Scientia Horticulturae 216 (2017) 38–44

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Essential oil of *Psidium guajava*: Influence of genotypes and environment

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ARTICLE INFO

Article history:
Received 22 September 2016
Received in revised form
12 December 2016
Accepted 28 December 2016
Available online 5 January 2017

Keywords:
Chemotype variability
Plant breeding
Bioactive compounds
Guava

ABSTRACT

The large amount of biomass resulting of successive prunings in guava crop, can add value to the culture by the extraction of their essential oil, which possess compounds of commercial value. For the first time, the stability of the essential oil from *Psidium guajava* L. was studied in field experiments considering the effects of 22 guava genotypes and two environments. The essential oils from leaves of adult plants, cultivated in experimental design, were characterized via FID-GC–MS analysis and sixteen volatile substances were identified as major compounds. The environments exerted influence in the essential oil profiles from guava, being more expressive in some genotypes (C7, C13 and C17) and more stable in others (C1, C5, C16 and PET). In addition, the oil composition varied among genotypes. The genotypes C10, C13 and SEC showed high levels of *(E)-trans*-Caryophyllene; C3 and C6 of *alpha*-Humulene; and C2, C8, C12, C15 and C16 of *beta*-Bisabolol. These compounds had little influence of environmental and as have biological activity proven, which makes it feasible to use these genotypes in breeding programs, in selection and crossing approaches.

1. Introduction

Ethnopharmacological studies demonstrate the use of *Psidium guajava* L. (Myrtaceae) for medicinal purposes in many parts of the world. In folk medicine, the leaves are used in raw state as well as in the preparation of decoctions, teas and baths (Roth, 2010; Gutiérrez et al., 2008). In Brazil, *P. guajava* is among the main plants of medicinal interest (BRASIL, 2014) owing to containing a range of bioactive compounds with diverse biological properties, such as anti-inflammatory (Ojewole, 2006), anticonvulsant, analgesic, antidiarrheal, antidiabetic and hypoglycemic, antitussive, antihyperlipidemic (Gutiérrez et al., 2008), insecticide (Rajendran and Sriranjini, 2008), antimicrobial (Mohamed et al., 2012; Biswas et al., 2013.) and antioxidant principles (Chen and Yen, 2007).

Most of the biological properties of *P. guajava* are related to its essential oil (Chen et al., 2007), which is generally considered as a unique chemotype for the species. However, the existence of chemotypic variability caused by genetic, environmental and physiological factors must be considered (Alves et al., 2013; Botrel et al., 2010; Bakkali et al., 2008). Different chromatographic profiles have been reported for the essential oil of *P. guajava*, which presupposes the existence of chemotypic variation in the species (Khadhri et al., 2014; Chen et al., 2007; Solórzano-Santos and Miranda-Novales, 2011).

The chemical variations caused by genetic factors contribute decisively to the occurrence of chemical variability in the species. In addition, those caused by physiological and environmental factors, such as climate, soil composition, plant organ, age, seasonality

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http://dx.doi.org/10.1016/j.scienta.2016.12.026
0304-4238/

and circadian cycle, affect the quality and quantity of the plant's essential oil composition (Alves et al., 2013; Botrel et al., 2010; Bakkali et al., 2008). The knowledge about chemotypic variation directs the plant's utilization; this way, characterization and identification of chemotypes from a plant material are required for fine chemistry purposes, pharmacological and agronomic applications (Sandasi et al., 2013; Paula et al., 2011; Potzernheim et al., 2006). In addition can guide strategies of domestication and plant breeding programs (Stesevic et al., 2014; Radulovic and Dekic, 2013). In this context, it allows the identification of genotypes favorable to agronomic fruit production and exploration of the essential oil, which adds value to the crop and makes it more sustainable.

In this work the chemical composition and chromatographic profile of essential oil of 22 *P. guajava* genotypes was studied in two distinct environments, aiming to gather knowledge about existing chemotypic variation in the species.

2. Materials and methods

2.1. Experimental design, plant material and preparation

A randomized block experimental design with four replications and two plants per allotment was adopted in each environment tested. A spacing of 6 m between rows and 4 m between plants was used. Twenty-two genotypes of *P. guajava* were evaluated, of which five were commercial genotypes: Paluma (PAL), Pedro Sato (PS) Século XXI (SEC), Roxa (ROX) and the variety Petri (PET), and 17 were selected from cross-pollination orchard in the state of Espírito Santo (ES, Brazil), denominated: C3–Cortibel 3; C5–Cortibel 5; C7–Cortibel 7; C9–Cortibel 9; C10–Cortibel 10; C11–Cortibel 11; C12–Cortibel 12; C13–Cortibel 13; C16–Cortibel 16; C17–Cortibel 17; C4–Cortibel Branca LG; C8–Cortibel Branca RM; C1– Cortibel LG; C2–Cortibel LM; C14–Cortibel RG; C6–Cortibel RM; C15–Cortibel SLG.

The plants were grown in two environments, with cultivation treatment aiming for fruit production. One environment was located in the South of Espírito Santo in Mimoso do Sul (M) at latitude of 21° 01′ 12.99″ S, longitude of 41° 17′ 13.48″ W and elevation of 250 m; the second site was located in the North of Espírito Santo in Linhares (L) at latitude of 19° 23′ 27″ S, longitude of 41° 04′ 17″ W and altitude of 30 m.

Leaf collection for extraction of the essential oil from the 22 genotypes was carried out during the first production pruning, in two-year old plants, on June 15, 2013 in Linhares and June 21, 2013 in Mimoso do Sul, between 7.00 and 9.00 a.m. Approximately 200 leaves were collected from each of the two plants of the allotments in each of the four repetitions for every genotype. Collection occurred at breast height (1.3 m) and around the crown circumference. The material was packed into paper bags, labeled and transported to the Laboratory of Applied Chemistry of the IFES, Campus of Alegre. Drying of the plant material was accomplished at room temperature in the shade for one week, for moisture removal and stabilization of the enzyme content. The dried leaves were weighed, packed into sealed plastic bags, totaling 176 samples, and stored in refrigerator at −20 °C until essential oil extraction.

2.2. Essential oil extraction

The essential oil was obtained by hydrodistillation in a Clevenger apparatus, according to methodology recommended by the Brazilian Pharmacopoeia (BRASIL, 2010). Whole dried leaves (100 g) were distilled with water (500 mL) for 4 h. The hydrolate was collected and subjected to centrifugation at 5200 × *g* for 10 min. After centrifugation, the oil was removed with the aid of a micropipette. The extraction yield was determined in triplicate for each genotype in all experimental repetitions; the results were expressed in percentage of essential oil mass (g) in the biomass of dry leaf (g) (%, m/m). The extracted essential oils were stored in flasks protected from light at 0 °C for further analyses (Chen et al., 2007).

2.3. Essential oil quantification and characterization

The essential oil samples were analyzed by gas chromatography with flame ionization detection (GC-FID) (Shimadzu GC-2010 Plus) and gas chromatography coupled to mass spectrometry (GC–MS) (Shimadzu GC–MS-2010). The following chromatographic components and conditions were applied in both analyses: fused-silica capillary columns (30 m × 0.25 mm) with RTX-5MS as stationary phase (0.25 μm thick film); N_2 (in GC-FID analysis) or He (in GC–MS analysis) as carrier gas with flow of 3.0 mL min^{-1}; oven temperature program of one minute at initial temperature of 60 °C, followed by gradual increases of 5 °C min^{-1} until reaching 220 °C, remaining at this temperature for 10 min; injector temperature of 240 °C; detector temperature of 240 °C; and split ratio of 1:30. A volume of 1.0 μL of solution was injected, containing 3% essential oil dissolved in hexane with DMA 0.1 mol L^{-1} (external standard for reproducibility control) for the GC-FID analyses, or 3% essential oil in dichloromethane for the GC–MS analyses. The latter analyses were performed in equipment operating by electronic impact, with impact energy of 70 eV; scanning speed of 1000; scanning interval

Table 1
Identification of the major compounds found in the essential oil of 22 *Psidium guajava* genotypes grown in two environments (Linhares – ES and Mimoso do Sul – ES, Brazil) by IR and GC–MS.

n.	Compounds	RT	IRCal	IRTab	*m/z* (Relative Intensity)
1	**Limonene**	7.906	1031	1029	M+· = 136; 121(23); 107(22); 93(72); 67(72); 41(18); 68(100)
2	**Eucalyptol**	7.993	1035	1031	M+· = 154; 139 (35); 108 (53); 81 (65); 43 (100)
3	**trans-Caryophyllene**	18.672	1420	1418	M+· = 204; 189(19); 133(89); 69(87); 41(76); 93(100)
4	**α-Humulene**	19.518	1454	1452	M+· = 204; 147(23); 121(25); 80(32); 93(100)
5	**γ-Muurolene**	20.156	1479	1478	M+· = 204; 161 (13); 134 (16); 105 (39); 93 (59); 79 (19); 119 (100)
6	**β-Selinene**	20.341	1486	1486	M+· = 204; 189 (44); 161 (63); 121 (61); 93 (96); 79 (71); 41 (49); 105 (100)
7	**α-Selinene**	20.546	1495	1494	M+· = 204; 161 (55); 133 (79); 107 (78); 93 (87); 81 (62); 41 (39); 189 (100)
8	**β-Bisabolene**	20.802	1506	1506	M+· = 204; 161 (26); 119 (36); 109 (33); 93 (95); 41 (65); 69 (100)
9	**trans-Nerolidol**	22.071	1560	1561	161 (24); 136 (27); 107 (43); 93 (71); 41 (62); 69 (100)
10	**Caryophyllene oxide**	22.686	1585	1583	161 (36); 121 (46); 109 (64); 93 (81); 79 (75); 55 (48); 43 (100)
11	**epi-β-Cubenol**	23.750	1633	1635	M+· = 222; 204 (22); 161 (23); 105 (30); 93 (42); 59 (25); 119 (100)
12	**epi-α-Cadinol**	23.824	1637	1638	200 (21); 157 (20); 135 (98); 132 (43); 91 (39); 69 (37); 43 (100)
13	**Hinesol**	23.935	1642	1643	204 (52); 134 (17); 119 (26); 105 (36); 95 (45); 81 (32); 59 (13); 161 (100)
14	**14-hydroxy-9-epi-(E)-Caryophyllene**	24.383	1661	1660	204 (70); 189 (47); 161 (53); 135 (77); 95 (64); 43 (99); 81 (100)
15	**β-Bisabolol**	24.632	1672	1671	M+· = 222; 204 (20); 119 (43); 111 (44); 93 (67); 69 (43); 82 (100)
16	**α-Bisabolol**	24.933	1685	1685	M+· = 222; 204 (20); 109 (15); 93 (67); 69 (43); 43 (27); 119 (100)

RT (retention time – minutes); IRcal (Calculated Retention Index); IRtab (Tabular Retention Index) and *m/z* (mass/charge and relative intensity).