Antimicrobial activity improvement after fractionating organic extracts from Lasiodiplodia sp. Fermentation

Melhoria da atividade antimicrobiana após fracionamento de extratos orgânicos de Lasiodiplodia sp. fermentação

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ABSTRACT
Endophytes constitute a promising source of bioactive substances with therapeutic potentialities. Thereby, an endophytic fungi was isolated from Handroanthus impetiginosus leaves and classified as Lasiodiplodia sp. by DNA sequence analysis and phylogenetic inference in this study. Chlorophorm (Ld-Chlo) and ethyl acetate extracts (Ld-EAm, Ld-AE and Ld-EA⁺) obtained from fungus fermentation broth have been fractionated, whose extracts and fractions have been tested for assessing their antimicrobial activity against four Gram-positive, two Gram-negative and three yeast strains. It was observed an antimicrobial profile regarding crude extracts against Gram-positive and yeast pathogens. The major inhibition was achieved by Ld-Chlo (MIC of
12.5-25 µg.mL⁻¹) and Ld-EAm (MIC of 12.5-25 µg.mL⁻¹), both against Candida parapsilosis. The extracts were more efficient against Listeria monocytogenes and C. parapsilosis pathogens. Fractionation increased the antimicrobial activity of fractions if compared to crude extracts, probably due to a higher concentration of bioactive compounds. Gass Chromatography (GC-MS) was performed using fractions from Ld-Chlo, through which it was possible to identify four known compounds with recognized antimicrobial activity: (Z)-docos-13-enamide (1), methyl (Z)-octadec-9-enoate (2), (Z)-octadec-9-enamide (3) and dodecanamidde (4). Thence, it is suggested that the fractionation of crude extracts improve antibacterial and antifungal activities and that the identified bioactive compounds are at the helm of the antimicrobial activity presented by some fractions.

**Keywords:** antimicrobial activity, Lasiodiplodia sp., endophytic fungi, fermentation, bioactive compounds.

1 INTRODUCTION

The need for finding and developing new effective antibiotics has aroused the interests of many researchers and laboratories so as to mitigate drug resistance to pathogens [1, 2] and improve the treatment of some neglected infectious diseases [3, 4]. Not only humans, but also animals and the environment are seriously affected and threatened by multidrug-resistant microorganisms, mainly due to an excessive use of antimicrobials in animals and humans, among other important factors [5].
Endophytic fungi have become an interesting class of microorganisms, as they are usually non-pathogenic to their hosts and establish an intimate intra- and intercellular association with them for ensuring competence, survival and reproduction. Endophytes have important agricultural and industrial applications on account of assisting in plant growth, acting as biocontrol agents and secondary metabolite producers, in addition to have a mechanism against pathogenic intrusion. Therefore, they have become rich sources of novel bioactive natural products with medical importance which can be used as therapeutic agents with anticancer, antiviral, antibiotic, anti-inflammatory and antioxidant properties [6-11].

The genus *Lasiodiplodia* (*L.*) belongs to the family Botryosphaeriaceae and comprises 31 species [12]. It is quite probable that some of these species are hybrid, e.g. *L. viticola, L. missouriana, L. laeliocattletae* and *L. brasiiliense*, as suggested by Cruywagen [13]. Some *Lasiodiplodia* genera are considered pathogens for specific hosts, with great relevance on grapevine crops [14, 15]. Recently, Felix [16] demonstrated a potential pathogenicity of some *Lasiodiplodia theobromae* strains isolated from a mammalian tissue of a patient in Jamaica, and Ali [17] considered this species as an emerging threat in cocoa crops. Although there are many studies related to its phytopathogenicity, little is known about the bioactive metabolites that *Lasiodiplodia* genus is capable of producing under different fermentation conditions.

Nevertheless, antimicrobial and antiploriferative activities are important biological features found in natural products isolated from this genus. Lasiodiplodins and preussomerins isolated from *L. theobromae* have been tested so as to assess their antibacterial [18, 19, 20] and anti-trypanosomal [21] activities, and promising results have been achieved thereof. Moreover, an important therapeutic anticancer, i.e. Taxol, has also been isolated from *L. theobromae* [22], among some other compounds isolated from this species with antitumor activity against specific cancer cell lines. The known compound 1H- Dibenzo[b,e] [1, 4]dioxepin-11-one,3,8-dihydroxy-4-(methoxy- methyl)-1,6-dimethyl showed activity against mouse lymphoma cell line L5178Y [19] and several preussomerins also exhibited cytotoxic activity against adenocarcinomic human alveolar basal epithelial cells A549, breast cancer cell line MCF-7 and human liver cancer cell line HepG2 [20]. Interestingly, the lasiodiplodin 2,4-dihydroxy-6-nonylbenzoate, isolated from *Lasiodiplodia* sp., revealed a cytotoxic activity against pituitary tumor cell lines MMQ and GH3 [23].
A continuous evaluation of antibiotic properties of organic extracts and pure compounds isolated from a variety of endophytes has evoked optimal responses that justify further studies on endophytic fungi \[24, 25\]. It is believed that screening of antimicrobial compounds from endophytes is a promising way to overcome the increasing threat of drug-resistant strains of human, animal and plant pathogens. Therefore, the present work aims to show an improved antimicrobial activity of *Lasiodiplodia* sp. organic extracts after their fractionation.

2 METHODS

**Plant collection and isolation of endophytic fungi**

Samples of healthy leaves from *Handroanthus impetiginosus* (Mart. ex DC.) Mattos were collected in December, 2016, Alfenas/Minas Gerais (S21°25′16.95″, W45°57′05.15″), Brazil. The plant was registered under the number 2745 at the herbarium of the Federal University of Alfenas.

A sterilization of leaves has been carried out according to Devarajan [26]. The samples have been placed into some solutions during a given time as follows: 70% alcohol solution for 1 min, 4% sodium hypochlorite solution for 6 min and 70% alcohol for 1 min. Finally, they were washed with sterile water, which was used as sterility control. The sterilized leaves were cut into 0.5 cm x 0.5 cm fragments, placed on potato dextrose agar (PDA) Petri dishes and kept at room temperature. The colonies that grew from plant fragments were cultivated in PDA successively, until obtaining pure colonies. The isolated strains were stored in bottles cleansed with sterile water according to Castellani’s method [27].

**Endophytic fungus identification**

The endophyte was identified as *Lasiodiplodia* sp. at the Multidisciplinary Center for Chemical, Biological and Agricultural Research (CPQBA), in association with the State University of Campinas. Fungus genomic DNA extraction was in accordance with the protocol described by Raeder and Broda [28]. The Internal Transcribed Spacer (ITS) region was amplified through the Polymerase Chain Reaction (PCR) methodology using genomic DNA extracted directly from the sample as template.

The primers used in the PCR were ITS-1 and ITS-4. The amplified fragments were further purified and directly subjected to ABI3500XL Series (Applied Biosystem) automated sequencing. Partial sequences in the ITS region obtained by using different
primers have been assembled into a contig and compared to organism sequences represented on the Genbank (http://www.ncbi.nlm.nih.gov) and Centraalbureau voor Schimmelcultures, Fungal Biodiversity Center (CBS) (http://www.cbs.knaw.nl/).

Sequences were aligned using the CLUSTAL X [29] and phylogenetic analyzes were conducted using the MEGA version 4.0 [30]. Evolutionary distance matrices were calculated using Kimura’s model [31] and the phylogenetic tree from the evolutionary distances was designed by the Neighbor-Joining method [32]. Initialization values have been calculated from 1000 resamples through the MEGA 4.0 as well.

**Fungal fermentation and liquid-liquid extractions**

*Lasiodiplodia* sp. has been grown in PDA for seven days at 25ºC. Two mycelium fragments (0.5 cm discs) were cut and inoculated into 1 liter erlenmeyers containing 150 mL of Czapek broth as fermentation medium (30.0 g glucose, 2.0 g NaNO₃, 1.0 g K₂HPO₄, 0.5 g MgSO₄.7H₂O, 0.5 g KCl, 0.01 g FeSO₄.7H₂O, 1.0 g yeast extract in 1000 mL distilled water), which have been incubated in static mode at room temperature for 21 days.

After the fermentation period, the culture medium was vacuum filtered to remove mycelium. Then, the broth (4 liters) was subjected to liquid-liquid extractions using different solvents (3 extractions x 900 mL for each solvent) with increasing polarity (hexane (Ld-Hex), chloroform (Ld-Chlo) and ethyl acetate (Ld-EA)) in order to obtain crude organic extracts.

After ethyl acetate extractions, the broth pH was modified (pH = 3) using a solution of HCl (0.1112 mol.L⁻¹) and a new extraction with ethyl acetate (Ld-EA⁺) was performed (3 x 900 mL). All mycelium obtained after filtration has been macerated in ethyl acetate (200 mL) for 24 hours at room temperature and then filtered (90 mm 80 G), thus yielding another EA crude extract (Ld-EAm).

**Extracts fractionation**

All crude extracts were fractionated, except for Ld-Hex. The three EA crude extracts (Ld-EA, Ld-EA⁺ and Ld-EAm) were fractionated in a glass column filled with Sephadex LH 20 (40 cm x 2 cm) at a flow rate of 2.9 mL.min⁻¹ and using ethyl acetate:methanol (7:3, v:v) as isocratic eluent. For chloroform extract (Ld-Chlo), a pressure-free Water Sep-Pak® Vac 20cc Diol-5g cartridge was used with a solvent system (hexane, chloroform and methanol) for gradient elution. The fractions were
grouped according to their similarity after evaluation by thin layer chromatography (TLC).

**Determination of antibacterial and antifungal activity. Minimum Inhibitory Concentration (MIC) and Minimum Microbicidal Concentration (MMC) tests**

The antimicrobial activity of crude extracts, fractions and subfractions (AE3-(A-I), EA+ 2-(A-G) and EA+ 3-(A-H)) have been determined in accordance with CLSI [33, 34] standards for yeast and bacteria, but with a few modifications. The test was performed in a Muller Hinton medium (HIMEDIA, India) so as to assess activity against *Staphylococcus aureus* (ATCC 6538), *Staphylococcus epidermidis* (ATCC 12228), *Bacillus cereus* (ATCC 11778), *Listeria monocytogenes* (ATCC 4555), *Salmonella typhimurium* (ATCC 6017) and *Escherichia coli* (ATCC 25922) and in RPMI-1640 (Roswell Park Memorial Institute) medium (Sigma Aldrich) for activity against *Candida albicans* (ATCC 10231), *Candida tropicalis* (ATCC 750) and *Candida parapsilosis* (ATCC 2209). Extracts were tested against all those pathogens; however, fractions were tested only against *Listeria monocytogenes* and *Candida albicans*.

Samples were dissolved in 125 µL absolute ethanol until reaching a final concentration of 8 mg.mL^{-1} and placed in 96-well microplates under serial dilutions (400-3,125 mg.mL^{-1}). The inoculum was prepared as suspension that was standardized in a spectrophotometer at 660 nm and 75% transmittance, corresponding to 1.5 x 10^8 CFU.mL^{-1} (colony forming units per mL). Amoxicillin and streptomycin (10 - 0.078 mg.mL^{-1}) for bacterial (Gram positive and Gram negative, respectively) and fluconazole (80-0.625 mg.mL^{-1}) for yeast were also used as positive controls. In addition, culture medium and pathogens were employed as controls. The microplates have been incubated at 37 °C for 24 h. A 0.2% aqueous resazurin solution was used as developer so as to determine inoculum viability, which has been revealed by a color change from blue to pink [35]. The MIC value was determined as the lowest concentration, with no variation on the developer color. All extracts and fractions were studied in triplicate.

In order to determine MMC, a 25 µL aliquot was removed from the wells that showed no color change (for samples and antimicrobials) and placed onto the surface of a Petri dish containing Muller Hinton agar (HIMEDIA, India) for bacteria and Sabouraud agar (HIMEDIA, India) for yeast. Each plate was incubated at 37°C for 24h. Bacterial or fungal growth on the plaque indicates that the extract exerted no bactericidal/fungicidal action. Otherwise, absence of growth is an indicative of bactericidal/fungicidal action.
Gas chromatography-mass spectroscopy (CG-MS)

Fractions obtained from the chloroform crude extract were analyzed on a Gas Chromatograph (GC-2010 Gas Chromatograph, Shimadzu Corporation, Kyoto, Japan) coupled with a Mass Spectrometer (MS-QP-5050A mass spectrometer, Shimadzu Corporation, Kyoto, Japan). Capillary column specifications: Rtx® Crossbond® 5% diphenyl 95% dimethylpolysiloxan capillary column (30 m x 0.25 mm x 0.25 μm film, Restek). GC Method specifications: The injector temperature was kept at 270°C and the detector temperature was 240 °C. The column temperature was set between 80°C -290°C, as follows: 1) kept at 80°C for 2 min; 2) from 80°C to 200°C at 20 °C.min⁻¹ rate; 3) from 200°C to 280°C at 10 °C.min⁻¹ rate and kept at 280°C for 10 min and 4) from 280 °C to 290°C at 2 °C.min⁻¹ rate and kept at 290°C for 10 min. Helium was used as carrier gas at 0.6 mL.min⁻¹. Samples were prepared in CHCl₃ HPLC grade and 1.0 μL was injected at splitless mode. MS method specifications: Ionization mode: Electron Ionization (70 eV), MS interface and ion source temperatures were kept at 300°C. Mass scan range was m/z 40-500 amu. Constituents were identified by comparing their mass spectra with standard ones using NIST (National Institute of Standards and Technology, U.S. Department of Commerce) compounds.

3 RESULTS AND DISCUSSION

Obtention of extracts and fractions

After fermentation, five different crude extracts were obtained (table 1): 1) hexane (Hex); 2) chloroform (Chlo), 3) ethyl acetate (EA), 4) ethyl acetate after broth acidification (EA⁺) and 5) ethyl acetate from mycelium maceration (EAm). Masses and the number of fractions obtained from each extract are shown in Table 1.

| Extracts | Ld-Chlo (65.2 mg) | Ld-EA (68.4 mg) | Ld-EA⁺ (335.6 mg) | Ld-EAm (173.3 mg) |
|----------|-----------------|----------------|--------------------|-------------------|
| LdChlo-A | LdEA-1          | LdEA⁺-1        | LdEAm-1            |
| (8.6 mg) | (7 mg)          | (11 mg)        | (23.8 mg)          |
| LdChlo-B | LdEA-2          | LdEA⁺-2        | LdEAm-2            |
| (9.8 mg) | (4.4 mg)        | (21.8 mg)      | (7 mg)             |
| LdChlo-C | LdEA-3          | LdEA⁺-3        | LdEAm-3            |
| (7.5 mg) | (10.2 mg)       | (82.8 mg)      | (37.7 mg)          |
| LdChlo-D | LdEA-4          | LdEA⁺-4        | LdEAm-4            |
| (5.1 mg) | (5.1 mg)        | (13.3 mg)      | (18.8 mg)          |

Table 1 Masses of crude extracts and fractions obtained after fungal fermentation and chromatography
Determination of antimicrobial activity

The antimicrobial activity of crude extracts (table 2) was determined using Gram-positive pathogen strains: *Staphylococcus aureus* (ATCC 6538), *Staphylococcus epidermidis* (ATCC 12228), *Bacillus cereus* (ATCC 11778) and *Listeria monocytogenes* (ATCC). 4555); Gram-negative: *Escherichia coli* (ATCC 25922) and *Salmonella typhimurium* (ATCC 6017); and yeast: *Candida albicans* (ATCC 10231), *Candida tropicalis* (ATCC 750) and *Candida parapsilosis* (ATCC 2209). *Candida albicans* and *Listeria monocytogenes* were selected so as to evaluate the antimicrobial potential of fractions due to their high sensibility and ease of cultivation.

The degree of antimicrobial activity was based on Holetz [38], who establishes antimicrobial action as strong (<100 µg.mL\(^{-1}\)), moderate (100 - 500 µg.mL\(^{-1}\)), weak (500 - 1000 µg.mL\(^{-1}\)) and inactive (≥ 1000 µg.mL\(^{-1}\)). These criteria are very similar to that established by Kuete [39] and they are both quite restrictive, since it was a requirement for this work regarding the antimicrobial activity evaluation of crude extracts and fractions.

Ld-Chlo and Ld-EAm extracts showed optimal MIC and MMC values against Gram-positive pathogens (table 2), unlike Ld-Hex, Ld-AE and Ld-EA\(^+\) crude extracts, which showed no activity at the maximum concentrations (MIC > 400 µg.mL\(^{-1}\)). Concerning yeast tests, the extract Ld-EA, along with Ld-Chlo and Ld-EAm, showed remarkable MIC and MMC values, thus highlighting activity against *Candida parapsilosis*.

Among all extracts, Ld-Chlo extract showed the highest antimicrobial activity due to providing an inhibitory effect of up to 25-50 µg.mL\(^{-1}\) against *Listeria monocytogenes*.
and bactericidal effect for this pathogen and *Bacillus cereus*. Furthermore, MIC values for *Candida parapsilosis* reached up to 12.5-25 µg.mL⁻¹ with MMC of 100-200 µg.mL⁻¹ (Table 2).

The most susceptible pathogens were *Listeria monocytogenes* and *Candida parapsilosis* on account of showing strong inhibitory action (MIC <100 µg.mL⁻¹) and the highest bactericidal action with MMC values of 200-400 µg.mL⁻¹ and 100-200 µg.mL⁻¹, respectively. In this sense, an antimicrobial profile of crude extracts against Gram-positive pathogens and yeast is glimpsed, since the results obtained for Gram negative pathogens produced no positive responses to tested concentrations (MIC> 400 µg.mL⁻¹).

Finally, by taking into account the variety of ways to obtain the aforementioned crude extracts, such results might indicate that, during *Lasiodiplodia* sp. cultivation, a wide range of antimicrobial secondary metabolites of moderate polar nature (crude extracts obtained from ethyl acetate) and apolar (crude extracts obtained from chloroform) have been produced. On the other hand, the antimicrobial activity observed for *Lasiodiplodia* sp. extracts is in accordance with results presented by other authors. Wei [18] isolated lasiodiplodin E from *Lasiodiplodia pseudotheobromae* and tested its antibacterial activity against *Streptococcus* sp., *Bacteroides vulgates*, *Peptostreptococcus* sp. and *Veillonella parvula* clinical strains with MIC range of 0.12–0.25 µg.mL⁻¹. Umeokoli [19] also isolated a lasiodiplodin ((+)-(R)-de-O-methyl-lasiodiplodin) from *Lasiodiplodia theobroma* whose antibacterial activity (MIC of 25 µg.mL⁻¹) was tested against *Staphylococcus aureus* and *Enterococcus faecium*.

Table 2 Minimal inhibitory concentration (MIC) and minimal microbicidal concentration (MMC) of crude extracts

| Pathogens / Extracts | Ld-Hex | Ld-Chol | Ld-EA | Ld-EA⁺ | Ld-EAm | Antimicrobials |
|----------------------|--------|---------|-------|--------|--------|----------------|
| *Staphylococcus aureus* |        |         |       |        |        | 0.078 - 0.156 |
| MIC                  | >400   | 100 - 200 | >400 | >400   | >400   | 200 - 400 |
| MMC                  | >400   | >400     | >400  | >400   | >400   | 200 - 400 |
| *Staphylococcus epidermidis* |        |         |       |        |        | 0.078 - 0.156 |
| MIC                  | >400   | 100 - 200 | >400 | >400   | >400   | 200 - 400 |
| MMC                  | >400   | >400     | >400  | >400   | >400   | 200 - 400 |
| *Bacillus cereus*    |        |         |       |        |        | 2.5-5         |
| MIC                  | >400   | 100 - 200 | >400 | >400   | >400   | 200 - 400 |
| MMC                  | >400   | >400     | >400  | >400   | >400   | 200 - 400 |

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| Pathogens / Extracts | Ld-Hex | Ld-Chol | Ld-EA | Ld-EA⁺ | Ld-EAm | Antimicrobials |
|----------------------|--------|---------|-------|--------|--------|----------------|
| *Staphylococcus aureus* |        |         |       |        |        | 0.078 - 0.156 |
| MIC                  | >400   | 100 - 200 | >400 | >400   | >400   | 200 - 400 |
| MMC                  | >400   | >400     | >400  | >400   | >400   | 200 - 400 |
| *Staphylococcus epidermidis* |        |         |       |        |        | 0.078 - 0.156 |
| MIC                  | >400   | 100 - 200 | >400 | >400   | >400   | 200 - 400 |
| MMC                  | >400   | >400     | >400  | >400   | >400   | 200 - 400 |
| *Bacillus cereus*    |        |         |       |        |        | 2.5-5         |
| MIC                  | >400   | 100 - 200 | >400 | >400   | >400   | 200 - 400 |
| MMC                  | >400   | >400     | >400  | >400   | >400   | 200 - 400 |
The evaluation of antimicrobial activity for fractions (table 3) clearly showed an activity improvement by obtaining higher MIC and MMC values, when compared to that obtained for extracts against *Listeria monocytogenes* and *Candida albicans*.

Table 3 Antimicrobial activity of fractions obtained from the respective crude extracts

| Samples   | *Listeria monocytogenes* | *Candida albicans* |
|-----------|--------------------------|--------------------|
|           | MIC MMC                   | MIC MMC             |
| Ld-Chlo   | 25-50 200-400             | 100-200 >400       |
| Ld-Chlo-A | >400 >400                 | >400 >400          |
| Ld-Chlo-B | >400 >400                 | 100-200 200-400    |
| Ld-Chlo-C | 25-50 25-50               | 50-100 100-200     |
| Ld-Chlo-D | 50-100 200-400            | 200-400 200-400    |
| Ld-Chlo-E | >400 >400                 | >400 >400          |
| Ld-Chlo-F | >400 >400                 | 200-400 200-400    |
| Ld-Chlo-G | >400 >400                 | >400 >400          |
| Ld-Chlo-H | 1.562-3.125               | >400 >400          |
| Ld-EAm    | 50-100 >400               | 200-400 >400       |
| Ld-EAm-1  | >400 >400                 | 200-400 >400       |
| LdEAm-2 | >400 | >400 | >400 | >400 |
| LdEAm-3 | >400 | >400 | 25-50 | 50-100 |
| LdEAm-4 | >400 | >400 | 200-400 | >400 |
| LdEAm-5 | >400 | >400 | >400 | >400 |
| LdEAm-6 | 200-400 | >400 | >400 | >400 |
| LdEAm-7 | 12.5-25 | >400 | 25-50 | >400 |
| LdEAm-8 | 6,250-12.5 | >400 | 12.5-25 | 100-200 |
| LdEAm-9 | 100-200 | 200-400 | 200-400 | >400 |
| Ld-EA | >400 | >400 | 200-400 | >400 |
| LdEA-1 | 200-400 | >400 | >400 | >400 |
| LdEA-2 | 200-400 | >400 | >400 | >400 |
| LdEA-3 | 200-400 | >400 | 100-200 | 100-200 |
| LdEA-4 | >400 | >400 | >400 | >400 |
| LdEA-5 | 200-400 | >400 | >400 | >400 |
| LdEA-6 | 200-400 | >400 | 200-400 | 200-400 |
| LdEA-7 | 200-400 | >400 | 200-400 | 200-400 |
| LdEA-8 | >400 | >400 | >400 | >400 |
| LdEA-9 | >400 | >400 | 200-400 | 200-400 |
| LdEA-10 | 200-400 | >400 | 200-400 | 200-400 |
| Ld-EA* | >400 | >400 | 200-400 | >400 |
| LdEA*-1 | >400 | >400 | >400 | >400 |
| LdEA*-2 | 200-400 | >400 | 50-100 | 50-100 |
| LdEA*-3 | 200-400 | >400 | 50-100 | 200-400 |
| LdEA*-4 | >400 | >400 | >400 | >400 |
| LdEA*-5 | >400 | >400 | >400 | >400 |
| LdEA*-6 | >400 | >400 | >400 | >400 |
| LdEA*-7 | >400 | >400 | >400 | >400 |
| LdEA*-8 | >400 | >400 | >400 | >400 |
| LdEA*-9 | >400 | >400 | >400 | >400 |
| LdEA*-10 | >400 | >400 | >400 | >400 |
| Amoxicillin | 0.078-0.156 | 0.312-0.625 | -- | -- |
| Fluconazole | -- | -- | 0.312-0.625 | 20-40 |

MIC and MMC values are expressed in µg.mL⁻¹.

Fig. 3 and Fig. 4 represent the antimicrobial activity of fractions that showed the most potent effects if compared to crude extracts for both MIC and MMC tests. In order to graphically represent the ranges of MIC and MMC, it was established the following scale to be plotted on the Y axis: 0.781 = (0.781-1.562 µg.mL⁻¹), 1.562 = (1.562-3.125 µg.mL⁻¹), 3.125 = (3.125-6.25 µg.mL⁻¹), 6.25 = (6.25-12.5 µg.mL⁻¹), 12.5 = (12.5-25 µg.mL⁻¹), 25 = (25-50 µg.mL⁻¹), 50 = (50-100 µg.mL⁻¹), 100 = (100-200 µg.mL⁻¹), 200 = (200-400 µg.mL⁻¹) and 400 = (>400 µg.mL⁻¹).

Fig. 3a shows that LdChlo-H (MIC of 1,562-3,125 µg.mL⁻¹) exceeded the degree of inhibition when compared to Ld-Chlo (MIC of 25-50 µg.mL⁻¹) against Listeria.
monocytogenes. In addition, the bactericidal effect of the LdChlo-C fraction (MMC 25-50 µg.mL⁻¹) was greater than the MMC of crude extract Ld-Chlo (200-400 µg.mL⁻¹). LdChlo-C and Ld-Chlo had the same high MIC value (25-50 µg.mL⁻¹). Some fractions obtained from a fractionation of the crude extract Ld-EAm (MIC 50-100 µg.mL⁻¹) have also led to an increase in the degree of inhibition against Listeria monocytogenes (fig. 3b) and MIC 12.5-25 µg.mL⁻¹ for LdEAm-7 and 6.25-12.5 µg.mL⁻¹ for LdEAm-8 which showed the most potent inhibition, thus LdEAm-9 was capable of developing a bactericidal effect (MMC 200-400 µg.mL⁻¹).

LdChlo-C fraction (MIC 50-100 µg.mL⁻¹) also overcame the inhibition of Candida albicans produced by Ld-Chlo extract (MIC 100-200 µg.mL⁻¹) and had a fungicidal effect (MMC 100-200 µg.mL⁻¹) that was not observed for the crude extract. LdChlo-B, LdChlo-D and LdChlo-F also showed a fungicidal effect in a MMC range of 200-400 µg.mL⁻¹ (fig. 3c). Furthermore, LdEAm-7 (MIC 25-50 µg.mL⁻¹) and LdEAm-8 (MIC 12.5-25 µg.mL⁻¹) fractions showed stronger inhibition if compared to the crude extract Ld-EAm (MIC 200-400 µg.mL⁻¹). In this case, the LdEAm-3 fraction (MIC 25-50 µg.mL⁻¹ and MMC 50-100 µg.mL⁻¹) was the most effective against Candida albicans (Fig. 3d).

The fractioning effect was very significant for the crude extracts Ld-EA and Ld-EA+ and was particularly relevant for Ld-EA-derived fractions, due to the fact that 7 out of the 10 fractions tested produced moderate inhibition against Listeria monocytogenes.
(fig. 4a) and 5 fractions showed moderate inhibition (accompanied by a fungicidal effect) against *Candida albicans* (fig. 4c). Regarding fractions derived from the crude extract Ld-EA+, only LdEA+-2 and LdEA+-3 showed moderate inhibition (MIC 200-400 µg.mL$^{-1}$) against *Listeria monocytogenes* (fig. 4b), but these fractions showed strong growth inhibition (MIC <100 µg.mL$^{-1}$) against *Candida albicans* (fig. 4d) and both had fungistatic effect (MMC 50-100 µg.mL$^{-1}$ and MMC 200-400 µg.mL$^{-1}$ respectively).

Fig. 4 Antimicrobial activity comparison among crude extracts Ld-EA, LdEA+ and its fractions: a) and b) activity against *Listeria monocytogenes*, c) and d) activity against *Candida albicans*

Etame et al. [40, 41] stated that an increased concentration of bioactive compounds due to fractionation causes an improvement in bioactivity of fractions when compared to crude extracts. It has been evidenced for all crude extracts processed herein concerning their antimicrobial activity against *Listeria monocytogenes* and *Candida albicans*. On the other hand, Voukeng [42] reported an increase in the antimicrobial activity of some extracts due to a synergistic effect exerted among compounds, which should also be considered in this paper by taking into account that fractions also have mixtures of compounds. Apparently, there are limits so that the mixtures of compounds can have a synergistic effect, once the presence of a larger amount of compounds in crude extracts did not allow the expression of a superior antimicrobial activity if compared to fractions. In this case, the effect observed was antagonist. It is important to highlight that the authors cited above used crude extracts derived from plants, not crude extracts derived from endophytic fungi cultivation. It is known that a fermentation of these microorganisms as production systems for valuable metabolites has many large-scale
production benefits: faster growth speeds, reduced space, and ease of modification, thus expressing the biotechnological potentialities of such microorganisms [43].

The most sensitive pathogen to fractions was *Candida albicans*, which was inhibited by many fractions when compared to *Listeria monocytogenes* (table 3). This is a very important result since *Candida* species have been considered by the US National Health Safety Network as the fourth most common group of pathogens in all types of healthcare-associated infections and the second most common catheter-associated urinary tract infections [44]. Furthermore, an increased resistance to azoles has been detected when such medicines had been prophylactically used during prolonged antibiotic, steroid and chemotherapy treatments [45].

In studies on the prevalence of candidemia (hematogenous candidiasis), *Candida albicans* is considered as the most frequent agent of that infection. However, there is a consensus on an increased participation of invasive infections caused by *Candida* non-albicans (CNA). In this scenario, North America and northern European countries usually present *Candida glabrata* as the main pathogen among CNAs [46]. On the other hand, according to Colombo [47], Pfaller [48], Hinrichsen [49] and Pinhati [50] there is a predominance of *Candida parapsilosis* and *Candida tropicalis* in Latin America, including Brazil, and Asia.

In this regard, Pinhati [50] described an outbreak of candidemia caused by fluconazole-resistant *Candida parapsilosis* strains in intensive care units patients of a tertiary care hospital for the first time in Brazil. They registered a mortality rate of 42.9 %, which was particularly high for this kind of pathogen and contradicts the current consensus established by other studies that state a higher mortality rate for infections with *Candida albicans* and *Candida tropicalis* [48].

Another data of great interest has been provided by the Centers for Disease Control and Prevention (CDC) through its report on the threats of antibiotic resistance in the United States, in which resistant *Candida* species have been ranked among the Serious Threats. Special attention has been devoted to *Candida auris* which has been included among the Urgent Threat pathogens. Methicillin-resistant *Staphylococcus aureus*, also related to the present work, was another pathogen reported as a Serious Threat [51].

Obtaining new options for the treatment of infections caused by these bacteria and yeast is a current priority, and this work showed a feasible antibacterial and antifungal potential for both crude extracts and fractions. It must be explored and improved through
the purification of bioactive substances in fractions. It should be noted that the most promising results were obtained for Ld-Chlo and Ld-EAm extracts and their fractions.

**Processing the fractions obtained from Ld-Chlo through CG-MS**

Fractions of Ld-Chlo were analyzed by GC-MS. The results are shown in Table 4.

A similarity percentage of 90% or so was the criteria for including the detected compounds.

| Fraction | Compound | % pic area/SI | Molecular formula | Retention time (min) |
|----------|----------|--------------|-------------------|----------------------|
| LdChlo-A | Dodecane | 0.22 / 93% | C_{14}H_{30} | 8.60 |
|          | (6Z)-3,7,11-Trimethyldodeca-1,6,10-trien-3-ol | 0.13 / 90% | C_{15}H_{26}O | 9.15 |
|          | Pentadecane | 0.16 / 95% | C_{15}H_{32} | 9.35 |
|          | 4,6-dimethyldodecane | 0.16 / 90% | C_{14}H_{30} | 10.26 |
|          | Octadecane | 0.14 / 95% | C_{20}H_{42} | 10.93 |
|          | Hexadecane | 0.18 / 94% | C_{16}H_{34} | 12.60 |
|          | 2,6,10,14-Tetramethylheptadecane | 0.80 / 92% | C_{21}H_{44} | 13.47 |
|          | (Z)-docos-13-enamide | 4.01 / 93% | C_{22}H_{43}NO | 20.47 |
| LdChlo-B | 4,6-dimethyldodecane | 0.20 / 93% | C_{14}H_{30} | 8.60 |
|          | Hexadecane | 0.13 / 94% | C_{16}H_{34} | 9.35 |
|          | Tetradecanal | 0.17 / 90% | C_{14}H_{28}O | 12.84 |
|          | Octadecanal | 0.17 / 90% | C_{18}H_{36}O | 12.84 |
|          | (Z)-octadec-9-enamide | 2.30 / 91% | C_{18}H_{36}NO | 20.60 |
| LdChlo-C | (Z)-docos-13-enamide | 5.30 / 94% | C_{22}H_{43}NO | 20.60 |
| LdChlo-D | Methyl (Z)-octadec-9-enoate | 1.48 / 95% | C_{19}H_{36}O_2 | 13.51 |
|          | (Z)-docos-13-enamide | 1.71 / 94% | C_{22}H_{43}NO | 20.46 |
|          | 2-pentyl-2,3-dihydropyran-6-one | 0.05 / 90% | C_{10}H_{16}O_2 | 6.96 |
|          | (3,3,5-trimethylcyclohexyl)2-hydroxybenzoate | 0.02 / 93% | C_{18}H_{22}O_3 | 11.93 |
|          | Octadecanal | 0.03 / 91% | C_{18}H_{36}O | 12.82 |
|          | Tetradecanal | 0.03 / 90% | C_{14}H_{28}O | 12.82 |
|          | 1-ethanoxyoctadecane | 0.05 / 91% | C_{20}H_{40}O | 13.46 |
|          | 2-hexyloctan-1-ol | 0.05 / 91% | C_{14}H_{30}O | 13.46 |
|          | Dodecanamide | 3.09 / 90% | C_{12}H_{25}NO | 14.21 |
|          | Hexadecanamide | 0.09 / 90% | C_{16}H_{33}NO | 14.21 |
|          | (Z)-docos-13-enamide | 6.29 / 92% | C_{22}H_{43}NO | 20.70 |
| LdChlo-E | (3,3,5-trimethylcyclohexyl)2-hydroxybenzoate | 0.84 / 92% | C_{18}H_{22}O_3 | 11.94 |
|          | Octadecanal | 0.19 / 92% | C_{18}H_{36}O | 12.82 |
|          | methyl (Z)-octadec-9-enoate | 2.75 / 96% | C_{19}H_{36}O_2 | 13.50 |
|          | (Z)-docos-13-enamide | 17.53 / 92% | C_{22}H_{43}NO | 20.44 |
It was detected several compounds in the applied chromatography (GC-MS) with known antimicrobial activity (fig. 5), which presented a high percentage of similarity and a high pic area percentage. This could explain the antimicrobial activity found for these fractions, mainly for LdChlo-C, LdChlo-D and LdChlo-H (table 4). The LdChlo-H fraction was active only against *Listeria monocytogenes*, thus indicating selectivity against the Gram-positive pathogen and showing high antibacterial activity.

LdChlo-A, LdChlo-B, LdChlo-E, LdChlo-F and LdChlo-G fractions also presented the compounds mentioned above, but probably the presence of other inhibitory compounds resulted in more discreet or null MIC and MMC values for the tested concentrations (Table 3). Thus, the effect of fractioning on the biological activity of these fractions was smaller.

Fig. 5 Structure of compounds identified by CG-MS in fractions from Ld-Chlo extract with known antimicrobial activity
The antimicrobial effect of (Z)-docos-13-enamide (1), one of the most abundant constituent detected in the studied fractions known as erucamide has been studied by Adnam and co-workers [52], they detected the formation of hydrogen bridges between erucamide and amino acid residues of tubulin and glucosamine-6-P synthase, which could explain their anthelmintic and antibacterial actions, respectively. In other studies, antibacterial, antifungal and nematicidal activities has been attributed to methyl (Z)-octadec-9-enoate (2) [53-55] and an antibacterial activity for (Z)-octadec-9-enamide (3) [56]. On the other hand, dodecanamide (4) and some of its derivatives have been isolated from marine organisms and present an important antibacterial and antifungal activity [57-59].

The production of those bioactive compounds by Lasiodiplodia sp. and the antimicrobial activity observed for crude extracts and their fractions both show the potential of Handroanthus impetiginosus to provide endophytes that are bioactive compounds producers, as reported by Gómez and Luiz [60]. Furthermore, the identification of medicinal valuable secondary metabolites from plant-isolated Lasiodiplodia fungus is scarce, leading us to further studies related to the isolation of these substances and not only to study the phytopathogenic effects of that fungus. Based on the results presented herein, these studies mainly involve fractions obtained from crude extracts of Ld-Chlo and Ld-EAm. Regarding the fractionation of crude extracts, there was an improvement on their antibacterial (Gram positive) and antifungal (yeast) activities and revealed that fractions must be purified in a sequence that leads to the isolation of substances with antimicrobial activity.

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REFERENCES

WHO (2017) Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics. World Health Organization. https://www.who.int/medicines/publications/WHO-PPL-Short_Summary_25Feb-ET_NM_WHO.pdf?ua=1. Accessed 26 November 2019

Pogue JM, Kaye KS, Cohen DA, Marchaim D (2015) Appropriate antimicrobial therapy in the era of multidrug-resistant human pathogens. Clin Microbiol Infect 21:302–312. https://doi.org/10.1016/j.cmi.2014.12.025

Debroy S, Prosper O, Mishoe A, Mubayi A (2017) Challenges in modeling complexity of neglected tropical diseases: A review of dynamics of visceral leishmaniasis in resource limited settings. Emerg Themes Epidemiol 14:10. https://doi.org/10.1186/s12982-017-0065-3

Akhoundi M, Kuhls K, Cannel A, Votýpka J, Marty P, Delaunay P, Sereno D (2016) A historical overview of the classification, evolution, and dispersion of Leishmania parasites and sandflies. PLoS Negl Trop Dis 10:1–40. https://doi.org/10.1371/journal.pntd.0004349

Aslam B, Wang W, Arshad MI, Khurshid M, Nisar MA, Alvi RF, Aslam MA, Qamar MU, Salamat MKF, Baloch Z (2018) Antibiotic resistance: a rundown of a global crisis. Infect Drug Resist 11:1645–1658. https://doi.org/10.2147/IDR.S173867

Rodríguez-Gálvez E, Maldonado L, Alves A (2015) Identification and pathogenicity of Lasiodiplodia theobromae causing dieback of table grapes in Peru. Eur J Plant Pathol 141:477–489. https://doi.org/10.1007/s10658-014-0557-8

Félix C, Salvatore MM, DellaGrecia M, Meneses R, Duarte AS, Salvatore F, Naviglio D, Gallo M, Jorrín-Nov o JV, Alves A, Andolfi A, Esteves AC (2018) Production of toxic
metabolites by two strains of *Lasiodiplodia theobromae*, isolated from a coconut tree and a human patient. Mycol 110:642-653. https://doi.org/10.1080/00275514.2018.1478597

Ali SS, Asman A, Shao J, Balidion JF, Strem MD, Puig AS, Meinhardt LW, Bailey BA (2020) Genome and transcriptome analysis of the latent pathogen *Lasiodiplodia theobromae*, an emerging threat to the cacao industry. Genome 63:37-52. http://dx.doi.org/10.1139/gen-2019-0112

Wei W, Jiang N, Mei YM, Chu YN, Ge HM, Song YC, Ng SW, Tan RX (2014) An antibacterial metabolite from *Lasiodiplodia pseudeotheobromae* F2. Phytochem 100:103–109. http://dx.doi.org/10.1016/j.phytochem.2014.01.003

Umeokoli BO, Ebrahim W, El-Neketi M, Müller WEG, Kalscheuer R, Lin W, Liu Z, Proksch P (2018) A new depsidone derivative from mangrove sediment derived fungus *Lasiodiplodia theobromae*. Nat Prod Res. https://doi.org/10.1080/14786419.2018.1496430

Chen S, Chen D, Cai R, Cui H, Long Y, Lu Y, Li C, She Z (2016) Cytotoxic and antibacterial preussomerins from the mangrove endophytic fungus *Lasiodiplodia theobromae* ZJ-HQ1. J Nat Prod 79:2397-2402. http://dx.doi.org/10.1021/acs.jnatprod.6b00639

Kamal N, Viegelmann CV, Clements CJ, Edrada-Ebel RA (2016) Metabolomics-guided isolation of anti-trypanosomal metabolites from the endophytic fungus *Lasiodiplodia theobromae*. Planta Med 83:565-573. http://dx.doi.org/10.1055/s-0042-118601

Pandi M, Kumaran RS, Choi YK, Kim HJ, Muthumary J (2011) Isolation and detection of taxol, an anticancer drug produced from *Lasiodiplodia theobromae*, an endophytic fungus of the medicinal plant *Morinda citrifolia*. Afr J Biotechnol 10:1428-1435. https://doi.org/10.5897/AJB10.950

Huang J, Xu J, Wang Z, Khan D, Niaz SI, Zhu Y, Lin Y, Li J, Liu L (2017) New lasiodiplodins from mangrove endophytic fungus *Lasiodiplodia* sp. 318#. Nat Prod Res 31:326–332. https://doi.org/10.1080/14786419.2016.1239096

Martinez-Klimova E, Rodriguez-Peña K, Sánchez S (2017) Endophytes as sources of antibiotics. Biochem Pharmacol 134:1–17. http://dx.doi.org/10.1016/j.bcp.2016.10.010

Huang C, Chen T, Yan Z, Guo H, Hou X, Jiang L, Long Y (2019) Thiocladospole E and cladospamide A, novel 12-membered macroclide and macroclide lactam from mangrove endophytic fungus *Cladosporium* sp. SCNU-F0001. Fitoter 137:104246. https://doi.org/10.1016/j.fitote.2019.104246

Devarajan PT, Suryanarayanan TS, Geetha V (2002) Endophytic fungi associated with the tropical seagrass *Halophila ovalis* (Hydrocharitaceae). Indian J Mar Sci 31:73–74. https://pdfs.semanticscholar.org/1ad8/d3f7a72e072176287e97356875f4a641a478.pdf. Accessed 20 November 2019

Capriles CH, Mata S, Middelveen M (1989) Preservation of fungi in water (Castellani): 20 years. Mycopathol 106:73-79. https://doi.org/10.1007/BF00437084

Raeder J, Broda P (1985) Rapid preparation of DNA from filamentous fungi. Lett Appl Microbiol 1:17-20. https://doi.org/10.1111/j.1476-756X.1985.tb01479.x

Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: Improving the sensitivity of progressive multiple alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673-4680. https://doi.org/10.1093/nar/22.22.4673

Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol Biol Evol 24:1596-1599. https://doi.org/10.1093/molbev/msm092
Kimura MA (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16:111-120. https://doi.org/10.1007/BF01731581
Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406-425. https://doi.org/10.1093/oxfordjournals.molbev.a040454
Clinical and Laboratory Standards Institute (2008). Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts-3rd edition. CLSI Approved Standard M27-A3. Clinical and Laboratory Standards Institute, Wayne, PA, USA
Clinical and Laboratory Standards Institute (2015). Methods for Antimicrobial Susceptibility Testing of Aerobic Bacteria-10th edition. CLSI Approved Standard M07-A10. Clinical and Laboratory Standards Institute, Wayne, PA, USA
Sarker SD, Nahar L, Kumarasamy Y (2007) Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the in vitro antibacterial screening of phytochemicals. Methods 42:321-324. https://doi.org/10.1016/j.ymeth.2007.01.006.
Phillips AJL, Alves A, Abdollahzadeh J, Slippers B, Wingfield MJ, Groenewald JZ, Crous PW (2013) The Botryosphaeriaceae: genera and species known from culture. Stud Mycol 76:51–167. https://doi.org/10.3114/sim0021
Slippers B, Roux J, Wingfield MJ, Van der Walt FJJ, Jami F, Mehl JWM, Marais GJ (2014) Confronting the constraints of morphological taxonomy in the Botryosphaeriaceales. Pers 33:155–168. 10.3767/003158514X684780
Holetz FB, Pessini GL, Sanches NR, Cortez DA, Nakamura CV, Filho BP (2002) Screening of some plants used in the Brazilian folk medicine for the treatment of infectious diseases. Mem Inst Oswaldo Cruz 97:1027-1031. https://doi.org/10.1590/s0074-02762002000700017
Kuete V (2010) Potential of cameroonian plants and derived products against microbial infections: A Review. Planta Med 76: 1479 – 1491. https://doi.org/10.1055/s-0030-1250027
Etame RE, Mouoouke RS, Pouaha CLC, Kenfack IV, Tchientcheu R, Assam JPA, Poundeu FSM, Tiabou AT, Etoa FX, Kuiate JR, Ngane RAN (2018) Effect of fractioning on antibacterial activity of Enantia chlorantha Oliver (Annonaceae) Methanol Extract and Mode of Action. Evid Based Complement Altern Med. https://doi.org/10.1155/2018/4831593
Etame RE, Mouoouke RS, Pouaha CLC, Voukeng IK, Cidjeu CLP, Tiabou AT, Yaya AJY, Ngane RAN, Kuiate JR, Etoa FX (2019) Effect of fractioning on antibacterial activity of n-butanol fraction from Enantia chlorantha stem bark methanol extract. BMC Complement Altern Med 19:56 https://doi.org/10.1186/s12906-019-2459-y
Voukeng IK, Nganou BK, Sandjo LP, Celik I, Beng VP, Tane P, Kuete V (2017) Antibacterial activities of the methanol extract, fractions and compounds from Elaeophorbia drupifera (Thonn.) Stapf. (Euphorbiaceae). BMC Complement Altern Med 17:28. https://doi.org/10.1186/s12906-016-1509-y
Palem PPC, Kuriakose GC, Jayabaskaran C (2015) Correction: an endophytic fungus, Talaromyces radicus, isolated from Catharanthus roseus, produces vincristine and vinblastine, which induce apoptotic cell death. Appl Microbiol Biotechnol. https://doi.org/10.1371/journal.pone.0153111
Weiner LM, Webb AK, Limbago B, Dudeck MA, Patel J, Kallen AJ, Edwards JR, Sievert DM (2016) Antimicrobial-resistant pathogens associated with healthcare-associated infections: summary of data reported to the National Healthcare Safety Network at the
Centers for Disease Control and Prevention, 2011–2014. Infect Control Hosp Epidemiol 37:1288-1301. https://doi.org/10.1017/ice.2016.174

Paul S, Kannan I, Mohanram K (2019) Extensive ERG11 mutations associated with fluconazole-resistant Candida albicans isolated from HIV-infected patients. Curr Med Mycol 5:1-6. https://doi.org/10.18502/cmm.5.3.1739

Pfalzer MA, Diekema DJ, Gibbs DL, Newell VA, Ellis D, Tullio V, Rodloff A, Fu W, Ling TA (2010) Results from the ARTEMIS DISK global antifungal surveillance study, 1997 to 2007: a 10.5-year analysis of susceptibilities of Candida species to fluconazole and voriconazole as determined by CLSI standardized disk diffusion. J Clin Microbiol 48:1366–77. https://doi.org/10.1128/JCM.02117-09

Colombo AL, Nucci M, Park BJ, Noué SA, Arrthington-Skaggs B, da Matta DA, Warnock D, Morgan J (2006) Epidemiology of candidemia in Brazil: a nationwide surveillance of candidemia in eleven medical centers. J Clin Microbiol 44:2816–23. https://doi.org/10.1128/JCM.00773-06

Pfalzer MA, Andes DR, Diekema DJ, Horn DL, Reboli AC, Rotstein C, Franks B, Azie NE (2014) Epidemiology and outcomes of invasive candidiasis due to non-albicans species of Candida in 2,496 patients: data from the Prospective Antifungal Therapy (PATH) registry 2004–2008. PLoS One. https://doi.org/10.1371/journal.pone.0101510

Hinrichsen SL, Falcão E, Villieira TAS, Colombo AL, Nucci M, Moura L, Rêgo L, Lira C, Almeida L (2008) [Candidemia in a tertiary hospital in northeastern Brazil]. Rev Soc Bras Med Trop 41:394–8. https://doi.org/10.1590/s0037-86822008000400014

Pinhati HM, Casulari LA, Souza AC, Siqueira RA, Damasceno CM, Colombo AL (2016) Outbreak of candidemia caused by fluconazole resistant Candida parapsilosis strains in an intensive care unit. BMC Infect Dis. 10.1186/s12879-016-1767-9

CDC (2019) Antibiotic resistance threats in the United States, 2019. Atlanta, GA: U.S. Department of Health and Human Services, CDC. https://www.cdc.gov/drugresistance/pdf/threats-report/2019-ar-threats-report-508.pdf. Accessed 25 December 2019

Adnan M, Nazim Uddin Chy M, Mostafa Kamal ATM, Obeyedul Kalam Azad M, Paul A, Uddin SB, Barlow JW, Faruque MO, Park CH, Cho DH (2019) Investigation of the Biological Activities and Characterization of Bioactive Constituents of Ophiirrhiza rugosa var. prostrata (D.Don) & Mondal Leaves through In Vivo, In Vitro, and In Silico approaches. Mol 24:1367. https://doi.org/10.3390/molecules24071367

Chandrasekharan M, Kannathasan K, Venkatesalu V (2008) Antimicrobial activity of fatty acid methyl esters of some members of chenopodiaceae. Z Naturforsch C J Biosci 63:331-6. https://doi.org/10.1515/znc-2008-5-604

Lima LA, Johann S, Cisalpino PS, Pimenta LP, Boaventura MA (2011) In vitro antifungal activity of fatty acid methyl esters of the seeds of Annona cornifolia A.St.-Hil. (Annonaceae) against pathogenic fungus Paracoccidioides brasiliensis. Rev Soc Bras Med Trop 44:777-80. https://doi.org/10.1590/s0037-86822011000600024

Ali A, Javaid A, Shaqib A (2016) GC-MS analysis and antifungal activity of methanolic root extract. Planta Daninha. https://doi.org/10.1590/s0100-83582017350100046

Hussein HM, Hameed IH, Ibraheem OA (2016) Antimicrobial activity and spectral chemical analysis of methanolic leaves extract of Adiantum capillus-veneris using GC-MS and FT-IR Spectroscopy. Int J Pharmacogn Phytochem Res 8:369-385

Abdalha AA, Mekawey AAI (2013) Antimicrobial susceptibility of certain fungal and bacterial strains to dodecanamide and quinazolinone derivatives. World Appl Sci J 24:312-319. https://doi.org/10.5829/idosi.wasj.2013.24.03.509

Mojid MA, Jae H (2014) Antibacterial and antifeast compounds from marine-derived bacteria. Mar Drugs 12:2913–2921. https://doi.org/10.3390/md12052913
Jayalakshmi M, Vanitha V, Pushpabharathi N (2018) Biochemical screening of *Penaeus vannamei* shell waste by liquid chromatography-tandem mass spectrometry. Asian J Chem 30:1311-1316. https://doi.org/10.14233/ajchem.2018.21229

Gómez OC, Luiz JHH (2018) Endophytic fungi isolated from medicinal plants: future prospects of bioactive natural products from *Tabebuia/Handroanthus* endophytes. Appl Microbiol Biotechnol. https://doi.org/10.1007/s00253-018-9344-3