healing activity of Dragon's blood (*Croton* spp.), a traditional South American drug, and its constituents. Phytomedicine, 2(1):17-22.
Pimentel IC, Kuczkowski FR, Chime MA, Auer CG, Junior Albino G (2006). Fungos Endofíticos em Folhas de Erva-Mate (*Ilex paraguaruensis* A. St.-Hil.). Floresta, 36:123-128.
Prachya S, Wiyakrutta S, Sriubolmas N, Ngamrojanavanich N, Mahidol C, Ruchirawat S, Kittakoop P (2007). Cytotoxic mycoepoxydiene derivatives from an endophytic fungus *Phomopsis* sp. isolated from *Hydnocarpus anthelminthicus*. Planta Medica, 73(13):1418-1420.
Premalatha K, Kalra A (2013). Molecular phylogenetic identification of endophytic fungi isolated from resinous and healthy wood of *Aquilaria malaccensis*, a red listed and highly exploited medicinal tree. Fungal Ecology, 6(3):205-211.
Rossi D, Bruni R, Bianchi N, Chiarabelli C, Gambari R, Medici A, Lista A, Paganetto G (2003). Evaluation of the mutagenic, antimutagenic and antiproliferative potential of *Croton lechleri* (Muell. Arg.) latex. Phytomedicine, 10(2-3):139-144.
Rossi D, Guerrini A, Maietti S, Bruni R, Paganetto G, Poli F, Scalvenzi L, Radice M, Saro K, Sacchetti G (2011). Chemical fingerprinting and bioactivity of Amazonian Ecuador *Croton lechleri* Müll. Arg.(Euphorbiaceae) stem bark essential oil: a new functional food ingredient? Food Chemistry, 126(3):837-848.
Rossi D, Guerrini A, Paganetto G, Bernacchia G, Conforti F, Statti G, Maietti S, Poppi I, Tacchini M, Sacchetti G (2013). *Croton lechleri* Müll. Arg.(Euphorbiaceae) stem bark essential oil as possible mutagen-protective food ingredient against heterocyclic amines from cooked food. Food Chemistry, 139(1-4):439-447.
Rukachaisirikul V, Sommart U, Phongpaichit S, Sakayaroj J, Kirtikara K (2008). Metabolites from the endophytic fungus *Phomopsis* sp. PSU-D15. Phytochemistry, 69(3):783-787.
Siqueira VM (2008). Fungos endofíticos de folhas e caule de *Lippia sidoides* Cham. e avaliação de atividade antimicrobiana. Master's thesis, Universidade Federal de Pernambuco.
Souza AQL, Souza ADL, Astolfi Filho S, Pinheiro MLB, Sarquis MIM, Pereira JO (2004). Antimicrobial activity of endophytic fungi isolated from amazonian toxic plants: *Palicourea longiflora* (aubl.) Rich and *Strychnos cogens* Bentham. Acta Amazonica, 34(2):185-195.
Strobel G, Daisy B, Castillo U, Harper J (2004). Natural products from endophytic microorganisms. Journal of Natural Products, 67(2):257-

268.
Tayung K, Sarkar M, Baruah P (2012). Endophytic Fungi Occurring in *Ipomoea carnea* Tissues and their Antimicrobial Potentials. Brazilian Archives of Biology and Technology, 55(5):653-660

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Antibacterial activity of endophytic fungi from the medicinal plant *Uncaria tomentosa* (Willd.) DC

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This study was designed to determine the diversity and antibacterial activity of endophytic fungi isolated from *Uncaria tomentosa*. Leaf and stem were disinfected superficially and inoculated in PDA and SDA medium, with and without plant extract and incubated at 18 and 28°C for isolation of endophytic fungi. Endophytic fungi were inoculated in BD medium and the metabolites extracted with ethyl acetate. Endophytic fungi extracts were tested for antibacterial activity by the disk diffusion test. One hundred and seventy endophytic fungi were isolated and identified as *Aspergillus*, *Asterosporium*, *Aureobasidium*, *Botrytis*, *Colletotrichum*, *Curvularia*, *Didymostilbe*, *Fusarium*, *Guignardia*, *Nigrospora*, *Penicillium*, *Pestalotiopsis*, *Phomopsis*, and sterile mycelium. *Staphylococcus aureus* was the most resistant bacterium, with only two fungal extracts inhibiting its growth, while the most sensitive was *Escherichia coli*, with 23 extracts inhibiting its growth. Five extracts inhibited *Enterococcus faecalis* and four *Klebsiella pneumoniae*. No fungal extract was able to inhibit the four tested bacteria. Extracts from endophytic fungi isolated from *U. tomentosa* showed *in vitro* antibacterial activity against gram-positive and gram-negative bacteria.

Key words: Cat's claw, microbial ecology, antibiotics.

INTRODUCTION

Endophytes are microorganisms that colonize internal tissues of plants for at least part of their life cycle without causing disease symptoms in their hosts (Petrini, 1991). Fungal endophytes can inhabit host tissues in different organs, including leaves, stems, barks, roots, fruits, flowers, and seeds (Stone et al., 2004). In this symbiotic relationship, fungal endophytes receive protection and nutrients from the host, while the host plant receives protection against natural enemies, such as pathogens and herbivores (Azevedo et al., 2000), promoting plant growth (Hamayun et al., 2010) and increasing its resistance to abiotic stress factors (Khan et al., 2014).

Many medicinal plants are known to harbor endophytic fungi, which are producers of important bioactive secondary metabolites for the industry. Therefore, efforts have been made to characterize and identify endophytic fungi isolated from medicinal plants (Strobel et al., 2004). *Uncaria tomentosa* (Willd.) DC belongs to the

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Rubiaceae family, being a medicinal plant widely used by Amazon peoples. This species is used to treat infections, rheumatism, gastritis, cancer, asthma, cirrhosis, fever, and has a wide range of other medicinal applications (Keplinger et al., 1998; Dreifuss et al., 2013). Several chemical compounds, such as oxindole alkaloids and quinolinic acids, have anti-inflammatory (Akhtar et al., 2011), anticancer (Dietrich et al., 2014), and antimicrobial activity (Sá et al., 2014). However, there are no studies on the endophytic community of this plant. Thus, this study was designed to determine the diversity and antibacterial activity of endophytic fungi isolated from *U. tomentosa*.

MATERIALS AND METHODS

Plant samples

Healthy and mature plant tissues were collected from three *U. tomentosa* trees in Rio Branco, Acre, Brazil (10°01′ S and 67°42′ W) in September 2015. Voucher specimens were deposited in the Herbarium of the Universidade Federal do Acre under the identification number 22.002. Leaves and stems were collected from each plant and brought directly to the laboratory, being processed within 24 h after collection (Azevedo et al., 2010).

Isolation of endophytic fungi

Each sample of plant material was washed with running water and surface sterilized with 70% ethanol for 1 min, followed by treatment with 2.5% active chlorine solution for 3 min, 70% ethanol for 30 s, and final rinsing in sterile water (Pereira et al., 1993). Prior to surface decontamination, the ends of stem fragments were sealed with paraffin to prevent the entry of germicidal agents into the plant tissue and thus inhibit the death of endophytic fungi. To assess whether the disinfection method was effective in the removal of fungi from the surface, 200 µL wash water were inoculated in the same culture media used for the isolation of endophytic fungi, and these plates were observed for emergent fungi (Azevedo et al., 2010).

After superficial disinfection, two plates of potato dextrose agar (PDA), Sabouraud dextrose agar (SDA), PDA+plant extract, and SDA+plant extract, supplemented with chloramphenicol (100 µg mL^{-1}), each of them containing 10 fragments of plant material, were prepared for each of the two types of samples (leaf and stem) and maintained in the dark at 18 and 28°C. For producing the plant tissue extract, 100 g of fresh tissue were ground in 500 mL distilled water, filtered on filter paper, and 500 mL of an infusion of 200 g of potato were added to prepare PDA+extract medium or 500 mL distilled water for SDA+extract (Lima et al., 2011).

Fragments of mycelium emerging from plant fragments were transferred to new PDA plates without chloramphenicol to obtain pure cultures for identification (Azevedo et al., 2010).

Identification of endophytic fungi

Fungal cultures were maintained at ambient temperature (22 to 25°C) under natural photoperiod for 14 days and then visually examined regarding macroscopic (morphology, size, mycelial and agar color) and microscopic (presence of spores or other reproductive structures) characteristics (Barnett and Hunter, 1998). Isolates with similar morphological characteristics were grouped into morphospecies. Each morphospecies is represented by several isolates, being an isolate representative of each selected for microscopic identification and antibacterial activity (Azevedo et al., 2010).

Antibacterial test

A fungus from each morphospecies was inoculated in PDA medium and incubated at 28°C for 14 days, and ten 5 × 5 mm plugs were inoculated into 20 mL potato dextrose broth (PD) incubated at 28°C, without agitation, for 14 days. Moreover, 2 mL of medium containing fungal metabolites were extracted by a liquid-liquid partition with ethyl acetate and solubilized in 300 µL dimethylsulfoxide 99.9%(DMSO) (Azevedo et al., 2010).

Antibacterial activity of fungal extracts was performed by the disc diffusion method against the pathogenic bacteria *Staphylococcus aureus* (ATCC 12598), *Streptococcus pneumoniae* (ATCC 11733), *Enterococcus faecalis* (ATCC 4083), *Escherichia coli* (ATCC 10536), and *Klebsiella pneumoniae* (ATCC 700603) (CLSI 2003).

Pathogenic bacteria were cultured at 3°C for 4 to 6 h and their turbidity adjusted to 0.5 McFarland scale. Bacteria were inoculated into Petri dishes containing Muller-Hinton (MH) medium, deposited on these paper discs, and then 20 µL of endophytic fungal extracts and incubated at 37°C for 24 h. The endophytic extract that did not allow bacterial growth around the disc was considered as having antibacterial activity and the inhibition halos produced were measured in millimeters (CLSI, 2003). Antibacterial tests were done in triplicate.

Statistical analysis of data

The infection index (FI) was calculated from the relationship between the number of fragments from which the endophytic fungi emerged and the total number of fragments used in the experiment (Azevedo et al., 2010).

The relative frequency of isolation (RF) was calculated as the number of isolates of a species divided by the total number of isolates, being expressed as a percentage.

For the diversity analysis of the endophytic community of *U. tomentosa*, the number of dominant species was calculated by using the Simpson and Shannon indices. The formula for calculating the Simpson diversity index is $1 - \Sigma (pi)^2$. Shannon-Wiener diversity (H') = $-\Sigma$ pi ln pi, where pi is the proportion of species colonization frequency in a sample. Equivalence of Eveness (E) was calculated by using the following formula: E = H / ln S, where S is the number of species in the sample (Bezerra et al., 2015).

RESULTS AND DISCUSSION

Isolation and identification of endophytic fungi

A total of 170 isolates belonging to 101 morphospecies, including isolates from sterile mycelium, were obtained from leaves and stems of *U. tomentosa* (Table 1). Isolation frequency of endophytic fungi was 89.6%, being higher in leaves (93.7%) than in stem (85.6%).

More endophytes were recovered from leaves (54.12%) than stems (45.88%) (Table 1). This difference may be related to the anatomical characteristics of *U. tomentosa*, which is a liana vine with more stems than leaves, facilitating the entry of microorganisms by

Table 1. Number and relative frequency percentages of endophytic fungi isolated from *Uncaria tomentosa* according to plant tissue, culture medium, and temperature.

Genus	Plant tissue		Culture medium						Temperature		T	RF(%)
	Leaf	Stem	PDA	PDA+ Leaf	PDA+Stem	SDA	SDA+Leaf	SDA+Stem	18 °C	28 °C		
Penicillium	7	8	5	2	-	5	-	3	4	11	15	8.82
Nigrospora	12	1	1	-	-	10	2	-	5	8	13	7.65
Colletotrichum	10	2	-	3	-	5	4	-	4	8	12	7.06
Pestalotiopsis	1	10	4	1	1	3	-	2	4	7	11	6.47
Curvularia	2	8	5	-	2	1	1	1	2	8	10	5.88
Phomopsis	9	-	5	-	-	2	2	-	3	6	9	5.29
Fusarium	1	4	5	-	-	-	-	-	4	1	5	2.94
Guignardia	3	-	-	-	-	3	-	-	-	3	3	1.76
Aspergillus	-	2	1	-	-	-	-	1	-	2	2	1.18
Asterosporium	-	1	-	-	-	1	-	-	1	-	1	0.59
Aureobasidium	-	1	1	-	-	-	-	-	1	-	1	0.59
Botrytis	-	1	-	-	1	-	-	-	-	1	1	0.59
Didymostilbe	1	-	-	1	-	-	-	-	-	1	1	0.59
Unknown	46	40	27	7	11	15	13	13	50	36	86	50.59
Total	92	78	54	14	15	45	22	20	78	92	170	-
RF (%)	54.12	45.88	31.76	8.24	8.82	26.47	12.94	11.76	45.88	54.12	-	-

T: Total identified in the sample; RF: relative frequency of endophytic fungi (%).

stomata and leaf grooves, as well as some fungi with hyphal growth on the leaf surface (Wagner and Lewis, 2000).

Among the total isolated species, 28.23% were Hyphomycetes, 21.18% were Coelomycetes, and 50.59% were sterile mycelium. Among the endophytic species, *Penicillium* (8.82%), *Nigrospora* (7.65%), *Colletotrichum* (7.06%), and sterile mycelium (50.59%) predominated. As specialists and isolated only once, *Asterosporium*, *Aureobasidium*, *Botrytis*, and *Didymostilbe* were observed. These fungi indicated an intimate relationship with this plant, which suggests a genotypic interaction between fungus and plant, which may depend exclusively on the plant for its survival (Malcolm et al., 2013).

The highest recovery rate of endophytic fungi of *U. tomentosa* may also be related to the variation in the used nutritional and environmental conditions (Huang et al., 2007; Putra et al., 2015).

A different genus of endophytic fungi was isolated. Some of them are common in tropical regions and are often isolated in this type of study, being called generalists. However, other endophytic fungi are not very frequent and are known as specialists. Those with a preference for a particular culture medium, tissue, and/or temperature were classified as specialists of this isolation condition (Toju et al., 2013).

Among the culture media used, the highest fungal recovery occurred in PDA regardless of the type of tissue used, with 54 isolates (31.76%), followed by SDA, with 45 isolates (26.47%) (Figure 1). Some fungal genera showed to be specialists in relation to the culture medium. *Fusarium* was isolated only in PDA medium, *Botrytis* and *Didymostilbe* in PDA+extract, and *Guignardia*, *Asterosporium*, and *Aureobasidium* in SDA, showing the need to use different nutritional sources to increase the recovery rate and richness of endophytic fungi. Studies on endophytic fungal isolation usually use only PDA medium (Hilarino et al., 2011; Katoch et al., 2014; Bezerra et al., 2015).

Another factor of relevance in this study was the isolation temperature. Lower temperatures, such as 18°C, allow the development of fastidious fungi (Souza Leite et al., 2013). However, for *U. tomentosa*, a temperature of 28°C provided the highest number of endophytes with 90 isolates (52.94%).

Some fungal genera also presented specificity to the isolation temperature, being isolated only at 18 or 28°C. *Asterosporium* and *Aureobasidium* were isolated only at 18°C, while *Guignardia*, *Aspergillus*, *Botrytis*, and *Didymostilbe* only at 28°C. Studies on endophytic fungi generally use temperatures between 25 and 28°C (Premalatha and Kalra, 2013; Campos et al., 2015; Ferreira et al., 2015).

Some fungi were not identified due to the absence of reproductive structures, called sterile mycelium. In *U. tomentosa*, 86 isolates could not be identified, representing 50.59%.

The diversity of the endophytic community isolated from different *U. tomentosa* tissues was compared by using α-diversity indices. Simpson diversity of endophytic

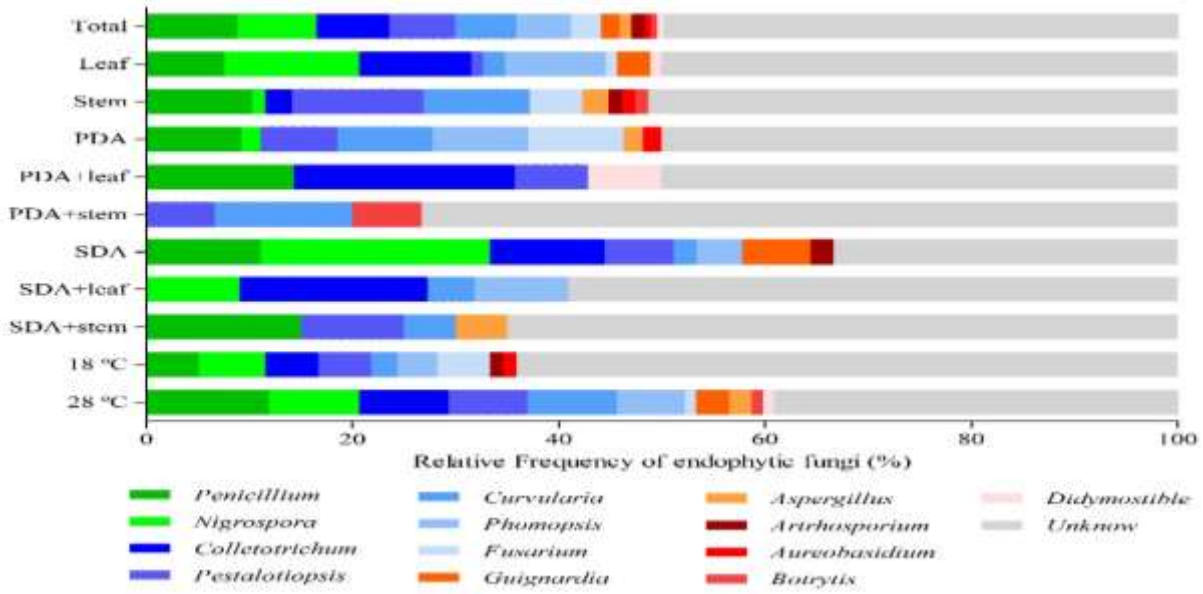

Figure 1. Endophytic fungal communities isolated from *Uncaria tomentosa* according to plant tissue, culture medium, and temperature.

Table 2. Diversity indices of endophytic fungi from *Uncaria tomentosa* according to plant tissue, culture medium, and temperature.

Diversity index	Abundance	Species richness	Shannon-Wiener diversity	Simpson diversity	Species evenness
Tissue					
Leaf	92	49	3.79	0.98	0.84
Stem	78	52	3.85	0.98	0.88
Culture medium					
PDA	54	36	3.47	0.97	0.87
PDA+leaf	14	08	1.97	0.85	0.75
PDA+stem	15	10	2.21	0.88	0.82
SDA	45	24	3.07	0.95	0.81
SDA+leaf	22	11	2.33	0.90	0.75
SDA+stem	20	12	2.39	0.90	0.80
Temperature					
18°C	78	46	3.72	0.98	0.85
28°C	90	54	3.88	0.98	0.86
Total sample	170	101	4.51	0.99	0.88

fungi was the same for both tissues. Both the Shannon-Wiener diversity and Eveness indices were higher in the stem. Species richness was also higher in the stem (Table 2).

Antibacterial activity

Among the 98 endophytic fungal extracts selected for testing against pathogenic strains, 23 were positive against at least one of the tested pathogenic bacteria. Five extracts were active against *E. faecalis*, two against *S. aureus*, four against *K. pneumoniae*, and 23 against *E. coli* (Table 3).

Extracts from *Penicillium* spp. 2 (2.378), *Penicillium* spp. 4 (2.4055), and *Fusarium* spp. 1 (2.3952) showed antibacterial activity against gram-positive and gram-negative bacteria (*S. aureus*, *E. coli*, and *K. pneumoniae*)

Table 3. Antibiosis results of extract from endophytic fungi isolated from of *U. tomentosa*, which presented some activity against pathogenic strains.

Endophytic fungus	Isolate	Antagonistic activity against*			
		Efa	Sau	Eco	Kpn
Colletotrichum spp. 1	2.4078	–	-	20.3±0.5	-
Colletotrichum spp. 2	2.3916	13.7±0.4	-	-	-
Colletotrichum spp. 3	23916	-	-	13.7±0.4	-
Colletotrichum spp. 4	2.3837	-	-	9.7±0.4	-
Colletotrichum spp. 5	2.4042	-	-	10.0±0.0	-
Colletotrichum spp. 6	2.3895	-	-	9.0±0.0	-
Nigrospora spp. 1	2.3831	-	-	18.0±0.0	-
Nigrospora spp. 2	2.3972	-	14.0±0.0	-	-
Nigrospora spp. 3	2.3907	-	-	9.7±0.4	-
Nigrospora spp. 4	2.3909	-	-	8.0±0.0	-
Nigrospora spp. 5	2.3799	-	-	7.7±0.4	-
Nigrospora spp. 6	2.4088	-	-	6.0±0.0	-
Penicillium spp. 1	2.3964	19.3±0.5	-	-	-

Concl.

Endophytic fungus	Isolate	Antagonistic activity against*			
		Efa	Sau	Eco	Kpn
Penicillium spp. 2	2.3788	17.7±0.4	-	-	10.0±0.0
Penicillium spp. 3	2.3964	-	16.0±0.0	-	-
Penicillium spp. 4	2.4055	-	-	17.7±0.4	11.0±0.0
Penicillium spp. 5	2.3828	-	-	8.3±0.4	-
Penicillium spp. 6	2.3758	-	-	7.0±0.0	-
Pestaloptiopsis spp. 1	2.4084	-	-	18.0±0.0	-
Pestaloptiopsis spp. 2	2.3794	-	-	10.7±0.4	-
Pestaloptiopsis spp. 3	2.3800	-	-	–	10.0±0.0
Curvularia spp. 1	2.4034	-	-	21.0±0.0	-
Curvularia spp. 2	2.3761	-	-	10.0±0.0	-
Fusarium spp. 1	2.3952	16.7±0.4	-	25.0±0.0	-
Fusarium spp. 2	2.3949	-	-	6.0±0.0	-
Phomopsis spp. 1	2.3934	-	-	-	10.0±0.0
Aspergillus spp. 1	2.3959	-	-	11.0±0.0	-
Unknown spp. 1	2.4090	-	-	20.7±0.4	-
Unknown spp. 2	2.3903	20.0±0.0	-	–	-
Unknown spp. 3	2.3773	-	-	19.7±0.4	-
Unknown spp. 4	2.4061	-	-	19.0±0.0	-
Unknown spp. 5	2.4014	-	-	-	-
Unknown spp. 6	2.3815	-	-	-	-
Unknown spp. 7	2.3903	-	-	-	-
Unknown spp. 8	2.3951	-	-	-	-
Chloramphenicol	-	13.3±0.4	19.3±0.5	26.0±0.0	13.7±0.5
Total		5	2	23	4

*Efa: *Enterococcus faecalis*; Spn: *Streptococcus pneumoniae*; Sau: *Staphylococcus aureus*; Eco: *Escherichia coli*; Kpn: *Klebsiella pneumoniae*.

(Figure 2). The extracts with the best antibacterial activity against *S. aureus* and *K. pneumoniae* were *Penicillium* spp. 3 (2.3964) and *Penicillium* spp. 4 (2.4055), respectively. The extract from *Fusarium* spp. 1 (2.3952) was the best for *E. coli*, while for *E. faecalis*, the fungus Unknown species 2 (2.3903), which did not produce

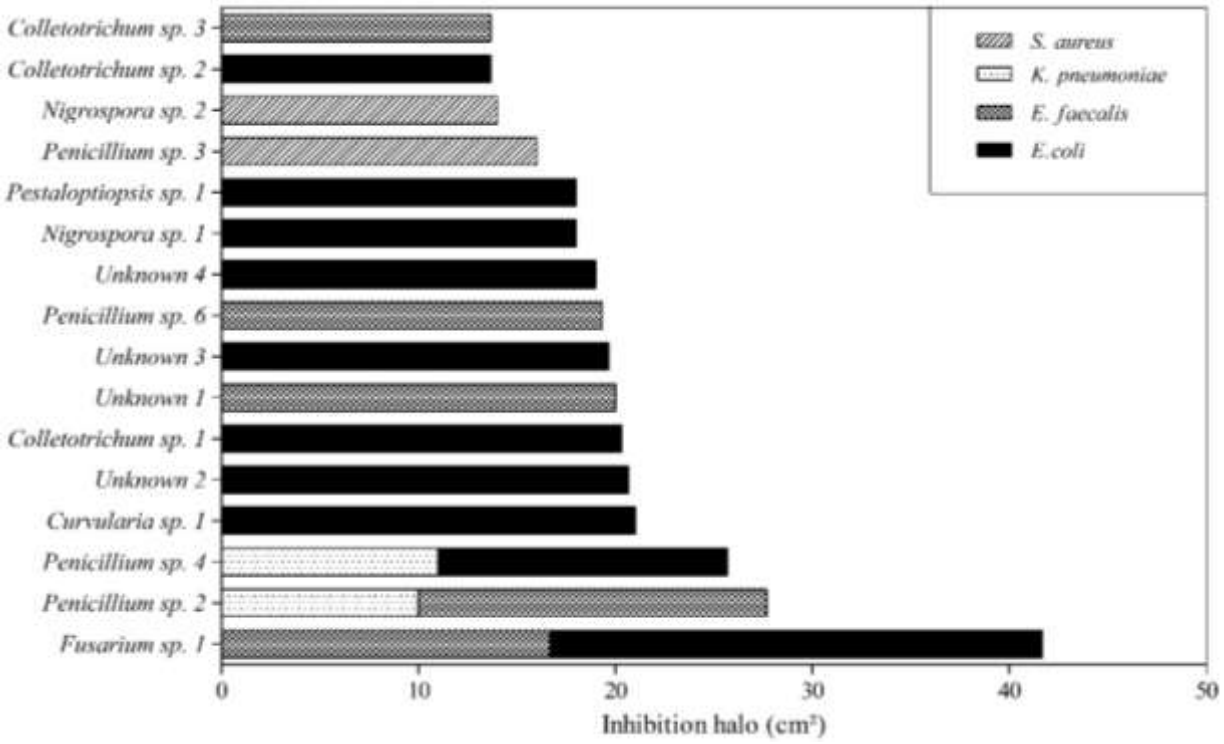

Figure 2. Antimicrobial activity (inhibition halo, in cm^2) against gram-positive and gram-negative bacteria presented by endophytic fungi isolated from *Uncaria tomentosa*. Each value is expressed as the average of three independent.

reproductive structures. Any fungal extracts presented antibacterial activity against the four bacteria tested.

Penicillium spp. is the most studied bioprospecting fungus since penicillin was discovered and produces several defense metabolites with several biological activities such as antibacterial and antifungal agents (Supaphon et al., 2013). Endophytic *Penicillium* was observed with antibacterial activity in other studies (Jouda et al., 2004; Padhi and Tayung, 2015).

Colletotrichum isolated as endophytic fungus also showed antibacterial activity against gram-positive, gram-negative, and *Candida albicans* bacteria (Katoch et al., 2014; Ferreira et al., 2015).

Nigrospora has not been a fungus commonly reported as a producer of antibiotics. However, in this study, six morphospecies presented this activity and *Nigrospora* spp. 1 (2.3831) presented strong activity against *E. coli*.

The endophytic fungus *Pestalotiopsis* spp. proved to be an important producer of antibacterial substances (Banhos et al., 2014; Pinheiro et al., 2017).

Antimicrobial activity is frequently detected among species of the genera *Fusarium* and *Phomopsis* (Radić and Štrukelj, 2012), as confirmed in this study.

Conclusion

This study demonstrated the diversity of endophytic fungi in the medicinal species *U. tomentosa* as the first report of endophytic studies for this plant. Crude extracts prepared from endophytic fungi isolated from leaves and stems demonstrated *in vitro* antibacterial activity against gram-positive and gram-negative bacteria.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

Akhtar N, Miller MJ, Haqqi TM (2011). Effect of a Herbal-Leucine mix on the IL-1β-induced cartilage degradation and inflammatory gene expression in human chondrocytes. BMC Complementary and Alternative Medicine, 11:66.

Azevedo JL, Araújo WL, Lacava PT, Marcon J, Lima AOS, Sobral JK, Pizzirani-Kleiner AA (2010). Meios de cultura utilizados para o estudo de microrganismos. In: Pizzirani-Kleiner AA et al. (eds) Guia prático:

isolamento e caracterização de microrganismos endofíticos. CALO, Piracicaba, P 167.
Azevedo JL, Maccheroni Jr W, Pereira JO, Araújo WL (2000). Endophytic microorganisms: a review on insect control and recent advances on tropical plants. Electronic Journal of Biotechnology, 3(1):15-16.
Banhos EF, Souza AQL, Andrade JC, Souza ADL, Koolen HHF, Albuquerque PM (2014). Endophytic fungi from *Myrcia guianensis* at the Brazilian Amazon: distribution and bioactivity. Brazilian Journal of Microbiology, 45:153-161.
Barnett HL, Hunter BB (1998). Illustrated Genera of Imperfect Fungi. 4 edn. Macmillan, New York. Available at: http://www.scirp.org/(S(lz5mqp453edsnp55rrgjct55))/reference/ReferencesPapers.aspx?ReferenceID=1114517
Bezerra J, Nascimento C, Barbosa R, Silva D, Svedese V, Silva-Nogueira E, Gomes B, Paiva L, Souza-Motta C (2015). Endophytic fungi from medicinal plant *Bauhinia forficata*: Diversity and biotechnological potential. Brazilian Journal of Microbiology, 46(1):49-57.
Campos FF, Junior S, Policarpo A, Romanha AJ, Araújo MS, Siqueira EP, Resende JM, Alves T, Martins-Filho OA, Santos VL, Rosa CA (2015). Bioactive endophytic fungi isolated from *Caesalpinia echinata* Lam.(Brazilwood) and identification of beauvericin as a trypanocidal metabolite from Fusarium sp. Memórias do Instituto Oswaldo Cruz, 110(1):65-74.
Dietrich F, Kaiser S, Rockenbach L, Figueiró F, Bergamin LS, da Cunha FM, Morrone FB, Ortega GG, Battastini AMO (2014). Quinovic acid glycosides purified fraction from Uncaria tomentosa induces cell death by apoptosis in the T24 human bladder cancer cell line. Food and Chemical Toxicology, 67:222-229.
Dreifuss AA, Bastos-Pereira AL, Fabossi IA, dos Reis Lívero FA, Stolf AM, de Souza CEA, de Oliveira Gomes L, Constantin RP, Furman AEF, Strapasson RLB (2013). Uncaria tomentosa exerts extensive anti-neoplastic effects against the Walker-256 tumour by modulating oxidative stress and not by alkaloid activity. PLoS One, 8(2):e54618.
Ferreira MC, Vieira MDA, Zani CL, Alves TMD, Sales PA, Murta SMF, Romanha AJ, Gil L, Amanda GDC, Zilli JE, Vital MJS, Rosa CA, Rosa LH (2015). Molecular phylogeny, diversity, symbiosis and discover of bioactive compounds of endophytic fungi associated with the medicinal Amazonian plant *Carapa guianensis* Aublet (Meliaceae). Biochemical Systematics and Ecology, 59:36-44.
Hamayun M, Khan SA, Khan AL, Rehman G, Kim YH, Iqbal I, Hussain J, Sohn EY, Lee IJ (2010). Gibberellin production and plant growth promotion from pure cultures of Cladosporium sp. MH-6 isolated from cucumber (*Cucumis sativus* L.). Mycologia, 102(5):989-995.
Hilarino MPA, Oki Y, Rodrigues L, Santos JC, Corrêa Junior A, Fernandes GW, Rosa CA (2011). Distribution of the endophytic fungi community in leaves of *Bauhinia brevipes* (Fabaceae). Acta Botanica Brasilica, 25(4):815-821.
Huang WY, Cai YZ, Xing J, Corke H, Sun M (2007). A potential antioxidant resource: endophytic fungi from medicinal plants. Economic Botany, 61(1):14-30.
Jouda J-B, Kusari S, Lamshöft M, Talontsi FM, Meli CD, Wandji J, Spiteller M (2014). Penialidins A–C with strong antibacterial activities from Penicillium sp., an endophytic fungus harboring leaves of Garcinia nobilis. Fitoterapia, 98:209-214.
Katoch M, Salgotra A, Singh G (2014). Endophytic fungi found in association with *Bacopa monnieri* as potential producers of industrial enzymes and antimicrobial bioactive compounds. Brazilian Archives of Biology and Technology, 57(5):714-722.
Keplinger K, Laus G, Wurm M, Dierich MP, Teppner H (1998). Uncaria tomentosa (Willd.) DC.-ethnomedicinal use and new pharmacological, toxicological and botanical results. Journal of Ethnopharmacology, 64(1):23-34.
Khan AL, Waqas M, Kang S-M, Al-Harrasi A, Hussain J, Al-Rawahi A, Al-Khiziri S, Ullah I, Ali L, Jung H-Y (2014). Bacterial endophyte Sphingomonas sp. LK11 produces gibberellins and IAA and promotes tomato plant growth. Journal of Microbiology, 52:689-695.
Lima AM, Salem JI, Souza JVB, Cortez ACA, Carvalho CM, Chaves FCM, Veiga VF (2011). Effects of culture filtrates of endophytic fungi obtained from *Piper aduncum* L. on the growth of *Mycobacterium tuberculosis*. Electronic Journal of Biotechnology, 14(4):8-8.
Malcolm GM, Kuldau GA, Gugino BK, Jiménez-Gasco MdM (2013). Hidden host plant associations of soilborne fungal pathogens: an ecological perspective. Phytopathology, 103(6):538-544.
National Committee for Clinical Laboratory Standards (NCCLS) (2003). Performance standards for antimicrobial disk susceptibility tests. National Committee for Clinical Laboratory Standards.
Padhi S, Tayung K (2015). In vitro antimicrobial potentials of endolichenic fungi isolated from thalli of Parmelia lichen against some human pathogens. Beni-Suef University Journal of Basic and Applied Sciences, 4:299-306.
Pereira JO, Azevedo JL, Petrini O (1993). Endophytic fungi of *Stylosanthes*: a first report. Mycologia, 85:362-364.
Petrini O (1991). Fungal Endophytes of Tree Leaves. In: Andrews JH, Hirano SS (eds) Microbial ecology of leaves. 1 edn. Springer, New York. pp. 179-197.
Pinheiro EA, Pina JR, Feitosa AO, Carvalho JM, Borges FC, Marinho PS, Marinho AM (2017). Bioprospecting of antimicrobial activity of extracts of endophytic fungi from Bauhinia guianensis. Revista Argentina de microbiologia, 49(1):3-6.
Premalatha K, Kalra A (2013). Molecular phylogenetic identification of endophytic fungi isolated from resinous and healthy wood of *Aquilaria malaccensis*, a red listed and highly exploited medicinal tree. Fungal Ecology, 6(3):205-211.
Putra IP, Rahayu G, Hidayat I (2015). Impact of Domestication on the Endophytic Fungal Diversity Associated With Wild Zingiberaceae at Mount Halimun Salak National Park. HAYATI Journal of Biosciences, 22(4):157-162.
Radić N, Štrukelj B (2012). Endophytic fungi-The treasure chest of antibacterial substances. Phytomedicine, 19:1270-1284.
Sá DS, Ribeiro GE, Rufino LRA, Oliveira NdMS, Fiorini JE (2014). Atividade Antimicrobiana da Uncaria Tomentosa (Willd) DC. Revista de Ciências Farmacêuticas Básica e Aplicada 35:53-57.
Souza Leite T, Cnossen-Fassoni A, Pereira OL, Mizubuti ESG, de Araújo EF, de Queiroz MV (2013). Novel and highly diverse fungal endophytes in soybean revealed by the consortium of two different techniques. Journal of Microbiology, 51:56-69.
Stone JK, Polishook JD, White JF (2004). Endophytic fungi. Biodiversity of Fungi Elsevier Academic Press, Burlington. pp. 241-270.
Strobel G, Daisy B, Castillo U, Harper J (2004). Natural products from endophytic microorganisms. Journal of Natural Products, 67:257-268.
Supaphon P, Phongpaichit S, Rukachaisirikul V, Sakayaroj J (2013). Antimicrobial potential of endophytic fungi derived from three seagrass species: Cymodocea serrulata, Halophila ovalis and Thalassia hemprichii. PLoS One, 8(8):e72520.
Toju H, Yamamoto S, Sato H, Tanabe AS, Gilbert GS, Kadowaki K (2013). Community composition of root-associated fungi in a Quercus-dominated temperate forest:"codominance" of mycorrhizal and root-endophytic fungi. Ecology and Evolution, 3(5):1281-1293.
Wagner BL, Lewis LC (2000). Colonization of corn, Zea mays, by the entomopathogenic fungus Beauveria bassiana. Applied and Environmental Microbiology, 66(8):3468-3473.

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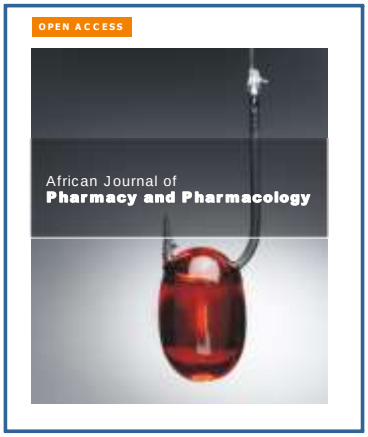

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