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Chemical composition and *in vitro* leishmanicidal, antibacterial and cytotoxic activities of essential oils of the Myrtaceae family occurring in the Cerrado biome

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ABSTRACT

The essential oils of *Eugenia uniflora* L. (EuEO), *Plinia cauliflora* (Mart.) (PcEO) and *Syzygium cumini* (L.) (ScEO), of the Myrtaceae family, were analyzed in gas chromatography with mass spectrometry in order to identify their volatile components, as well as their *in vitro* leishmanicidal, antibacterial and cytotoxic activities. A total of 34 compounds were identified from EuEO. The major compounds found in EuEO were germacrone (8.52%), spathulenol (8.20%), α-selinene (7.50%) and (Z)-β-elemenone (4.88%). PcEO contained 52 compounds, with the major compounds being (E)-cariophene (14.69%), β-bisabolene (9.36%), (E, E)-α-farnecene (8.07%) and globulol (7.86%). ScEO contained 38 compounds, with the major compounds being α-pinene (21.20%), globulol (15.30%), eugenol (11.20%), and α-terpineol (8.88%). Our results demonstrated that EuEO, PcEO and ScEO, tested against *Leishmania amazonensis*, affected promastigote growth in a dose-dependent manner. The IC_{50} of EuEO, PcEO and ScEO were 0.99, 0.46 and 8.78 μg/mL, respectively, while the IC_{50} of Amphotericin B was 0.60 μg/mL. All the EO of the species evaluated presented moderate inhibitory activity, with Minimum Inhibitory Concentration (MIC) variation of 100 to 400 μg/mL for the following bacteria tested: *Streptococcus mutans*, *Streptococcus mitis*, *Streptococcus sanguinis*, *Streptococcus sobrinus*, *Streptococcus salivarius*, *Actinomyces naeslundii*, *Bacteroides fragilis*, *Bacteroides thetaiotaomicron*, *Prevotella nigrescens* and *Porphyromonas gingivalis*. Our results showed that the IC_{50} values for the treated tumor cell lines ranged from 76.5 to 106.2 μg/mL for EuEO and 76.6 to 116.2 μg/mL for PcEO. ScEO was not tested on the tumor cell lines because it presented an $IC_{50} > 400$ μg/mL on normal cell lines. These results highlight the variability of the chemical composition of essential oils and the high potential of their bactericidal and leishmanicidal activities.

1. Introduction

The Cerrado is a large biome in Brazil that spans over more than 2,000,000 km^2, an area equivalent to the size of Portugal, Spain, France, the Netherlands, Belgium, Luxembourg, Italy, Germany, Switzerland, and the Czech Republic combined (Françoso et al., 2016). The Brazilian Cerrado is a biodiversity hotspot recognized as a conservation priority for being rich in a variety of endemic botanical species (Myers et al., 2000). In the Brazilian Cerrado, the Myrtaceae family is the most reported family in floristic and phytosociological studies and is present in more than 80% of localities (Françoso et al., 2016). Some exotic fruit Myrtaceae species are very common, such as, *Eugenia uniflora* L., *Plinia cauliflora* (Mart.) and *Syzygium cumini* (L.). The fruits of these species are valued and consumed in natura, in juices or

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jellies. Meanwhile, the leaves are used in traditional medicine against diseases (Ayyanar and Subash-Babu, 2012; Coelho de Souza et al., 2004).

Protozoan parasites of the *Leishmania* genus are responsible for a spectrum of diseases collectively known as leishmaniasis, which affect the skin, membranes, mucous membranes and internal organs (http://www.who.int/leishmaniasis/en/). More than 350 million people are currently infected worldwide, and it has been reported in 98 countries around the world (WHO, 2010). The increased incidence of leishmaniasis is associated with urban development, deforestation, and environmental changes as well as increased migration to areas where the disease is endemic (Leta et al., 2014).

The diseases caused by oral bacteria such as caries and periodontitis are a public health problem. Recent studies suggest that tooth loss, root caries, and periodontal disease may also be associated with increased odds of dying (Kim et al., 2013). Poor oral health may have a profound effect on general health, and several oral diseases are related to chronic diseases (*e.g.*, diabetes). The experience of pain and problems with eating, chewing, smiling and communication due to missing, discolored or damaged teeth have a major impact on people's daily lives and well-being. Furthermore, oral diseases restrict activities at school, at work and at home, causing millions of school and work hours to be lost each year throughout the world (Petersen et al., 2005).

In this sense, new active agents against diseases, as essential oils, have been researched due to the complexity of their chemical composition, as well as their lower toxicity and lower risk of resistance to microorganisms (Azevedo et al., 2014). Essential oils are normally composed of volatile compounds derived from two groups of secondary metabolites, terpenes and phenylpropanoids, which help in plant defense against phytopathogens (Iriti and Faoro, 2009).

Recent studies of the essential oils of the Myrtaceae species have shown that they have important properties, such as insecticidal (Kumar et al., 2012), parasiticidal (Rodrigues et al., 2015), fungicidal (Hamini-Kadar et al., 2014), bactericidal (Sousa et al., 2015), antimicrobial (Mouna and Segni, 2014), and antioxidant (Victoria et al., 2013), among others. In addition, essential oils can be used for pest control either by direct application or their isolated active principle (Pavela and Benelli, 2016). Thus, essential oils can serve as raw material for the discovery of new synthetic products, which may be of great importance for the treatment of diseases (Bajalan and Pirbalouti, 2014).

The objective of this work was to evaluate the *in vitro* leishmanicidal, antibacterial and cytotoxic activities of the essential oils of the following three Myrtaceae species occurring in the Brazilian Cerrado: *Eugenia uniflora* L., *Plinia cauliflora* (Mart.) and *Syzygium cumini* (L.).

2. Material and methods

2.1. Plant material

The species *E. uniflora* L. and *P. cauliflora* (Mart.) were collected in Santa Helena de Goiás city, State of Goiás, Brazil (17°48′35.9″, 17°48′32.8″ latitude and 50°365′26.2″, 50°35′04.6″ longitude). The specie *S. cumini* (L.) was collected in Rio Verde city (17°48′28″ latitude, 50°53′57 longitude).

The species were identified in the Herbário Jataiense Germano Guarim Neto and a voucher specimen was deposited in the herbarium with identification numbers 7441 (*P. cauliflora* (Mart.)), 7442 (*E. uniflora* L.) and 7443 (*S. cumini* (L.)). The leaves of each species were collected between the months of September 2015 and March 2016 at the same time at 6:00 pm on the day prior to extraction. After the collection, the leaves were washed, dried in room temperature and homogenized, and the essential oils were extracted. The essential oils were combined to compose of each species samples.

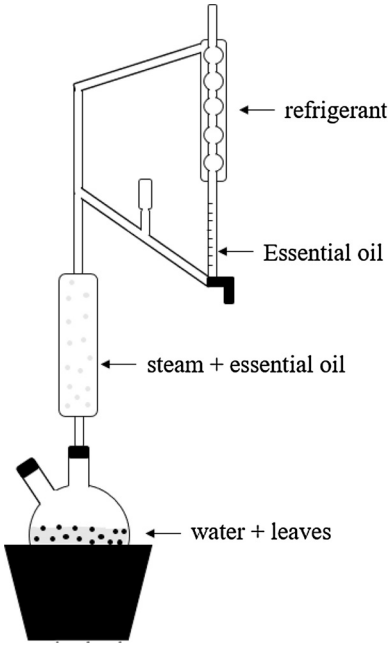

Fig. 1. Hydrodistillation using a Clevenger type-apparatus.

2.2. Extraction and yield of essential oils

Essential oils (EO) were extracted from the leaves (100 g) of *E. uniflora* L. (EuEO), *P. cauliflora* (Mart.) (PcEO), and *S. cumini* (L.) (ScEO) by a modified Clevenger-type apparatus (Fig. 1) and a 3-hour hydrodistillation. The oil was filtered using anhydrous sodium sulfate and stored in a refrigerator at 5 °C until the analysis. All the experiments were carried out in triplicate.

The yield (percentage content in fresh matter) of the EO was determined according to Eq. (1).

$$Yield\ (\%FM) = \left[\frac{oil\ mass\ (g)}{plant\ material\ mass\ (100g)}\right] \times 100 \quad (1)$$

2.3. Chemical composition of essential oils

The gas chromatography with mass spectrometry (GC–MS) analysis was performed on an Agilent Technologies 7820 A GC and MSD 5975 using a DB-5MS column (25 m x 0.25 mm, 0.25 mm in thickness). The carrier gas was He, at a rate of 1.2 mL/min and a pressure of 5.70 Psi. A 1-μL sample was injected in the splitless mode (5 mL/min). The injector and detector were both kept at 260 °C. The oven temperature was held initially at 50 °C. The temperature was increased to 240 °C at 4 °C/min and maintained for 5 min. The parameters of the MS detector were as follows: the transfer line temperature was 280 °C, the oven was 150 °C/min and the electronic impact technique was applied at 1635 MeV for the detection. Volatile compounds were identified by comparing the retention times obtained with linear hydrocarbon retention times (C8-C18), by comparing linear retention indices and mass spectra with the NIST 2.0 library (US National Institute of Standards and Technology, 2008), and was confirmed with data reported in the literature. The quantification was performed through peak area normalization measurements and expressed as a percentage (%).

2.4. Leishmanicidal activity of essential oil

The leishmanicidal activity was assessed with the promastigote *Leishmania amazonensis* (MHOM/BR/PH8) maintained in culture medium RPMI 1640 (Gibco, São Paulo, Brasil) supplemented with 10% fetal bovine serum. Then, 1×10^6 parasites were distributed in a 96-