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Chemical variability of essential oils of *Copaifera langsdorffii* Desf. in different phenological phases on a savannah in the Northeast, Ceará, Brazil

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ABSTRACT

In most of the ethnobotanical studies among species with greater versatility, the *Copaifera langsdorffii* Desf. stands out due to the high number of therapeutic properties attributed to the oil-resin of its trunks. This species is widely distributed occurring both in preserved and anthropogenic environments. Plants that occur in different environmental conditions present qualitative and quantitative variation of the chemical constituents responsible for the biological activity, moreover, physiological factors can alter the synthesis of these compounds. This study aims to contribute with information about the chemical profile of *C. langsdorffii* in two areas of cerrado (preserved and anthropic) in different phenological phases and seasons. The vegetative (leaf fall and budding) and reproductive phenophases (flowering and fruiting) were qualified as present and absent from August 2013 to July 2014. In order to obtain the essential oils, the oil-resin were subjected to hydrodistillation process in an adapted Clevenger type device. The identification of the essential oils' chemical components was performed by gas chromatography coupled mass spectrometry (GC/MS). Statistical analyses of the results were obtained by Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA). The reproductive phase occurred only in anthropic areas. The essential oils of both areas had yields ranging from 5.1 to 28.8%; with higher yields at the end of the rainy season coincided with the fruiting phenophase in anthropic areas and the presence of only leaves in the conserved area. Total of 42 chemical constituents were identified, 27 common to the two areas. The main constituents were β-caryophyllene, α-humulene, germacrene B and caryophyllene oxide which presented changes in levels during all collections. Cluster analysis confirmed that the volatile oil of *C. langsdorffii* was strongly influenced in its chemical composition by the different areas (preserved and anthropic). These informations collaborate to establish the most appropriate collection period to obtain higher yields of Copaifera langsdorffii's essential oil, as well as the desirable concentrations of the chemical compounds responsible for therapeutic activities.

1. Introduction

Many traditional communities have an extensive natural dispensatory due to the variety of plant resources, which are often used as a single therapeutic means available for the treatment and cure of diseases (Amorozo, 2002; Maciel et al., 2002). Part of these resources is included in natural environments occupied by these populations or anthropically altered environments (Amorozo, 2002).

Plants that occur in different environmental conditions present qualitative and quantitative variation of chemical constituents responsible for the biological activities (Hiruma-Lima et al., 2006; Jorge et al., 2004; Lima et al., 2006). Given that such variation results

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from the interaction between the plants and the environment, the biosynthesis of chemical compounds is often affected by environmental conditions (Bitu et al., 2015; Cerqueira et al., 2009; Fernando Rolim de Almeida et al., 2014).

Among the main factors that influence the overall chemical composition and the relative proportions of the chemicals present in the natural plant products are the seasonality (Santos et al., 2009; Zoghbi et al., 2007), the circadian rhythm (Blank et al., 2005), the phenological phase (Murakami et al., 2013), the soil's composition (Barbosa et al., 2012; Duarte et al., 2010) and solar radiation (Gouinguené and Turlings, 2002). Plants found in anthropic areas are excessively exposed to stressors due to habitat modification in which they are inserted, with increased levels of light, temperature, humidity and wind in the affected areas (Carvalho Filho et al., 2006).

Knowledge of the factors, growing conditions that determine the chemical variability or concentrations of compounds of each plant species is very important, especially for those of commercial and medicinal interest (Figueiredo et al., 2009). The *Copaifera langsdorffii* species is distinguished by its medical importance (Ribeiro et al., 2014) due to the high number of therapeutic properties attributed to the oil-resin present in the trunk of its subjects.

There are few studies that evaluate the chemical variations of species struck by environmental factors in conjunction with the phenological events (Murakami et al., 2013; Valentini et al., 2010). In the phenological stages of the plant species, there are biochemical and physiological alterations able to modify the development of biologically active substances, directly influencing the content and quality of the essential oils (Sousa et al., 2011). The study aims to contribute with information about the chemical profile of *C. langsdorffii* in savanna areas, both conserved and anthropized, assessing the chemical variations during the phenological phases and in different seasonal periods.

2. Materials and methods

2.1. Study area

The survey was conducted in two savannah areas inserted in Chapada do Araripe, at Crato, Ceará, Brazil (Fig. 1) tabular surface, preserved in a level of 800 m to 1000 m of altitude (da Costa et al., 2004). The red dystrophic latosol is prevalent in the area (Costa and da Araújo de, 2007). The climate is Aw according to Koppen classification. It is a hot and humid climate with 4–5 dry months and two distinct seasons: a rainy one (spring-summer) starting from November, then decreasing from May with higher rainfall peaking in March (de Souza and de Oliveira, 2008) and dry one (autumn-winter) from May to November.

The conserved area is known as Malhada Bonita (07° 21′5.0.5 "S and 039° 27′26.3" W), which is located in the National Forest of Araripe (Flona Araripe/Apodi), 10 km from the anthropic area named by Barreiro Grande (07° 21′37.4 "S and 039° 28′33.8" W). This area is characterized by forest management activities initiated in 2009, with some species preserved at the request of the Brazilian Institute of Environment and Renewable Natural Resources (IBAMA), including *Copaifera langsdorffii*, with isolated or grouped individuals.

2.2. Identification of botanical material

During the flowering period, five fertile samples of *Copaifera langsdorffii* were collected in February 2014. The plant material was prepared and treated according to the usual techniques of herborization (Mori et al., 1983). The witness material was incorporated into the collection of the Herbário Caririense Dárdano de Andrade-Lima of the Regional University of Cariri (HCDAL- URCA) – registered under number 10.645.

The authorization for the activities was provided by the Biodiversity Authorization and Information System (SISBIO) of the Brazilian Institute of Environment and Renewable Natural Resources (IBAMA). Registered under number 40171-1.

2.3. Phenology

Phenological observations were carried out monthly from August 2013 to July 2014, on 10 individuals randomly selected in each area. All selected individuals were marked with tags attached to trees by naylon wire. The rainfall data was obtained from the Crato station, monitored by the Cearense Foundation of Meteorology and Water Resources (FUNCEME) for the Crato post. Phenophases were qualitatively estimated as present or absent and quantitatively by the percentage of intensity of Fournier (Fournier et al., 1975).

2.4. Collection of oil-resin

For the collection of the oil-resin was used an adult tree, visually healthy. The extraction was made by sticking the tree trunk with a 2 cm diameter auger at a height of 1.30 m from the ground. A PVC pipe (¾) was introduced in the hole for the flow of oil-resin. The barrel was connected to a collecting vessel with 250 mL of capacity, through a plastic hose (¾). All collections were made always in the first 15 days of each month. After collecting the oil-resin, the pipes were removed and the holes sealed with clay. All extracted oil was packed in plastic pots and covered with foil for greater safety in transport to the laboratory. This method produces little oil, has high operating costs, but preserves the tree for future crops and is ecologically correct (Rigamonte-Azevedo et al., 2006).

2.5. Obtaining the essential oils

24 oil-resin samples from the two study sites were obtained for over a year, which were subjected to hydrodistillation process for 2 h, in adapted Clevenger type apparatus. The oil-water mixture was collected, treated with anhydrous sodium sulphate (Na_2SO_4), and the essential oils were separated and kept under refrigeration in temperature lower than 4 °C until analyzed.

2.6. Analysis of the chemical composition

The components' identifications of essential oils of *C. langsdorffii*'s oil-resin were carried out by gas chromatography coupled with mass spectrometry (GC/MS) on a Hewlett-Packard model 5971 spectrometer operating with ionization energy of 70 eV. It was used a fused silica capillary column DB-5 (30 m x 0.25 mm id, 0.25 mm thick film) and helium carrier gas with a flow of 1 mL/min. The injector and detector temperatures were programmed to 250 °C and 200 °C, respectively. The column temperature was determined from 35 °C to 180 °C at 4 °C/min and then 180 °C to 280 °C at 10 °C/min. Mass spectra were obtained on 30–450 *m*/*z*. The identification of rest compounds was performed by comparing their mass spectra with data from NIST05 (National Institute of Standards and Technology, US) and WILEY 275 libraries by their retention times, as well as by visual comparison of the fragmentation pattern with those reported in the literature (Adams, 2007).

2.7. Chemical variability multivariate analyses

The statistical multivariate analysis were procedure for available seasonal trends in the chemical composition. Statistical