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Spring alterations in the chromatographic profile of leaf essential oils of improved guava genotypes in Brazil

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ABSTRACT

Leaf essential oils of guava (*Psidium guajava* L., Myrtaceae) show intraspecific variation in chemical composition. The essential oils of 21 *P. guajava* genotypes, obtained in each season in 2015 and 2016, were analyzed via GC-FID and GC–MS to detect composition variability due to seasonal fluctuation and a total of 35 compounds were identified. Qualitative and semi-quantitative variations were observed in different genotypes and within same genotypes during different seasons. Overall, the genotypes showed a predominance of sesquiterpenes with a relative area > 70%. Spring was the most varied season, revealing a decrease in hydrogenated sesquiterpenes and an increase of oxygenates. Precipitation data and daily temperatures during the experiment were registered at the time of guava leaf sampling to verify whether this phenomenon was due to a seasonal condition. We found that climatic variations were not the primary influence on this result. Phenological factors like as flowering showed a decrease in compounds like (E)-Caryophyllene and α-Humulene in the essential oils of all studied genotypes and were determined to be influenced by that cyclic phenomenon. Hence, due to the variations that occur in the chemical compounds the harvesting of leaves for the extraction of essential oils should be performed according to the highest concentrations of the active principles.

1. Introduction

Species of the family Myrtaceae are distinctive not only for their production of essential oils, which are found in the plant's oil glands and products of the secondary metabolism (Goodger et al., 2016; Morais et al., 2014; Padovan et al., 2014) but also for its high diversity of genera and species. The essential oils produced by species of this family have an ecological importance that contributes to plant protection by repelling undesirable insects, attracting those that disperse pollen and seeds, and others (Moore et al., 2013). Additionally, these essential oils present phytotherapeutic action, are of commercial interest for the pharmaceutical industry (Almeida et al., 2016), and have a larvicidal effect on *Aedes aegypti* L., which is an important vector of human pathogens (Mendes et al., 2017a, 2017b).

The variability in the chemical composition of essential oils within species renders production at the industrial scale challenging (Keszei et al., 2010). These inter- and intraspecific variations are commonly reported in the literature and are attributed to different factors (Padovan et al., 2014; Souza et al., 2017). Among them, genetic components are considered to be a highly influential factor in the respective species (Külheim et al., 2015). Moreover, the age of the plant, its development stage, the availability of nutrients in the soil, abiotic factors such as light, temperature and pluviosity, and harvesting techniques that take into consideration the season and time of harvest may bring about significant alterations in the production of secondary metabolites (Morais, 2009).

Souza et al. (2017) recently demonstrated that the chemical profiles of essential oils from commercial and improved genotypes of guava (*Psidium guajava* L.) in Brazil predominantly consist of sesquiterpenes, with variation in their composition. The authors verified that the environmental conditions of *P. guajava* culture had little influence on the composition of leaf essential oils, except for one of the 22 evaluated genotypes. Nevertheless, no information on the existence of qualitative and semiquantitative variability of essential oils, as a result of seasonal variation in this species, is available as of yet. The guava culture is perennial and undergoes annual cycles of fruit production. Therefore,

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the effects of seasonality should be considered along with the metabolic alterations related to the processes of flowering and fructification (Lopes and Gobbo-Neto, 2007; Stefanello et al., 2010).

Factors like temperature and pluviosity, which are typical of each season, may influence the composition of essential oils and, if known, may benefit the commercial exploration of this product (Meira et al., 2012). In this context, we aimed to seasonally evaluate the chemical composition of essential oils from 21 commercial and improved guava genotypes. This study considered distinct genotypes in one species, which represent a large part of the genetic variability of guava cultivars in Brazil, and in previous studies have shown morphological, molecular (Coser et al., 2012), and chemotypic variability (Souza et al., 2017). Our results will allow the determination of strategic seasons for the sampling of the leaves that will be used for the extraction of essential oils and will help direct their industrial use based on chemical composition, which will potentiate their effects for their intended purposes.

2. Materials and methods

The plant samples were collected at the municipality of Alegre (20°45′S, 41°31′E; 254 m in elevation), Espírito Santo State (ES), Brazil. The experiments were conducted at the Laboratory of Plant Sample Preparation and the Laboratory of Plant Genetics and Breeding, at the Center for Agricultural Sciences and Engineering, Federal University of Espírito Santo (Centro de Ciências Agrárias e Engenharias da Universidade Federal do Espírito Santo- CCAE-UFES), and at the Laboratory of Applied Chemistry, Federal Institute of Espírito Santo (Instituto Federal do Espírito Santo - IFES). The experimental orchard used in this study has been located at Alegre since July 2013 and was maintained under natural conditions, without irrigation, shading or pruning.

2.1. Sampling and extraction of foliar essential oils from 21 P. guajava genotypes

Plant material – Leaves were collected in each of the four seasons from 21 genotypes of *P. guajava* from 11 commercial cultivars that cultivated the plants for fresh fruit production: Paluma (PAL); Século XXI (SEC); Pedro Sato (PS); Petri (PET); Cortibel LG (C1); Cortibel LM (C2); Cortibel Branca LG (C4); Cortibel RM (C6); Cortibel Branca RM (C8); Cortibel RG (C14); and Cortibel SLG (C15); and from 10 experimental genotypes: Cortibel 3 (C3); Cortibel 5 (C5); Cortibel 7 (C7); Cortibel 9 (C9); Cortibel 10 (C10); Cortibel 11 (C11); Cortibel 12 (C12); Cortibel 13 (C13); Cortibel 16 (C16); and Cortibel 17 (C17). The leaves were collected from the same plant of each genotype at 9.00 a.m. in days typical of the mid-seasons, April 24th, 2015 (autumn), August 11th, 2015 (winter), November 11th, 2015 (spring), and February 20th, 2016 (summer). The sampling was performed at chest height (1.3 m) and around the canopy diameter.

Approximately 100 fully developed leaves were collected for oil extraction and chemical characterization. The material was placed into paper bags, identified, and transported to the Laboratory of Plant Genetics and Breeding at CCAE-UFES. The leaves were dried in a shaded area at room temperature for one week. Along with other procedures, this process aimed to reduce distillation time and cost, increase the stability of the product, inhibit enzymatic activity that can cause decomposition or modification of the original aromatic principles, and stabilize color, smell, taste, and texture (Cerimele and Ringuelet, 2008). Subsequently, the dried leaves were placed into plastic bags and stored in a freezer at −9 °C until essential oil extraction.

Essential oil extraction – The essential oils were obtained at the Laboratory of Plant Sample Preparation (CCAE-UFES) via hydrodistillation in a Clevenger apparatus, during four hours of extraction, according to the methodology recommended by the Farmacopeia Brasileira for volatile oils (Brasil, 2010). Approximately 100 g of dried leaves in approximately 1000 mL of reverse osmosis water in round-bottom flasks with a volume of 2000 mL were used in the extractions. The vapors of water and oil were mixed and, following cooling, the molecules condensed and were separated based on solubility and density differences. The mixture of oil and water was placed into Eppendorf tubes, centrifuged, and the oil was removed with a micropipette and stored in a freezer at −20 °C, protected from light.

2.2. Physical characterization of the essential oils and climatic data of the experiment

Determination of density and refractive index of the essential oils – Essential oils can refract polarized light, and it has been found that each oil has a characteristic refractive index (η) that can be used as a measure to control oil purity (Cerimele; Ringuelet, 2008). A refractometer (Quimis Q767B) with four decimal places was used to determine η. This measurement was carried out at the Laboratory of Applied Chemistry (IFES) at 20 °C.

Oil density was determined at the Laboratory of Plant Sample Preparation (CCAE-UFES) at 20 °C by weighing in triplicate a precise oil volume measured using a micropipette. An analytical balance (Shimadzu AUY220) with four decimal places was used.

Climatic data – The data on climatic variations across the seasons were obtained from the Capixaba Institute for Research, Technical Assistance, and Rural Extension (Instituto Capixaba de Pesquisa, Assistência Técnica e Extensão Rural - INCAPER), consisting of pluviosity indices and temperature at the municipality of Alegre, during the periods of leaf sampling.

2.3. Chromatographic profile of essential oils

Identification of essential oil components – The samples of essential oils extracted from the leaves were analyzed by gas chromatography with flame ionization detector (GC-FID) (Shimadzu GC-2010 Plus) and gas chromatography coupled to mass spectrometry (GC–MS) (Shimadzu GCMS-QP2010 SE). The following conditions were employed for these analyses: Helium (He) as carrier gas in both detectors, with flow and linear speed of 2.80 mL min^{-1} and 50.8 cm sec^{-1} (GC-FID), and 1.98 mL min^{-1} and 50.9 cm sec^{-1} (GC–MS), respectively; 220 °C injector temperature in split ratio of 1:30; fused silica capillary column (30 m × 0.25 mm); stationary phase Rtx®-5MS (0.25 µm film thickness); oven program with initial temperature of 40 °C for 3 min, followed by gradual increments of 3 °C min^{-1} up to 180 °C, which was maintained for 10 min, for a total analysis time of 59.67 min; and 240 °C FID and 200 °C MS detector temperatures (Souza et al., 2017). The used samples were removed from the vials in 1 µL of a solution consisting of 3% essential oil dissolved in hexane with 0.1 mol.L^{-1} dimethylamine (DMA) (external standard for reproducibility control).

GC–MS analyses were carried out in electronic impact equipment with 70 eV impact energy; 1000 scan speed; 0.50 fragments.sec^{-1} scanning interval; and fragment detection from 29 to 400 (m/z). GC-FID analyses were performed using an H_2 flame and atmospheric air at 300 °C temperature. Flows used for H_2 and air were 40 mL.min^{-1} and 400 mL.min^{-1}, respectively. Ion detection occurs when the organic compounds of the sample are mixed with the carrier gas (He), and a current proportional to the amount of these compounds in the sample is generated. If only He and H_2 are mixed, a small current is produced between the electrodes.

The identification of the essential oil components was performed by comparing the obtained mass spectra with those available in the spectral library database (Wiley 7, NIST 05 and NIST 05 s) and the Kovats retention indices (KI). To calculate KI, a sample of saturated alkanes C_7–C_{40} (Supelco – USA) and the adjusted retention time of each compound, as obtained by GC-FID, were employed. Subsequently, the values calculated for each compound were compared to those in the literature (Adams, 2007; El-Sayed, 2016; NIST, 2011).

The relative percentage of each compound in the essential oil was