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Myrcia lundiana Kiaersk native populations have different essential oil composition and antifungal activity against *Lasiodiplodia theobromae*

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ABSTRACT

Myrcia lundiana Kiaersk (Myrtaceae) is a medicinal and aromatic shrub or small tree up to 6 m high, without studies of chemical diversity and its use to control plant pathogens. The objectives of this study were to characterize the chemical diversity of the essential oil from leaves of *M. lundiana* plants collected in the state of Sergipe, and to evaluate the antifungal potential of one representative plant of each chemical group against *Lasiodiplodia theobromae*. Essential oils of 23 plants were obtained by hydrodistillation and analyzed by GC/MS-FID. Twenty four compounds were identified in the essential oil of *M. lundiana* plants. Chemical diversity was observed among plants, which were distributed within chemical clusters. Three clusters were formed by cluster analysis. Cluster I, consisting of 10 plants, was characterized by presenting nerolic acid (24.44–43.97%) and 1,8-cineole (10.53–33.75%) as the major compounds. Cluster II, consisting of 4 plants, presented neral (9.74–24.25%) and geranial (13.71–32.64%) as the major compounds; Cluster III, consisting of 9 plants, presented isopulegol (24.23–41.06%) and iso-isopulegol (11.42–5.26%) as the major compounds. The essential oils of the representing plants of *M. lundiana*, clusters I, II and III, provided mycelial growth inhibition of *L. theobromae* in all tested concentrations, reaching 100.0% mycelial growth inhibition in the concentration of 0.5 $\mu L\,mL^{-1}$ (Cluster II), after 96 h of incubation.

1. Introduction

Myrcia lundiana Kiaersk is a species of the Myrtaceae family, found in tropical and subtropical areas of the southern hemisphere (Govaerts et al., 2008). Aqueous extracts obtained from leaves of species from the *Myrcia* genus have been used in folk medicine as astringents and diuretics, in the treatment of *diabetes mellitus*, to stop bleeding and to treat hypertension and ulcers (Russo et al., 1990).

Although there is little information regarding *M. lundiana* species, great potential is found for the use of its essential oil to control plant pathogens in agriculture, making it economically important. In 2013, a fungicide product obtained from *M. lundiana* essential oil to eliminate the fungus *Lasiodiplodia theobromae* was deposited as patent (Blank et al., 2013). *L. theobromae* fungus stands out in agriculture due to losses in tropical fruit species, both in the cultivation system and in post-harvest (Freire et al., 2011). The importance of food within a socio-environmental reality, together with the interest of consumers for good quality products have led to the search of alternatives to control these pathogens.

The essential oils consist of a mixture of a variable number of organic substances, which may reach hundreds of substances, in various concentrations, ranging from very low quantities to major compounds (Lavabre, 2001). Several factors can cause variations in the composition of essential oils, such as environmental, genetic characteristics, source of materials, agricultural practices, maturity, post-harvest treatment and extraction methods (Verma et al., 2010).

Studies have shown the presence of chemical diversity in the essential oil of aromatic species, such as *Ocimum basilicum* (Costa et al., 2015), *Lippia alba* (Blank et al., 2015), *Varronia curassavica* (Nizio et al., 2015), *Lippia sidoides* (Santos at al., 2015), *Chrysopogon zizanioides* (Celestino et al., 2015), *Pogostemon* sp. (Patchouli) (Blank et al., 2011), *Myrciaria cauliflora* (Duarte et al., 2012), *Eugenia dysenterica* (Duarte et al., 2010). Although *M. lundiana* occurs in the state of Sergipe, there is no information regarding the chemical diversity

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Table 1
Identification of *M. lundiana* plants collected in Areia Branca, Sergipe State, Brazil.

Plants	Georeferenced information
MLUN001	11°44′17.9″S; 37°52′0.80″W
MLUN002	10°44′57.0″S; 37°20′24.6″W
MLUN003	10°44′58.0″S; 37°20′26.2″W
MLUN004	10°44′58.0″S; 37°20′25.0″W
MLUN005	10°44′57.7″S; 37°20′24.6″W
MLUN006	10°44′57.5″S; 37°20′26.0″W
MLUN007	10°44′57.5″S; 37°20′26.1″W
MLUN008	10°44′56.2″S; 37°20′26.3″W
MLUN009	10°44′58.1″S; 37°20′26.1″W
MLUN010	10°45′00.0″S; 37°20′26.0″W
MLUN011	10°44′57.7″S; 37°20′24.3″W
MLUN012	10°44′56.2″S; 37°20′26.5″W
MLUN013	10°45′8.30″S; 37°20′27.5″W
MLUN014	10°44′57.5″S; 37°20′26.5″W
MLUN015	10°44′54.5″S; 37°20′27.9″W
MLUN016	10°44′56.5″S; 37°20′24.0″W
MLUN017	10°44′16.6″S; 37°52′00.5″W
MLUN018	10°44′58.2″S; 37°20′26.1″W
MLUN019	10°44′56.8″S; 37°20′24.0″W
MLUN020	10°44′57.5″S; 37°20′26.0″W
MLUN021	10°44′57.6″S; 37°20′26.1″W
MLUN022	10°45′8.10″S; 37°20′17.5″W
MLUN023	10°45′8.10″S; 37°20′18.5″W

of the essential oil of this species. Due to the potential of *M. lundiana* as raw material for the obtainment and commercialization of bioproducts for agriculture, it is important to know the chemical diversity of its essential oil. The information obtained in this study may be useful in establishing conservation and breeding strategies for *M. lundiana,* and in enabling the discovery of other bioactive compounds potentially useful to humans. The objectives of this study were to evaluate the essential oil chemical diversity of *M. lundiana* plants in the municipality of Areia Branca/SE, Northeast Brazil, and to evaluate the antifungal activity of the essential oil against *L. theobromae*.

2. Materials and methods

2.1. Plant material

Twenty-three *M. lundiana* plants were collected in the National Park of Itabaiana (Table 1). The Park is located in the municipalities of Areia Branca, Itabaiana, Laranjeiras, Itaporanga D'ajuda and Campo do Brito, in the state of Sergipe, northeastern Brazil (10°41′06″S lat., 37°25′31″W long., at 659 m). Its area covers 7966 ha and 87.25 km perimeter, comprising the areas of Serras Cajueiro, Comprida and Itabaiana. It is located in a transition zone between the Brazilian caatinga and the rainforest, and the predominant climate in the basin where it is located is semi-arid with annual rainfall ranging between 1100 and 1300 mm in well-defined seasons, and average monthly relative humidity of 84.6%. The climate consists of four to five months of drought, and it is a semi-arid climate, with temperatures between 34.5 °C and 35 °C, which is hotter than Aracaju, the capital of the state of Sergipe (IBAMA, 2006).

2.2. Extraction and analysis of essential oils

The freshly collected leaves of all plants were dried in an oven with forced air circulation, at 40 °C, for five days. The essential oils were extracted by hydrodistillation, using a modified Clevenger apparatus. Samples consisted of 50 g of dry leaves, distilled for 140 min (Ehlert et al., 2006). Three replications were carried out. The extracted essential oil was properly assessed, collected and stored in amber vials at −20 °C, until analysis.

The analysis of the chemical composition of the essential oils was carried out using a GC–MS/FID (QP2010 Ultra, Shimadzu Corporation, Kyoto, Japan), equipped with an autosampler AOC-20i (Shimadzu). Separations were accomplished using an Rtx®-5MS Restek fused silica capillary column (5%-diphenyl-95%-dimethyl polysiloxane) of 30 m × 0.25 mm i.d., 0.25 mm film thickness, at a constant helium (99.999%) flow rate of 1.2 mL min^{-1}. Injection volume of 0.5 μL (5 mg mL^{-1}) was employed, with a split ratio of 1:10. The oven temperature was programmed from 50 °C (isothermal for 1.5 min), with an increase of 4 °C/min to 200 °C, then 10 °C/min to 250 °C, ending with a 5 min isothermal at 250 °C.

The MS and FID data were simultaneously acquired employing a Detector Splitting System; the split flow ratio was 4:1 (MS:FID). A 0.62 m × 0.15 mm i.d. restrictor tube (capillary column) was used to connect the splitter to the MS detector; a 0.74 m × 0.22 mm i.d. restrictor tube was used to connect the splitter to the FID detector. The MS data (total ion chromatogram, TIC) were acquired in full scan mode (m/z of 40–350), at a scan rate of 0.3 scan/s, using the electron ionization (EI), with an electron energy of 70 eV. The injector temperature was 250 °C, and the ion-source temperature was 250 °C. The FID temperature was set to 250 °C, and the gas supplies for the FID were hydrogen, air, and helium at flow rates of 30, 300, and 30 mL min^{-1}, respectively. Quantification of each compound was estimated by FID peak-area normalization (%). Compound concentrations were calculated from the GC peak areas, and they were arranged in order of GC elution.

Identification of individual components of the essential oil was carried out by computerized matching of the acquired mass spectra with those stored in NIST21, NIST107 and WILEY8 mass spectral library of the GC–MS data system. A mixture of hydrocarbons (C_9H_{20}–$C_{19}H_{40}$) was injected under these same conditions and identification of constituents was then performed by comparing the spectra obtained with those of the equipment data bank, and by the retention index, calculated for each component, as previously described (Adams, 2007). Retention indices were obtained using the equation proposed by Van Den Dool and Kratz (1963).

2.3. Antifungal activity

Pure culture of the fungus *L. theobromae* was obtained in the Phytopathology laboratory of the Federal University of Sergipe. The experimental design was completely randomized with three replications. The essential oils used in the antifungal activity assay were obtained from plants representing each chemical group formed by the cluster analysis. MLUN005 plant represented Cluster I; MLUN022 plant represented Cluster II; MLUN019 plant represented Cluster III. Essential oils were solubilized in 1% DMSO and homogenized in PDA culture medium (Potato Dextrose Agar, HIMEDIA). For each sample, it was tested concentrations of 0.1; 0.5; 1; 5 and 10 (μL mL^{-1}) of essential oil. Then, solutions were poured into 9.0 cm diameter Petri dishes, and each dish was inoculated in the center, with a 7 mm diameter disk, containing mycelia of the fungus culture.

Dishes were sealed, identified and incubated in B.O.D. chamber at a temperature of 22 ± 3 °C, with a 12 h photoperiod. The evaluations were carried out by measurements of the mycelial diameter (mean of two diametrically opposed measures), using a caliper, 96 h after incubation. Petri dishes without essential oil, containing BDA culture medium and 1% DMSO, and Petri dishes containing only the BDA culture medium were used as controls. Viper 700 (0.07% w/v), a broad-spectrum fungicide, was used as positive control. At the end of the assessment, we estimated the percentage of growth inhibition of the fungus (PIG) of the treatments in relation to the control, using the expression: PIG = [(control diameter − treatment diameter)/(control diameter)] × 100.