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# Chemotype diversity of *Psidium guajava* L.

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ABSTRACT

The essential oil of *Psidium guajava* L. has been studied for pharmacological and industrial purposes, without considering the plant's genotype regarding the heterogeneity of its composition. The present study aimed to characterize the chemotype diversity of the essential oil extracted from the leaves of 22 genotypes of *P. guajava* grown in two different environments in the state of Espírito Santo, Brazil, and to identify the different chemical markers present in these plants. Essential oil from the leaves of the *P. guajava* genotypes was extracted by hydrodistillation, and its chemical composition was analyzed by gas chromatography-flame ionization detection (GC-FID) and gas chromatography-mass spectrometry (GC-MS). Thirty-three compounds were identified, comprising 87.5–99.0% of the total composition, with a prevalence of sesquiterpenes in all samples. The major compounds identified consisted of *(E)-trans*-Caryophyllene, *α*-Humulene, *trans*-Nerolidol, *β*-Bisabolene, *β*-Bisabolol, and Hinesol, the first of which was identified as a possible chemical marker for the species. Multivariate factor analysis of the chemical composition of *P. guajava* oil identified three chemotypes: Commercial - PAL, SEC, PS, PET, C7, C11, and C17MI, characterized by high levels of *β*-Selinene, *α*-Selinene, Hinesol, and 14-hydroxy-*epi-(E)*-caryophyllene, with *β*-Selinene and *α*-Selinene as the chemical markers; C10 and C13, exhibiting high levels of Elemol, *trans*-Nerolidol, *trans-β*-Eudesmol, and (*2Z, 6Z*)-Farnesol, which were indicated as chemical markers, and Cortibel - C1, C2, C3, C4, C5, C6, C8, C9, C12, C14, C15, C16, C17LI, which retained high levels of *α*-Cedrene, *cis-α*-Bergamotene, *α*-Humulene, Humulene epoxide, *epi-α*-Cadinol, *β*-Bisabolol, and *α*-Bisabolol, with *β*-Bisabolol and *α*-Bisabolol as the chemical markers. The use of guava genotypes with different chemotypes, that are agronomically favorable to fruit production and essential oil exploitation adds value to the crop and renders it more sustainable. Given guava crops produce large amounts of leaf biomass, resulting from successive prunings, the extraction of their essential oil, which retains commercially valuable compounds, can be feasible.

## 1. Introduction

*Psidium guajava* L. of the family Myrtaceae, has more than 160 cultivars worldwide, and is valued for the characteristic flavour, aroma and nutritional value of its fruit, in addition to being a potential source of phytochemicals, such as phenols, flavonoids, carotenoids, triterpenes, and essential oil constituents (Geidam et al., 2007; Govaerts et al., 2008; Gutiérrez et al., 2008; Kariawasam et al., 2017; Ngbolua et al., 2018). Some biological properties attributed to the species are related to its essential oil, which retains antimicrobial, larvicidal, insecticidal, and antioxidant activity (Lima et al., 2009; Joseph et al., 2010; Sacchetti et al., 2010; Biswas et al., 2013; Mailoa et al., 2014; Dias et al., 2015; Wang et al., 2017; Mendes et al., 2017). Also, the essential oil of *P. guajava* is predominantly composed of mono- and sesquiterpenes (Silva et al., 2003; Lima et al., 2009; Solórzano-Santos and Miranda-Novales, 2011; Khadhri et al., 2014).

Different chromatographic profiles have been reported regarding the essential oil of *P. guajava*, indicating chemotype variability (Silva et al., 2003; Chen et al., 2007; Cole and Setzer, 2007; Lima et al., 2009; Joseph et al., 2010; Solórzano-Santos and Miranda-Novales, 2011; Khadhri et al., 2014). This variability may be related to genetic factors that determine the occurrence of chemical polymorphism in the species, as well as physiological and environmental factors, which qualitatively and quantitatively affect essential oil composition, namely climatic

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factors, soil composition, plant organs, plant age, seasonality, and circadian cycle (Bakkali et al., 2008; Botrel et al., 2010; Alves et al., 2013).

Due to the occurrence of chemotype variability, awareness of the chemical profile of each genotype or group of genotypes with a similar chemotype, as well as knowledge of the most favorable physiological and environmental conditions, are necessary to assign the different genotypes to possible applications in traditional medicine, chemical and pharmaceutical industries, and for taxonomic studies.

Chemical analyses of essential oils reveal the available compounds, both quantitatively and qualitatively, even when they are present in minimal amounts (ppm). These oils may contain several substances, and their identification may aid in identifying discrepancies among genotypes, groups of similar genotypes (chemotypes), and potential chemical markers (Radulovic and Dekic, 2013; Stesevic et al., 2014; Yapia et al., 2014).

Multivariate analyses can be employed in studies on chemotype diversity of essential oils to investigate differences among genotypes and to select the primary descriptors for their discrimination (Valente et al., 2011; Oliveira et al., 2014). When the type of analysis is selected, the following factors should be considered: higher plants exhibit two metabolic pathways capable of synthesizing essential oil precursors (Phillips, 2008; Dewick, 2009), and these pathways are interrelated and may generate compounds with high correlations among them (multicollinearity) (Valente et al., 2011).

Factor analysis consists of determining a smaller number of new alternative uncorrelated variables that, in some way, summarize the main information of the original variables by identifying latent variables or factors through covariant relations (Belfiore et al., 2006; Bakke et al., 2008; Oliveira et al., 2014). The new groups of variables represent a single construct or factor, or 'super-characteristic', which is responsible for the observed correlations (Hair Junior et al., 2005; Bomfim et al., 2011; Damásio, 2012), thereby eliminating problems of multicollinearity and increasing result reliability.

The objective of the present study was to characterize the chemotype diversity of the essential oil extracted from the leaves of 22 genotypes of *P. guajava* grown in two distinct environments (L = Linhares, Espírito Santo state (ES), and M = Mimoso do Sul, ES), and to identify the different available chemical markers.

## 2. Results and discussion

### 2.1. Essential oil extraction and physical properties

The hydrodistillation process of the leaves from 22 genotypes of *P. guajava*, grown in experimental plantations in two different environments (Linhares and Mimoso do Sul, ES), resulted in slightly viscous, light-yellow essential oils, with a characteristic odor. Significant differences in the oil extraction yield, density, or refractive index were not observed regarding the assessed environments (Table 1).

Genotypes C6, C7, C11, and PAL retained higher average extraction yields (approximately 0.5%), corroborating reports for *P. guajava* genotypes from Tunisia (Khadhri et al., 2014) and China (Wang et al., 2017), while genotypes C10 and C13 showed lower values for the same variable. The average density at 20 °C varied from 0.9033 g $cm^{-3}$ (C7) to 1.0167 g $cm^{-3}$ (C17 and SEC), and the refractive index ranged from 1.4870 (C16) to 1.4995 (C5). These discrepancies, combined with the lack of significant differences between the analyzed environments, indicate a predominantly genotypic influence on the physical properties of the oils. Thus, the density and refractive index parameters may be used for the identification and quality control of essential oils (Bakkali et al., 2008).

### 2.2. Essential oil composition

The gas chromatography-flame ionization detection (GC-FID) and gas chromatography-mass spectrometry (GC-MS) analyses of the essential oil from the leaves, harvested in June of 2013, of the 22 genotypes of *P. guajava* grown in two distinct environments (Linhares - L and Mimoso - M), identified 33 compounds, comprising 87.5–99.0% of the total oil composition (Table 2).

**Table 1**
Average extraction yield and physical properties of essential oils from 22 genotypes of *P. guajava* grown in two different environments (Linhares and Mimoso do Sul, ES).

| Genotypes/ environments | Extraction yield* (% m/m) | | Density* 20 °C (g/$cm^3$) | | Refractive index 20 °C (η) |
|---|---|---|---|---|---|
| | L | M | L | M | |
| C1 | $0.36^{Ab}$ | $0.44^{Ab}$ | $0.9130^{Bd}$ | $0.9133^{Bd}$ | 1.4925 |
| C2 | $0.40^{Ab}$ | $0.44^{Ab}$ | $0.9512^{Bc}$ | $0.9500^{Bc}$ | 1.4965 |
| C3 | $0.50^{Ab}$ | $0.44^{Ab}$ | $0.9589^{Bb}$ | $0.9600^{Bb}$ | 1.4936 |
| C4 | $0.36^{Ac}$ | $0.33^{Ac}$ | $0.9603^{Bb}$ | $0.9633^{Bb}$ | 1.4910 |
| C5 | $0.31^{Ac}$ | $0.29^{Ac}$ | $0.9647^{Bb}$ | $0.9633^{Bb}$ | 1.4995 |
| C6 | $0.50^{Aa}$ | $0.51^{Aa}$ | $0.9300^{Bc}$ | $0.9333^{Bc}$ | 1.4940 |
| C7 | $0.58^{Aa}$ | $0.50^{Aa}$ | $0.9058^{Bd}$ | $0.9033^{Bd}$ | 1.4930 |
| C8 | $0.44^{Ab}$ | $0.44^{Ab}$ | $0.9737^{Bb}$ | $0.9767^{Bb}$ | 1.4950 |
| C9 | $0.41^{Ab}$ | $0.40^{Ab}$ | $0.9841^{Bb}$ | $0.9833^{Bb}$ | 1.4950 |
| C10 | $0.24^{Ad}$ | $0.20^{Ad}$ | $0.9622^{Bb}$ | $0.9620^{Bb}$ | 1.4920 |
| C11 | $0.49^{Aa}$ | $0.54^{Aa}$ | $0.9873^{Ba}$ | $0.9867^{Ba}$ | 1.4950 |
| C12 | $0.31^{Ac}$ | $0.32^{Ac}$ | $0.9904^{Ba}$ | $0.9900^{Ba}$ | 1.4910 |
| C13 | $0.22^{Ad}$ | $0.17^{Ad}$ | $0.9324^{Bc}$ | $0.9300^{Bc}$ | 1.4920 |
| C14 | $0.37^{Ac}$ | $0.30^{Ac}$ | $0.9800^{Bb}$ | $0.9800^{Bb}$ | 1.4970 |
| C15 | $0.41^{Ab}$ | $0.42^{Ab}$ | $0.9952^{Ba}$ | $0.9967^{Ba}$ | 1.4960 |
| C16 | $0.30^{Ac}$ | $0.29^{Ac}$ | $0.9505^{Bc}$ | $0.9500^{Bc}$ | 1.4870 |
| C17 | $0.43^{Ab}$ | $0.44^{Ab}$ | $1.0099^{Ba}$ | $1.0167^{Ba}$ | 1.4930 |
| PAL | $0.56^{Aa}$ | $0.52^{Aa}$ | $0.9928^{Ba}$ | $0.9933^{Ba}$ | 1.4890 |
| P.S | $0.39^{Ab}$ | $0.39^{Ab}$ | $1.0012^{Ba}$ | $1.0000^{Ba}$ | 1.4925 |
| SEC | $0.30^{Ac}$ | $0.33^{Ac}$ | $1.0127^{Ba}$ | $1.0167^{Ba}$ | 1.4925 |
| ROX | $0.37^{Ac}$ | $0.33^{Ac}$ | $0.9700^{Bb}$ | $0.9710^{Bb}$ | 1.4925 |
| PET | $0.40^{Ab}$ | $0.39^{Ab}$ | $0.9705^{Bb}$ | $0.9712^{Bb}$ | 1.4880 |

Extraction yield (% m/m, based on dry biomass); *Data submitted to ANOVA. Means followed by the same lowercase letter in a column do not differ between each other by the Scott & Knott test ($p < 0.05$). Means followed by the same capital letter in a row do not differ between each other by the Scott & Knott test ($p < 0.05$).

Among the 33 obtained substances (Table 2), volatile composites with relative areas greater than 10% were considered major compounds (*(E)-trans*-Caryophyllene, *α*-Humulene, *trans*-Nerolidol, *beta*-Bisabolene, Hinesol, and *beta*-Bisabolol). The essential oil showed a prevalence of sesquiterpenes (80–99%). Monoterpene concentrations were relatively low, except for the genotypes exhibiting elevated concentrations of limonene: C4 (Mimoso = 11.6%; Linhares = 11.6%), C6 (Mimoso = 8.9%; Linhares = 6.6%), C9 (Mimoso = 8.8%; Linhares = 9.7%), C12 (Mimoso = 11.7%; Linhares = 11.0%), and C17 (Mimoso = 5.5%; Linhares = 9.4%).

The chromatographic analysis revealed high concentrations of *(E)-trans*-Caryophyllene in all of the genotypes, regardless of the environment, reflecting their genetic similarity and implicating this compound as a potential chemical marker for the species, corroborating reports by Wang et al. (2017) in genotypes collected from different regions of China. The following substances were present in higher concentrations in the Cortibel genotypes: *β*-Bisabolol, *α*-Bisabolol, *α*-Humulene, Humulene epoxide, and *epi-α*-Cadinol. *β*-Selinene, *α*-Selinene, Hinesol, and 14-Hydroxy-*epi-(E)*-Caryophyllene were considerably abundant in the commercial genotypes (Table 2).

Previous studies have reported different chromatographic profiles regarding the essential oil of *P. guajava*, indicating chemotype variability, corroborating the present results. They also evidenced the importance of caryophyllene oxide (13.8%) (Lima et al., 2009), viridiflorol (36.4%) and *(E)-trans*-caryophyllene (5.9%) (Khadhri et al., 2014), *(E)-trans*-Caryophyllene (ranging from 17.17 to 31.38%, depending on the analyzed *P. guajava* genotype) (Wang et al., 2017), and *β*-bisabolol (19.5%) and Limonene (17.8%) (Mendes et al., 2017).

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