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Isolation and identification of endophytic microorganisms of *Campomanesia adamantium* (Cambess) O. Berg (Myrtaceae) and their metabolites

Isolamento e identificação de microorganismos endofíticos de Campomanesia adamantium (Cambess) O. Berg (Myrtaceae) e seus metabólitos

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ABSTRACT

Campomanesia adamantium (Cambess.) O. Berg (Myrtaceae) is a shrub used in folk medicine for stomach disorders and urinary tract infections. The aim of this study was to isolate and identify endophytic microorganisms from *C. adamantium* leaves and their metabolites. The botanical material composed of *C. adamantium* leaves was collected near Bela Vista, Goiás. Four actinobacteria, 10 bacteria and 60 fungi were isolated. Three fungi were able to maintain the feasibility of experimentation in the laboratory: *Cercospora zebrina*, *Agaricales* sp, and *Passarola daleae*. Crude ethyl acetate extract was obtained from strains cultured in Czapek broth, and the produced metabolites were analyzed by electrospray ionization mass spectrometry (ESI MS). 36 metabolites were identified. This work represents the first isolation of endophytic fungi and their metabolites from *C. adamantium* leaves, opening future perspectives of researches with biotechnological interest in medicine, industry or agriculture.

Keywords: Endophytic fungus, gabiroba, medicinal plant.

RESUMO

Campomanesia adamantium (Cambess.) O. Berg (Myrtaceae) é um arbusto usado na medicina popular para distúrbios do estômago e infecções do trato urinário. O objetivo deste estudo foi isolar e identificar microorganismos endofíticos das folhas de C. a*damantium* e seus metabolitos. O material botânico composto por folhas de *C. adamantium* foi coletado próximo a Bela Vista, Goiás. Foram isoladas quatro actinobactérias, 10 bactérias e 60 fungos. Três fungos foram capazes de manter a viabilidade da experimentação em laboratório: *Cercospora zebrina*, *Agaricales* sp e *Passarola daleae*. O extrato acetato de etila foi obtido a partir de linhagens cultivadas em caldo Czapek, e os metabólitos produzidos foram analisados por espectrometria de massa por ionização por electropulverização (ESI MS). 36 metabolitos foram identificados. Este trabalho representa o primeiro isolamento de fungos endofíticos e seus metabólitos das folhas de *C. adamantium*, abrindo perspectivas futuras de pesquisas com interesse biotecnológico na medicina, indústria ou agricultura.

Palavras-chave: Fungo endofítico, gabiroba, planta medicinal.

INTRODUCTION

Campomanesia adamantium (Cambess.) O. Berg (Myrtaceae) is a shrub, known in popular medicine as "guavira", "gabiroba", "guabiroba-do-campo", "guabiroba-do-cerrado", "guabiroba-lisa" and "guabiroba-branca". It occurs in the states of Goiás, Minas Gerais and Mato Grosso do Sul. The leaves are aromatic, subcoriaceous and glabrous as adults. They are popularly used for stomach disorders and urinary tract infections (LORENZI et al., 2006; ARANTES and MONTEIRO, 2002; PIVA, 2002).

The fruits of *C. adamantium* have a pleasant flavor, aroma and high vitamin contents (RAMOS et al., 2007) and are used in the production of liqueurs, juices, and candies (CARDOSO et al., 2010).

Scientific studies with the fruits of *C. adamantium* observed antimicrobial (PAVAN et al., 2009; CARDOSO et al., 2010), anti-inflammatory, antihyperalgesic, antidepressant activities (SOUZA et al., 2016) and positive hepatoprotectiveeffect on lesioned liver HepG2 cells, which may be associated with the antioxidant activity of phenolic compounds (FERNANDES et al., 2015). Pascoal et al. (2014) found antiproliferative and apoptotic activities in PC-3 human prostatecarcinoma cells from leaf and fruit extracts of *C. adamantium*. Sá et al. (2018) observed antimicrobial activity of the hexane fraction of *C. adamantium* leaves against *Staphylococcus aureus*, *S. epidermidis*, *Bacillus cereus*, *B. subtilis* and antifungal activity of the aqueous fraction and the aqueous extract concentrated in valoneic acid against *Candida albicans*, *C. krusei*, *C. parapsilosis*, *C. tropicalis*, *Cryptococcus neoformans.*

Endophytic microorganisms are those that live part or all of their life cycles inside plants without causing apparent damage to the host (DUTTA et al., 2014); receive nutrients and protection from the host plant while producing alkaloids, enzymes, antibiotics and other substances that protect and assist the plant under stress conditions (AZEVEDO et al., 2000). Endophytic fungi can be isolated from internal tissues, surface-sterilized plants (VESTERLUND et al., 2011).

Some phytotoxic natural products isolated from endophytic fungi, according to Macías-Rubalcava et al. (2014) and Schulz and Boyle (2005), can be used as prototypes of bioactive compounds particularly herbicides.

Due to the scarcity of studies of *C. adamantium* and its medicinal and industrial potential, the objective was to isolate and identify endophytic microorganisms of *C. adamantium* and their metabolites.

MATERIALS AND METHODS

Obtaining botanical material

The botanical material composed of healthy leaves with no apparent disease symptoms and pathogen-free was collected in the Cerrado region, Bela Vista, Goiás (-17,034972 S; -48,817428 W; 782 Alt.) of 20 different specimens, from February 2015 to October 2016. A voucher specie was deposited in the Herbarium of the Federal University of Goiás, number 43832.After collection, the leaves were stored in previously identified plastic bags. The transport was done in the icebox and then stored in a refrigerator at 4ºC for 24 hours.

Isolation of endophytic microorganisms

The vegetable samples were washed with soap, water and air dried on absorbent paper. After drying the leaves were weighed (1.0g), disinfected with 70% ethanol for 1 minute; 2% sodium hypochlorite for 4 minutes; washed in sterile distilled water three times; ultrasonic bath 40 kHz, for 10 minutes; washed with sterile distilled water three times; 70% ethanol for 30 seconds, followed by a final wash with sterilized distilled water twice (ARAÚJO et al., 2002).

The control of the plant surface disinfection process was carried out by inoculating three 1.0 mL aliquots of the last sample wash water into tubes containing nutrient broth or BHI (Brain Heart Infusion) broth and incubating at 30 ° C for 72 hours. The absence of turbidity in the culture medium was considered a positive response to the efficiency of the disinfection process. For confirmation, tube samples were inoculated into Petri dishes with Nutrient Agar and incubated at 30ºC for 48 hours. Non-colony development was indicative of disinfection efficiency. For the isolation of the endophytic microorganisms, the fragmentation technique was used: the previously disinfected samples were cut in 21 fragments of 1 cm² with the aid of a metal clamp, a scalpel, and a stainless steel scissors, both previously sterilized materials. The culture media: Nutrient agar (NA), TSA medium (Tryptone Soy Agar), King's Medium and Sabouraud Dextrose Agar (ASD) were used for isolation. Samples were individually inoculated into Petri dishes containing the above media. In this initial stage of isolation, two plates of each culture medium were used. Plates with the previously disinfected plant fragments were incubated at 30ºC for 15 days. After growth, the microorganisms were isolated and purified by depletion in the respective solid media. This procedure was repeated about five times, aiming to obtain pure colonies. After purification, the microorganisms were maintained in NA and stored in a refrigerator at 4°C. The isolates were preserved in 50% glycerol and stored in a freezer at -20°C.

Molecular identification of isolated fungi

DNA extraction, amplification of the 18S coding region by Polymerase Chain Reaction (PCR) and sequencing were performed by the company BPI Genotyping®, from samples in culture medium on sabouraud dextrose agar medium.

The sequences were analyzed in the CodonCode Aligner® software of CodonCode Corporation and compared to the GenBank NCBI database (http://blast.ncbi.nlm.nih.gov/Blast.cgi). For better identity and similarity patterns, the identification followed the methodology of Carvalho-Netto (2008). A phylogenetic tree was constructed with isolated endophytic fungi samples and the best fungi classified in the NCBI database for sequence comparison. The sequences were aligned by CLUSTAL W, and the tree constructed by the Neighbor-Joining method, Jukes-Cantor model with 1000 repetitions bootstrap in the Mega7® program. 18S DNA sequences were deposited in GenBank NCBI. Evolutionary analyzes (phylogeny) were performed in the MEGA7 program.

Cultivation in liquid medium of isolated endophytic microorganisms for the production of metabolites

After isolation and purification of *C. adamantium* endophytic fungi, CaF12, F12 and F20 strains were cultured in Czapek broth for 21 days at 30 ° C with a rotation of 120 rpm. Then the culture medium was filtered and the crude extract obtained was separated by liquid-liquid extraction with ethyl acetate and subsequent evaporation at room temperature

Analysis of Crude Extract by Electrospray Ionization Mass Spectrometry (ESI / MS)

To evaluate the positive mode of the ESI / MS, 1 ml of formic acid was added. Subsequently, the crude ethyl acetate extracts of the isolated endophytic fungi were diluted, filtered through a 0.45 mm membrane. Each sample was inserted directly into the apparatus by an injection pump with a continuous flow of 30 μL / min. The total acquisition time was 1 minute. The spectra obtained by ESI were acquired in positive and negative mode in a mass spectrometer (MS) and operating conditions: capillary voltage 3.0 kV; source temperature 80 ° C; desolvation temperature 80 ° C and a voltage cone equal to 35 V. The spectrum was obtained in the feed mass

ratio (m / z) 50-1200. Nitrogen was used as the drag gas (280°C). The samples were packed in amber glass containers, kept under refrigeration at approximately 4°C, and 50 mL of the samples were added to 950 mL of methanol. For positive mode evaluation, 1 mL of formic acid was added and for the negative mode, ammonium hydroxide was added. Subsequently, the diluted samples were filtered in a membrane of 0.45 mm. The exact mass of the peaks was calculated, and then a comparison was made with already identified secondary metabolites produced by endophytic microorganisms, through the literature consulting the SciFinder® database, in 2502 articles on microorganisms endophytic and 2727 secondary metabolites.

RESULTS

Isolation of endophytic microorganisms

In a first battery, 3 actinobacteria, 4 bacteria and 28 fungal strains of the *C. adamantium* leaves were isolated and morphologically identified. In a second battery, 1 actinobacterium, 6 bacteria and 6 fungi of *C. adamantium* leaves were isolated.

In a third isolation battery, two fungi were isolated. One with the feathery appearance of green color in the initial stage of growth and white in the stage of spore production, which produced volatile substances observed by the agglutination of droplets in the lid of the petri dish. Another fungus with dark purple coloration that secreted the substance of the same coloration on the agar. Both fungi isolated in the third step were and were cultured in the starch-casein agar medium. In a fourth isolation battery, 24 fungi were isolated. Due to the difficulty of growing the fungi in the laboratory condition, only three strains remained alive and in conditions to perform the experiments: CaF12, which produced a purple color substance diffused in the agar, F12 which produced volatile substances and F20 (Figure1). No bacteria or actinobacteria survived under the conditions used, probably because they were not genetically adapted.

A B C

Figure 1. Endophytic fungi strains isolated from *C. adamantium* leaves**. A-**CaF12 **B-**F12**. C-**F20

Production of substances in the liquid medium

7mg of crude extract was obtained in ethyl acetate from strain CaF12, 5.9mg from strain F20 and 8.7mg from strain F12. The extract CaF12 showed an intense red color, the extract F20 has yellowish coloration and the extract F12 white coloration.

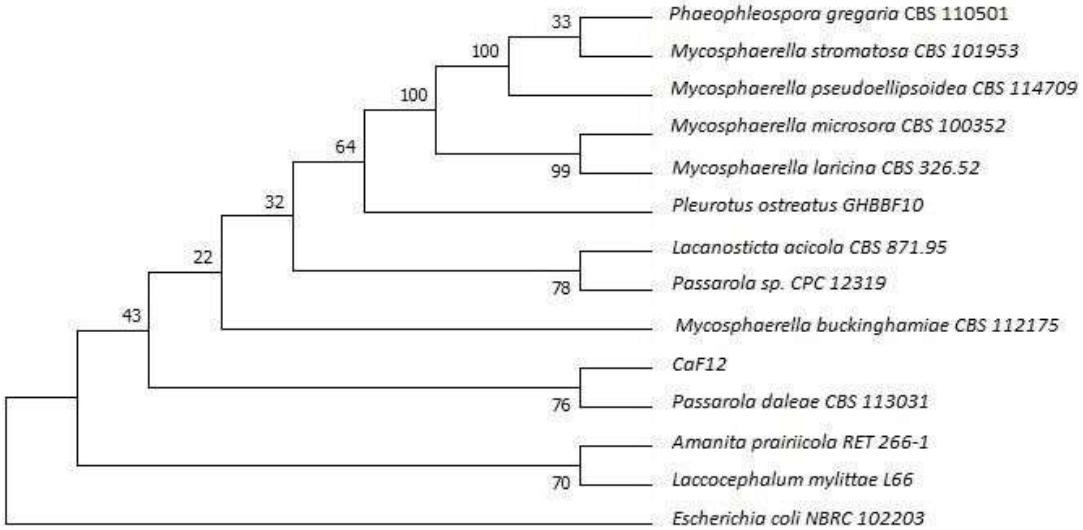
The extracts were analyzed by a mass spectrometer to identify the substances produced by the strains.

Identification of endophytic fungi

After DNA sequencing, sequence alignment and phylogenetic tree construction, strain F20 were identified as *Cercospora zebrina*, F12 as *Agaricales* sp and CafF12 as *Passarola daleae*. The ideal phylogenetic tree for each microorganism is shown in Figures 2, 3 and 4; using the best- ranked sample data from the NCBI GenBank

database for comparison. The percentage of replicate trees in which the associated rates grouped in the bootstrap test (1000 replicates) are shown next to the branches.

The evolutionary distances were calculated using the Maximum Probability Method and are in the units



of the number of base substitutions per site. The analyzes involved 7 nucleotide sequences for the fungus *C. zebrina*,7 nucleotide sequences for the fungus *Agaricales* sp and 5 nucleotide sequences for the fungus *Passarola daleae*. All positions with gaps and missing data were eliminated.

Figure 2. Phylogenetic tree of the isolated strain CaF122

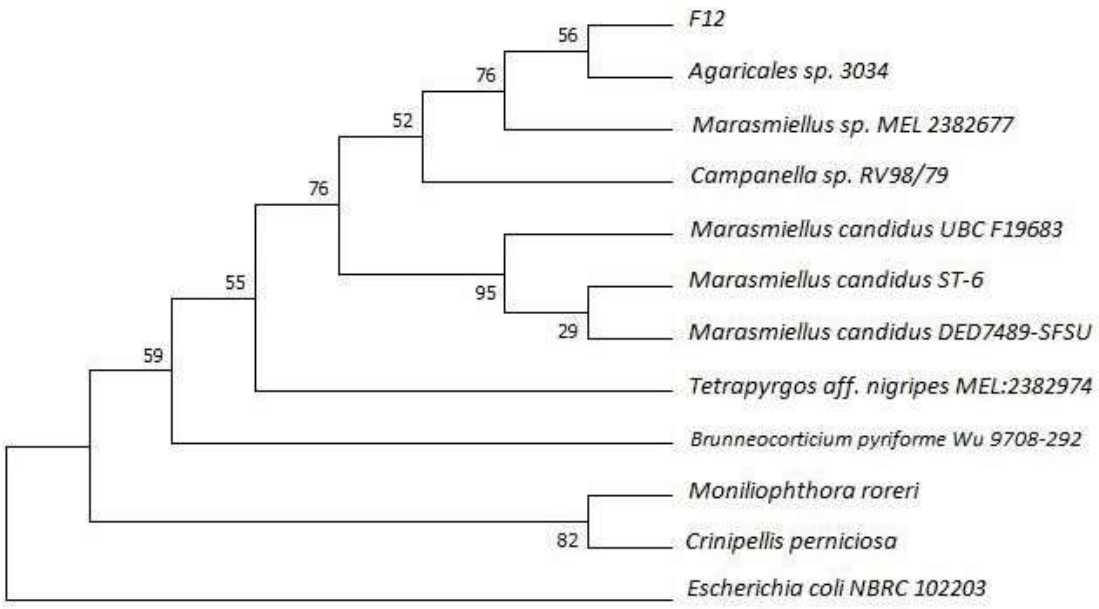


Figure 3. Phylogenetic tree of the isolated strain F12.

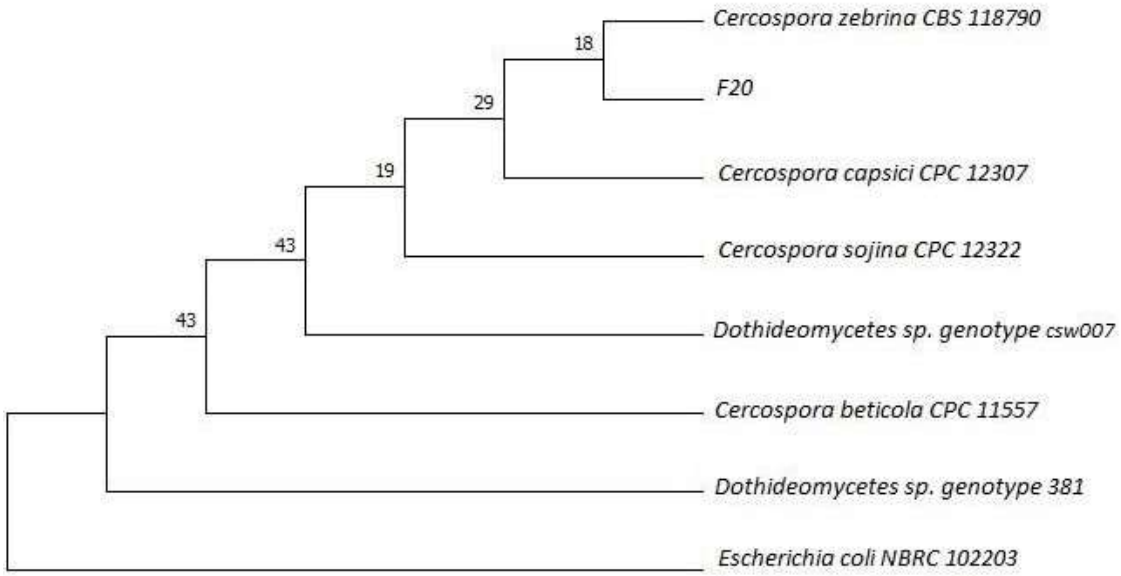
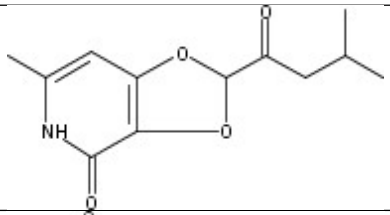
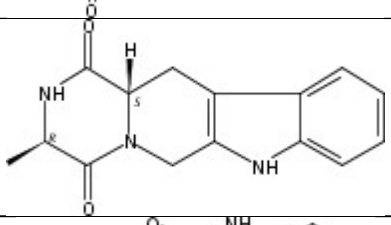
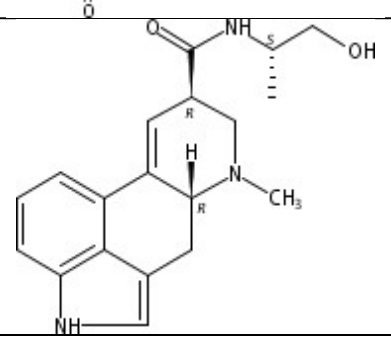


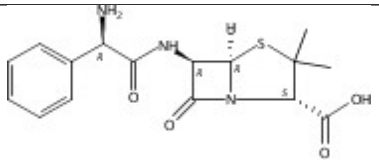
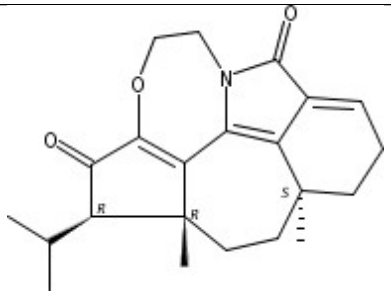
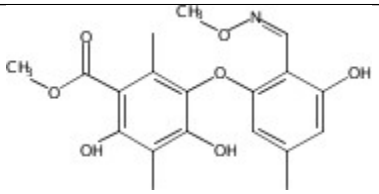
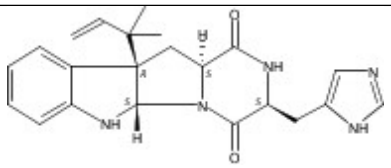
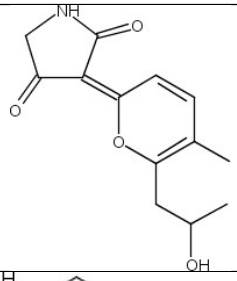
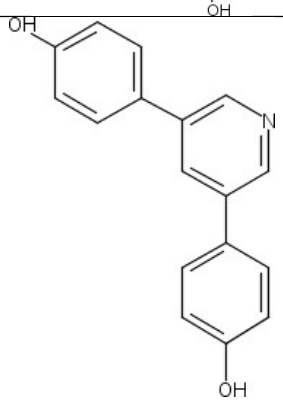
Figure 4. Phylogenetic tree of F20 isolated strain.

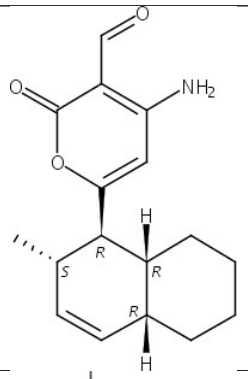
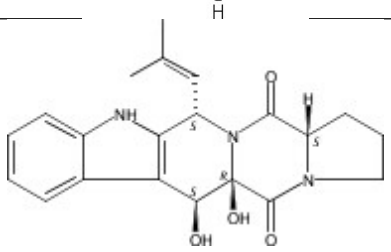
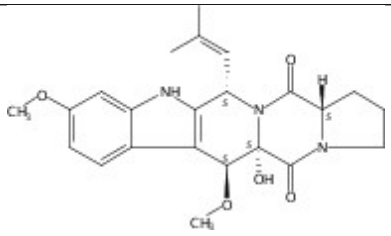
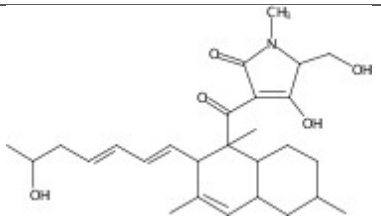
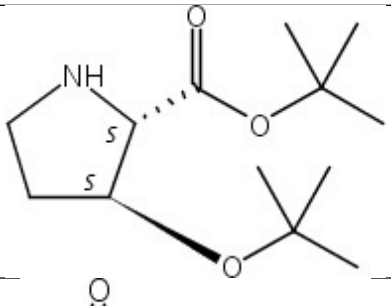
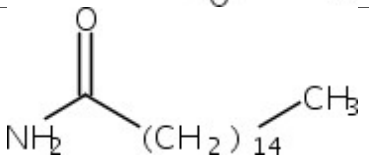
Analysis of crude extract by Electrospray Ionization Mass Spectrometry (ESI / MS)

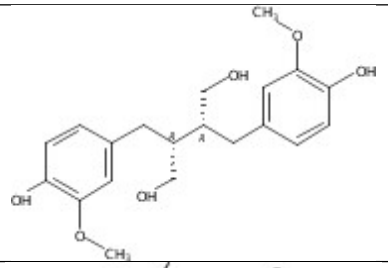
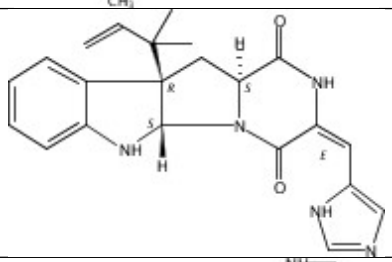
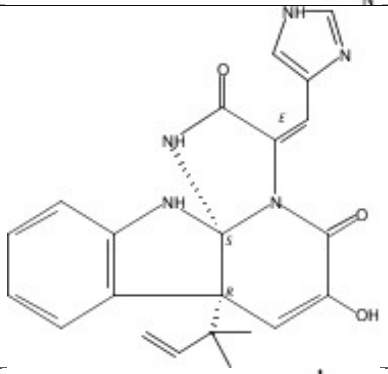
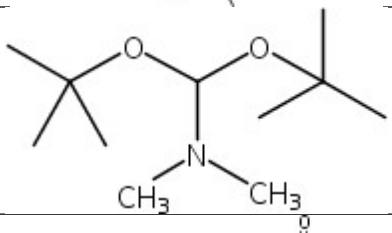
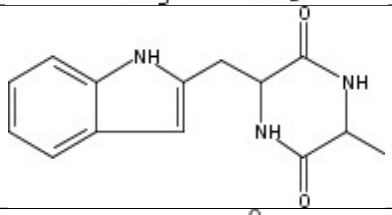
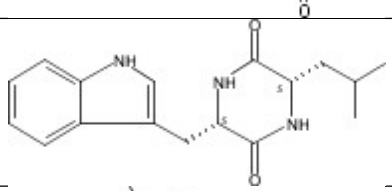
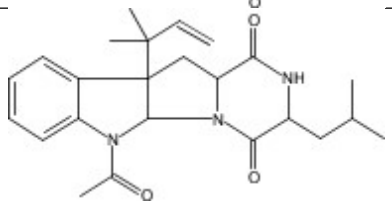
Through the ESI / MS, 36 metabolites were identified (Table 1): 13 of *P. daleae* (1-13); 15 of *C. zebrina* (**3,5,6,11,13,14-23**); 20 *Agaricales* sp (**3,4,5,13,18,20,23 24-36**) (Table 1).

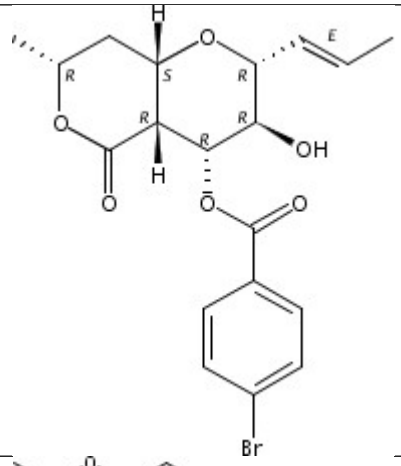
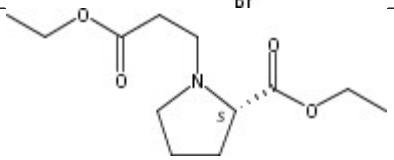
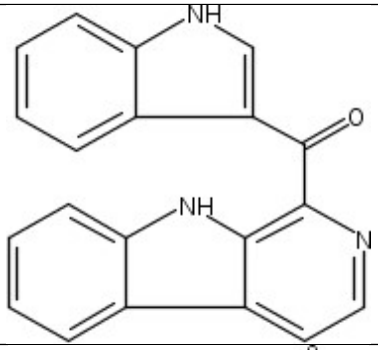
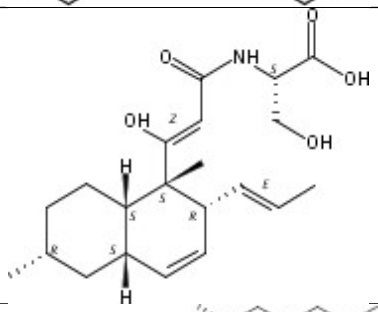
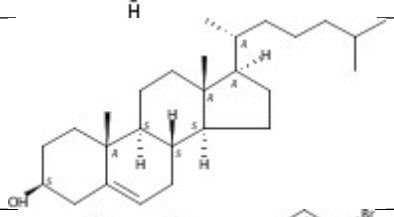
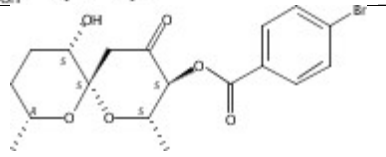
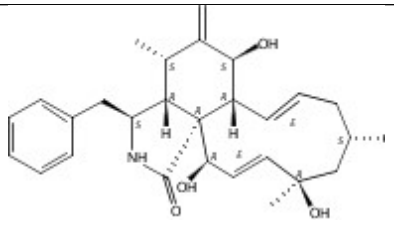
Table 1: Metabolites obtained by ESI / MS analysis and suggested structural formulas for *P. daleae* (**1-13**), *C. zebrina* (**3**,**5**,**6**,**11**,**13**,**14-23**) and *Agaricales* sp (**3**,**4**,**5**, **13**,**18**,**20**,**23**, **24-36**).

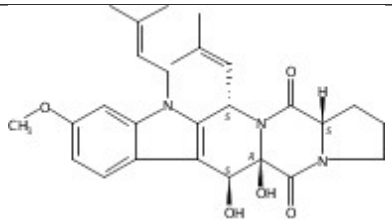
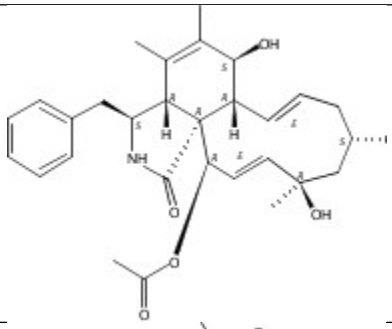
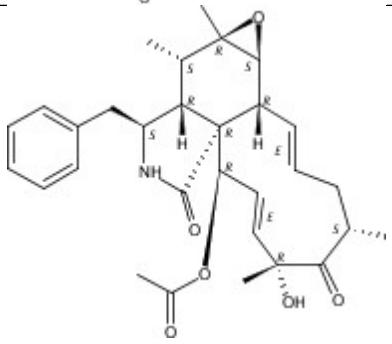
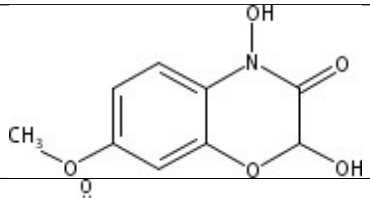
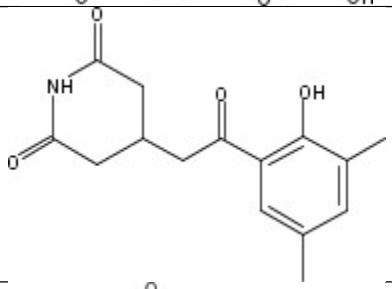
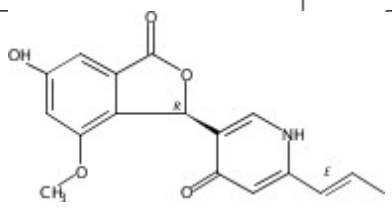
Nº	Molecular Formula / Molecular Weight	Names	Suggested structural formulas
1	**C12 H15 N O4** 237.25 (LOESGEN et al., 2011)	Dihidroflavipucine 1,3-Dioxolo[4,5-c]pyridin-4(5H)-one, 6- methyl-2- (3-methyl-1- oxobutyl)-	
2	**C15 H15 N3 O2** 269.30 (CHEN et al., 2008).	Pyrazino[1',2':1,6] pyrido[3,4-*b*]indole-1, 4-dione, 2,3,6,7,12, 12a-hexahydro-3- methyl-, (3*R*,12a*S*)-	
3	**C19 H23 N3 O2** 325.40 (JARMUSCH et al., 2016)	Ergobasina Ergoline-8- carboxamide, 9,10-didehydro-N-[(1S)-2-hydroxy-1- methylethyl]-6-methyl-, (8b)-	

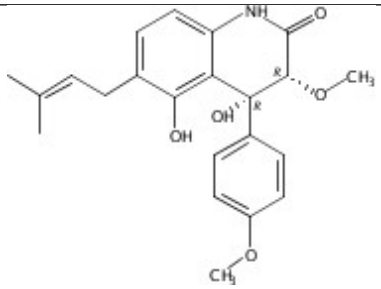
4	**C16 H19 N3 O4 S** 349.40	Ampicilina 4-Thia-1- azabicyclo[3.2.0]hepta ne-2-carboxylic acid, 6-[[(2R)-2-amino- 2-phenylacetyl]amino]- 3,3-dimethyl-7-oxo-, (2S,5R,6R)-	
5	**C22 H27 N O3** 353.45 (BRADY et al., 2001)	Guanacastepene D 1H-9-Oxa-11a-azacyclopent[ef]inden o[2,1,7-kla]heptalene-1,8(3H)- dione, 4,4a,5,6,6a,7,10,11-octahydro-4a,6a- dimethyl-7-(1-methylethyl)-, (4aS,6aR,7R)- (9CI)	
6	**C19 H21 N O7** 375.37 (KROHN et al., 2012)	Derivado da Phomosina A Benzoic acid, 2,4- dihydroxy-5-[3-hydroxy-2- [(methoxyimino) methyl]-5-methylphenoxy]-3,6- dimethyl-, methyl ester	
7	**C22 H25 N5 O2** 391.47 (TATA et al., 2015)	Roquefortine D 2*H*-Pyrazino[1',2':1,5] pyrrolo[2,3-*b*]indole-1, 4(3*H*,5a*H*)-dione, 10b- (1,1-dimethyl-2- propen-1-yl)-6,10b,11, 11a-tetrahydro-3-(1*H*- imidazol-5-ylmethyl)-, (3*S*,5a*S*,10b*R*,11a*S*)-	
8	**C13 H15 N O4** 249.26 (KUHNERT et al., 2014)	Hypoxyvermelhotin B O-Demethylhypoxyvermelhot in C 2,4-Pyrrolidinedione, 3-[6-(2-hydroxypropyl)-5- methyl-2H-pyran-2-ylidene]-	
9	**C17 H13 N O2** 263.29	Phenol, 4,4'-(3,5-pyridinediyl)bis-	

10	**C17 H21 N O3** 287.35 (WANG, 2014)	Solanapyrone G 2H-Pyran-3-carboxaldehyde, 4- amino-6-[(1R,2S,4aR,8aR)-1,2,4a,5,6,7,8,8a-octahydro-2-methyl-1-naphthalenyl]-2-oxo-	
11	**C21 H23 N3 O4** 381.42 (ZHANG et al., 2017)	Cyclotryprostatin C 5H,14HPyrrolo[1",2":4',5']pyrazino[1',2':1,6]pyrido[3,4-b]indole-5,14-dione, 1,2,3,5a,6,11,12,14aoctahydro- 5a,6-dihydroxy-12-(2-methyl-1- propen-1-yl)-, (5aR,6S,12S,14aS)-	
12	**C23 H27 N3 O5** 425.48 (ZHANG et al., 2017)	Cyclotryprostatin B 5H,14HPyrrolo[1",2":4',5']pyrazino[1',2':1,6]pyrido[3,4-b]indole-5,14-dione, 1,2,3,5a,6,11,12,14aoctahydro- 5a-hydroxy-6,9-dimethoxy-12-(2- methyl-1-propen-1-yl)-, (5aS,6S,12S,14aS)-	
13	**C27 H39 N O5**	2H-Pyrrol-2-one, 1,5-dihydro-4-hydroxy-5-(hydroxymethyl)-1-methyl-3-[[1,2,4a,5,6,7,8,8aoctahydro-2-(6-hydroxy-1,3-heptadien-1-yl)-1,3,6-trimethyl-1-naphthalenyl]carbonyl]-	
14	**C13 H25 N O3** 243.34 (FAULKNER et al., 2006)	L-Proline, 3-(1,1-dimethylethoxy)-, 1,1- dimethylethyl ester, (3S)-	
15	**C16 H33 N O** 255.44 (PREMJANU and JAYNTHY, 2014)	n- Hexadecanamida Hexadecanamide	

16	**C20 H26 O6** 362.42 (ARNEAUD AND PORTER, 2015)	Secoisolarisiresinol 1,4-Butanediol, 2,3-bis[(4- hydroxy-3-methoxyphenyl)methyl]-, (2R,3R)-	
17	**C22 H23 N5 O2** 389.45 (MADY et al., 2016)	Roquefortine C 2H-Pyrazino[1',2':1,5]pyrrolo[2,3-b]indole-1,4(3H,5aH)-dione, 10b-(1,1-dimethyl-2-propen- 1-yl)-6,10b,11,11a-tetrahydro-3-(1H-imidazol-5-ylmethylene)-, (3E,5aS,10bR,11aS)-	
18	**C22 H21 N5 O3** 403.43 (TATA et al., 2015)	Glandicoline A 1H,5H-Imidazo[1',2':1,2]pyrido[2,3-b]indole-2,5(3H)-dione, 7a-(1,1-dimethyl-2-propenyl)-7a,12-dihydro-6-hydroxy-3- (1H-imidazol-4-ylmethylene)-, (3E,7aR,12aS)- (9CI)	
19	**C11 H25 N O2** 203.32 (FAULKNER et al., 2006)	Di(ter- butoxy)(dimethylamino)methane Methanamine, 1,1-bis(1,1-dimethylethoxy)-N,Ndimethyl-	
20	**C14 H15 N3 O2** 257.29	2,5-Piperazinedione, 3-(1H-indol-2-ylmethyl)-6-methyl-	
21	**C17 H21 N3 O2** 299.37	2,5-Piperazinedione, 3-(1H-indol-3-ylmethyl)-6-(2-methylpropyl)-, (3S,6S)-	
22	**C24 H31 N3 O3** 409.52	2H-Pyrazino[1',2':1,5]pyrrolo[2,3-b]indole-1,4(3H,5aH)-dione, 6-acetyl-10b- (1,1-dimethyl-2-propen-1-yl)-6,10b,11,11a- tetrahydro-3-(2- methylpropyl)-, stereoisomer	

23	**C19 H21 Br O6** 425.27 (KROHN et al., 2008)	Dinomasone B 4-(p- bromobenzoate) Benzoic acid, 4-bromo-, (2R,3R,4R,4aR,7R,8aS)-hexahydro-3-hydroxy-7-methyl-5-oxo-2-(1E)-1-propen-1- yl-2H,5Hpyrano[4,3-b]pyran-4-yl ester	
24	**C12 H21 N O4** 243.30 (FAULKNER et al., 2006)	1-Pyrrolidinepropio nic acid, 2- carboxy-, diethyl ester, (-)- (8CI) 1-Pyrrolidinepropanoic acid, 2-(ethoxycarbonyl)-, ethyl ester, (2S)-	
25	**C20 H13 N3 O** 311.34	Pityriacitrina Methanone, 1H-indol-3-yl-9H-pyrido[3,4- b]indol-1-yl-	
26	**C21 H31 N O5** 377.47 (CHEIKH-ALI, 2015)	L-Serine, N-[(2Z)-3-hydroxy-3-[(1S,2R,4aS,6R,8aS)-1,2,4a,5,6,7,8,8aoctahydro-1,6-dimethyl-2-(1E)-1-propen-1-yl-1-naphthalenyl]-1-oxo-2-propen-1-yl]-	
27	**C27 H46 O** 386.65	Colesterol Cholest-5-en-3-ol (3b)-	
28	**C18 H21 Br O6** 413.26 (KROHN et al., 2008)	Dinemasone A 3- (p- bromobenzoate) Benzoic acid, 4-bromo-, (2S,3S,6S,8R,11S)-11-hydroxy-2,8-dimethyl-4-oxo-1,7-dioxaspiro[5.5]undec-3-yl ester	
29	**C28 H37 N O4** 451.60 (CHANG et al., 2018)	Cytochalasin J 1H-Cycloundec[d]isoindol-1-one, 2,3,3a,4,5,6,6a,9,10,11,12,15-dodecahydro-6,12,15-trihydroxy-4,10,12-trimethyl-5-methylene-3-(phenylmethyl)-, (3S,3aR,4S,6S,6aR,7E,10S,12R,13E,15R,15aR)-	

30	**C27 H33 N3 O5** 479.57 (ZHANG et al., 2017)	Fumitremorgin B 5H,14HPyrrolo[1",2":4',5']pyrazino[1',2':1,6]pyrido[3,4-b]indole-5,14-dione, 1,2,3,5a,6,11,12,14aoctahydro-5a,6-dihydroxy-9-methoxy-11-(3-methyl-2-buten-1-yl)-12-(2-methyl-1-propen-1- yl)-, (5aR,6S,12S,14aS)-	
31	**C30 H39N O5** 493.63	1H-Cycloundec[d]isoindol-1-one, 15-(acetyloxy)- 2,3,3a,6,6a,9,10,11,12,15-decahydro-6,12- dihydroxy-4,5,10,12-tetramethyl-3-(phenylmethyl)-, (3S,3aR,6S,6aR,7E,10S,12R,13E,15R,15aR)-	
32	**C30 H37 N O6** 507.62 (WEI and XU, 2015).	Cytochalasin Q 3H- cloundec[d]oxireno[f]isoindole-5,10(4H,11H)-dione, 9-(acetyloxy)-6,9,12,12a,13,13a,14a,14b-octahydro-6-hydroxy-4,6,13,13a-tetramethyl-12-(phenylmethyl)-, (1E,4S,6R,7E,9R,9aR,12S,12aR,13S,13aR,14aS,14bR)-	
33	**C9 H9 N O5** 211.17	2H-1,4-Benzoxazin-3(4H)-one, 2,4-dihydroxy-7- methoxy-	
34	**C15 H17 N O4** 275.30 (WARDECKI et al., 2015)	Actiphenol 2,6-Piperidinedione, 4-[2-(2- hydroxy-3,5- dimethylphenyl)-2-oxoethyl]-	
35	**C17 H15 N O5** 313.30 (GE et al., 2008)	Penicidone B 4(1H)-Pyridinone, 5-[(1R)-1,3- dihydro-5- hydroxy-7-methoxy-3-oxo-1-isobenzofuranyl]-2- (1E)-1-propen-1-yl-	

36	**C22 H25 N O5** 383.44 WUBSHET et al., 2013	Peniprequinolone 2(1H)-Quinolinone, 3,4-dihydro- 4,5-dihydroxy-3- methoxy-4-(4-methoxyphenyl)- 6-(3-methyl-2- buten-1-yl)-, (3R,4R)-	

DISCUSSION

Analysis of crude extract by Electrospray Ionization Mass Spectrometry (ESI / MS)

The metabolite **1** known as dihydroflavipucine was also found in the endophytic fungus *Phoma* sp., isolated from the plant *Salsola oppositifolia* (Amaranthaceae) and showed activity against *Phytophthora infestans* (LOESGEN et al., 2011).

The metabolite **2** has been identified in an endophytic fungus S26 of the plant *Cephalotaxus hainanensis* (Cephalotaxaceae) (CHEN et al., 2008). Metabolite **3** also known as Ergobasin have described by Jarmusch *et al*. (2016) in the fungus *Epichloe* spp., isolated from the *Achnatherum robustum* (Poaceae) plant. The metabolite **5** (Guanacastepten D) was isolated from the fungus CR115 and showed activity against *Staphylococcus aureus* and *Enterococcus faecalis* (BRADY et al., 2001). The metabolite **6** is a derivative of Phomosin A, and was identified by Krohn et al. (2012) in the fungus *Phomopsis* sp., and showed antimicrobial activity against *Escherichia coli* and *Bacillus megaterium*. The metabolite **7** (Roquefortine D) has already been identified in the endophytic fungi of the cacao tree *Thichoderma harzianum* and *Moniliphthora roreri* (TATA et al., 2015).

Compound **8** (O-Hypoxyvermelhotin B) was found in the fungus *Hypoxylon lechatii* sp. and showed moderate cytotoxic activity against L-929 fibroblast cells, in addition to weak activity against *Mucor hiemalis* (KUHNERT et al., 2014). Leyete-Lugo et al. (2012) found compounds of the same vermelhorin chemical family in the endophytic fungus MEXU 26343 isolated from the plant *Hintonia latiflora* (Rubiaceae).

According to Wang et al. (2014), compound **10** (Solanapyrone G) was isolated from the endophytic fungus *Alternaria tenuissima* sp-07 from a Chinese medicinal plant *Salvia przewalskii* (Lamiaceae), and showed strong antimicrobial activity against *C. perfringens* and *B. megaterium*.

According to Zhang et al. (2017), compound **11** (Cyclotryprostatin C), and compound **12** (Cyclotryprostatin B) were isolated from the endophytic fungus *Aspergillus fumigatus* from the plant *Astragalus membranaceus* (Fabaceae) and showed potent antimicrobial activity (MIC 1-32 mcg/mL) against *B. subtilis*, *S. aureus, Escherichia coli*, *Pseudomonas aeruginosa*, *C. albicans*, *Fusarium solani* and *Penicillium chrysogenum*. According to Faulkner et al. (2006), compounds **14**, **19** and **24** were found in endophytic fungi *Neotyphodium uncinatum*, and exert protective activity against herbivory in cold season grasses.

Premjanu and Jaynthy (2014) identified compound **15** (Hexadecanamide) as a metabolite of the endophytic fungus *Colletotrichum gloeosporioides* isolated from the plant *Lannea corammendalica* (Anacardiaceae), and found that it had antimicrobial activity against *S. aureus*.

Arneaud and Porter (2015) identified compound **16** (Secoisolarisiresinol), present in the endophytic fungus *Phialocephala podophylli* isolated from the plant *Podophyllum peltatum* (Berberidaceae) popularly known as the American Mandrake. This compound is a precursor of podophyllotoxin, an important precursor of known chemotherapeutic compounds.

According to Mady et al. (2016), compound **17** (Roquefortine C) is a metabolite of the endophytic fungus *Penicillium chrysogenum*, isolated from *Olea europaea* (Oleaceae), and has activity against human breast cancer cells.

Compound **18** (Glandicoline A) was found in the endophytic fungus *Moniliophthora roreri*, isolated from the cacao by Tata *et al.* (2015).

Compound **23** (Dinomasone B 4- (p-bromobenzoate)) was found as a metabolite of the fungus *Dinemasporium strigosum* isolated from the plant *Calystegia sepium* (Convolvulaceae) and showed activity against *Bacillus megaterium* and *Microbotryum violaceum* (KROHN et al., 2008). The compound **24** (1-Pyrrolidinepropionic acid, 2-carboxy-, diethyl ester, (-) - (8CI)), isolated by Faulkner et al. (2006) from the endophytic fungus *Neotyphodium uncinatum*, was found in cold season grasses and has activity against herbivory. Compound **25** (Pityriactrin) was isolated from the fungus *Exophiala* sp found in the plant *Microthlapsi perfoliatum* (Brassicaceae) (CHEIKH-ALI et al., 2015).

Compound **28** was described by Krohn et al. (2008) as Dinemasone A 3- (p-bromobenzoate), which was isolated from the fungus *Dinemasporium strigosum* found in the plant *Calystegia sepium* (Convolvulaceae) and its activity has not yet been tested. Compound **29** (Cytochalasin J) was isolated from the fungus *Phomopsis asparagi*, found in the plant *Peperomia sui* (Piperaceae) and has an activity of antagonism by adrenergic receptor (CHANG et al., 2018).

Compound **30** (Fumitremorgin B) was isolated from the endophytic fungus *Aspergillus fumigatus* found on the plant *Astragalus membranaceaeus* (Fabaceae), and has antimicrobial activity against *B. subtilis*, *S. aureus*, *E. coli*, *P. aeruginosa*, *C. albicans*, *Fusarium solani* and *Penicillium chrysogenum* (ZHANG et al., 2017).

Compound **32** (Cytochalasin Q) was found in the endophytic fungus *Xylaria* sp, isolated from the *Hypnum* sp plant (Hypnaceae) and has moderate activity against tumor cell lines (WEI and XU, 2015).

Compound **34** (Actiphenol) was found by Wardecki et al. (2015) in the fungus *Streptomyces* sp. isolated from the plant *Arnica montana* L. (Asteraceae) and has antimicrobial activity against *Candida parapsilosis* and *Fusarium verticillioides*. Compound 35 (Penicidone B) was found in the fungus *Penicillium* sp, isolated from the *Quercus variabilis* (Fagaceae) plant and has moderate cytotoxicity against cancer cells (GE et al., 2008).

Compound **36** (Peniprequinolone) was found in the fungus *Penicillium namyslowskii* isolated from the plant *Rhododendron tomentosum* (Ericaceae), and its activity has not yet been tested (WUBSHET et al., 2013).

Compound **4** was suggested by the method used as ampicillin, however; ampicillin is known to be semi-synthetic penicillin; thus it is suggested that compound **4** is another substance of the same molecular weight and not yet described in the literature.

Compound **27** (cholesterol), an important component of cell membrane structures.

No data were found in the literature on compounds **9**, **13**, **20**, **21**, **22**, **26**, **31** and **33** that relate them to endophytic fungi. In relation to these compounds, it is suggested *in silico* studies to discover new bioactive molecules.

Compound **31** is a Cytochalasin described as Cytochalasin H. Cytocalasins are secondary metabolites of fungi of the genera *Phomopsis*, Chaetominum, *Zygosporium* spp. and *Hypoxilon* sp. (ONDEYKA et al., 1992). Natarajan et al. (2000) verified the effects of Cytochalasin H on platelet function. They found that Cytochalasin H is an inhibitor of actin polymerization, an important component of the platelet cytoskeleton, showing itself to be a modulator of platelet aggregation.

Compound **33** occur mainly in rye and according Etzerodt et al. (2015) could play an important role against the accumulation of trichothecenes in wheat grain. Breeding or engineering of wheat with increased levels of benzoxazinoids could provide varieties with increased resistance against trichothecene contamination of grain and lower susceptibility to *Fusarium* head blight

Considering the activities against microorganisms of metabolites produced by endophytic fungi of *C. adamantium* leaves described above and those of leaf fractions of this species described by Sá et al. (2018), it can be inferred that antimicrobial activities may be due to both plant metabolites and endophytic fungi metabolites.

CONCLUSION

Four actinobacteria, 10 bacteria and 60 fungi from the leaves of *C. adamantium* were isolated. Of all the isolated microorganisms, only three fungi were able to maintain the feasibility of experimentation in the laboratory: *Cercospora zebrina*, *Agaricales* sp, and *Passarola daleae*. Thirty-six metabolites were identified, many of them with antimicrobial and anticancer activities described in the literature.

This work represents the first isolation of endophytic fungi and their metabolites from *C. adamantium* leaves, opening future perspectives of researches with biotechnological interest in medicine, industry or agriculture.

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