

Full Length Research Paper

Study of seasonality and location effects on the chemical composition of essential oils from *Eugenia uniflora* leaves

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The essential oils of *Eugenia uniflora* leaves possess several biological activities but a great variability in their chemical composition is observed. In the present study, gas chromatography and mass spectrometry were applied to examine the essential oil from leaves of four different specimens over the seasons of the year, two of which are located in a habitat of a Brazilian metropolis, and the other two in a natural reserve. The collected data allowed identifying twenty-nine compounds; an aliphatic ketone, sesquiterpenes, fatty acids, hydrocarbons, and phthalate derivatives. Sesquiterpene hydrocarbons and oxygenated sesquiterpenes were the chemical classes prevailing in most samples. The curzerene was observed in higher content (10.5-53.4%) in all samples in which the presence of sesquiterpenes class was confirmed. Phthalate derivatives were identified for the first time in the essential oil of *E. uniflora* leaves. The occurrence of a common chemical marker for four specimens was not observed. Besides, no compound was observed in the same specimen throughout the seasons, and specimens of the same habitat exhibited different essential oil chemical profiles. According to the multivariate analysis applied to the chemical profile for all individuals in different seasons, four different clusters were identified without dependence on season or location.

Key word: *Eugenia uniflora*, seasonality, location, essential oil, chemical composition.

INTRODUCTION

The Myrtaceae family is comprised of more than 140 genera and about 5744 species distributed in different regions of the world (The Plant List, 2013a). *Eugenia* is the largest genus containing about 1011 species, of

which 391 occur in Brazilian territory (COPPETEC-UFRJ, 2020; The Plant List, 2013b). *Eugenia uniflora* ('pitanga'), *Eugenia involucrata* ('cherry'), and *Eugenia caryophyllata* ('clove') are the most well-known species due to their

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importance in gastronomy and cosmetics. However, interesting pharmaceutical properties have been also reported involving their essential oils leaves. Specifically, *E. uniflora* is widely and popularly used due to its medicinal properties (Sobeh et al., 2019). Several pharmacological activities are reported for the essential oils from *E. uniflora* leaves such as cytotoxic, antibacterial (Figueiredo et al., 2019; Sobeh et al., 2016), antifungal (Siega, 2018; Souza et al., 2018), leishmanicidal (da Silva et al., 2018), molluscicide (Pinheiro et al., 2017), analgesic (Savegnago et al., 2015), antioxidant (Victoria et al., 2012), larvicide (Leite et al., 2009), repellent (Lobo et al., 2019), antiproliferative (Gomes et al., 2020), anti-inflammatory (Falcão et al., 2018), antinociceptive and hypothermic (Amorim et al., 2009). Some of these activities were associated with the leaf oil compounds, such as selina-1,3,7(11)-trien-8-one (36.37 %) and selina-1,3,7(11)-trien-8-one epoxide (27.32%) (dos Santos et al., 2018). The essential oil of *E. uniflora* leaf with red fruit, which inhibited the yeast form of *P. brasiliensis* at a concentration of 62.5 µg/ml, showed as major constituents curzerene (42.0-43.2%), germacrene D (8.7-9.0%), and germacrene A (5.9-8.9%) (Costa et al., 2010). The sesquiterpenes selina-1,3,7(11)-trien-8-one and oxidoselina-1,3,7(11)-trien-8-one isolated from the essential oil of *E. uniflora* leaves showed pro-oxidative activity and cytotoxicity in relation to the IMR90 cell line (Ascari et al., 2021).

Some studies have addressed the effect of the location or the leaves collection period on the chemical profile. For example, in the essential oil of *E. uniflora* leaves collected in Nigeria, was observed the presence of caryophyllene (5.7%), germacrene B (5.8%), seline oxide-1,3,7 (11)-trien-8-one (14%), seline-1,3,7 (11)-trien-8-one (17%) and furanediene (24%) (Weyerstahl et al., 1988). On the other hand, the essential oil leaves collected in the northeast region of Brazil showed seline-1,3,7 (11)-trien-8-one oxide (17.3%) and seline-1,35 (11)-trien (48.5%) as the main components (de Moraes et al., 1996). Moreover, the constitution of essential oil leaves collected at Belém (PA, Brazil) presented germacrene B (15.6%), germacrene and curzerene (30%) (Maia et al., 1999). According to Amorim et al. (2009), the essential oil also collected at Rio de Janeiro showed mainly the presence of atracylone, 3-furanoeudesmene, spathulenol, β-elemene, γ-elemene, globulol, ledene, and β-caryophyllene. The other side, the essential oil collected at Rio de Janeiro too (Brazil), presented as major compounds (+/-seline-1,3,7 (11)-trien-8-one and (+/-)-seline-1,3,7 (11)-trien-8-one epoxide (Marques et al., 2018). Due to the great variability in the essential oil chemical composition in different locations, some studies involving the search for understanding this problem can be found in the literature. However, the multivariate data analysis, specifically, Principal Component Analysis (PCA) has been extensively used to establish similarities and differences within groups of complex mixtures such as essential oils (Stashenko et al., 2010). Considering the

use and the potential of *E. uniflora* essential oils in cosmetics area and or for ethnomedicinal purposes, it is important to understand the secondary metabolites production for the pharmaceutical and therapeutic industry benefits. In this sense, this work aims to evaluate the chemical profile of oils leaves over a year and try to establish correlations between different habitats of individuals.

MATERIALS AND METHODS

Plant material

E. uniflora leaves (about 60-70 g) from four specimens were collected on the tenth day after the beginning of each season 2018, two of them occurring near the Ayrton Senna highway in the metropolitan city of Sao Paulo, adjacent to the School of Arts, Sciences and Humanities, the University of Sao Paulo (EACH-USP), according to the geographical coordinates: Lat: 23°29'091" S, Long: 46°30'301" O and Alt: 734 m for **Eu1** and Lat: 23°29'097" S, Long: 46°30'306" O (voucher SPFE 711) and Alt: 732 m for **Eu3** (voucher SPFE 712). The other two selected specimens are located in a preserved region in the Municipal Park of Mogi das Cruzes (SP), according to the coordinates: Lat: 23°29'294" S, Long: 46°11'683" O and Alt: 867m for **Eu2** (voucher SPFE 713) and Lat: 23°29'286" S, Long: 46°11'684" O and Alt: 848 m for **Eu4** (voucher SPFE 714). The botanical identification was carried out by Dra. Fabiana Pioker (EACH, USP- East Campus), and dried samples of *E. uniflora* are deposited at the SPF Herbarium of the School of Arts, Science, and Humanities.

Leaf essential oils extraction

The hydrodistillation technique was applied to obtain the volatile oil from the fresh leaves using a Clevenger-type apparatus. To extract the essential oils from each sample studied, 50 g of fresh leaves were ground and mixed with 1 L of water. The mixture was transferred to a 3 L round-bottom flask placed on a heating mantle connected to a condenser. After 4 h of heating, the essential oil was collected and dried over anhydrous sodium sulfate (ca. 1 g) (Moraes et al., 2019). All these processes were performed once a time with each leaf sample. The essential oils were maintained in sealed vials in a freezer at - 27°C to preserve the chemical constituents.

Chromatographic analysis

The chromatographic analyses of the leaf essential oils were performed in a chromatographic system coupled to Shimadzu and Model QP 2020 mass spectrometer. The optimized operating conditions for these analyses were: ZB5HT capillary chromatographic column (30 × 0.25 mm × 0.25), mobile phase flow rate (He): 2.5 ml/min; injector Split ratio 1/25, and the injection volume of 3 µl. The operating temperatures of the equipment are as follows: injector at 260°C, detector at 280°C, and column at programmed temperature starting at 60°C for 1 min, 3°C/min rise to 220°C, later rise from 10°C/min to 280°C and remaining for 1.67 min. The conditions of the mass spectrometer employed were scan detector 1,000; scanning interval of 0.50 fragments and fragments detected in the range of 40 to 550 Da. To identify the chemical composition of the essential oil, two NIST107 and NIST21 libraries were used to compare the mass spectra data. The identification of each peak was assigned only when the similarity was above 80%.

The retention index was calculated by reference to a solution of

the C₈-C₂₂ homologous series by the Van den Dool and Kratz equation (van Den Dool and Dec. Kratz, 1963). To obtain the relative percentages (%) by peak area normalization of the identified compounds, all samples were analyzed in gas chromatography with a flame ionization detector (GC-FID) using an Agilent CG 6850 system. GC was equipped with the same chromatographic conditions used in the CG-MS analysis.

Principal components analysis (PCA) and hierarchical cluster analysis (HCA) for identified chemical compounds in extracts

PCA and HCA from chemical profile (% w/w) of all essential oil obtained in different seasons and locations (Table 1) were performed. Both analyses aimed to identify similarities and differences among the samples, as well as the chemical reasons for samples' grouping. Scaling and mean-centering to the data were applied to the data before PCA. Wards method was used for HCA. The multivariate data analyses were executed in SIMCA 16 software (Trial version, Sartorius Stedim Data Analytics AB Umeå, Sweden).

RESULTS AND DISCUSSION

Descriptive analysis of primary data

To evaluate the chemical profile of essential oil leaves among individuals of the same habitat, the identified chemical constituents and their contents are presented in Table 1. Thirty compounds present in the essential oil leaf of specimens Eu1, Eu2, Eu3, and Eu4 were identified in the four seasons of the year by CG-MS analysis. The essential oils predominantly contain sesquiterpenes, phthalate derivatives, hydrocarbons, fatty acids, and some of their methyl esters. According to the chemical constituents' data, the essential oils of *E. uniflora* leaves indicate the expressive presence of sesquiterpenes, while the Wi3 and Wi4 samples showed the identification of only hydrocarbons and/or phthalate derivatives. The Sp1 oil is comprised 38.1% of sesquiterpene hydrocarbons and 49.7% of oxygenated sesquiterpenoids. Nine components in total were identified in the Sp2, which were equal to 61.0% of its content. The chemical profile of the Sp2 oil was constituted mostly by 51.3% of oxygenated sesquiterpenes and 13.4% of sesquiterpene hydrocarbons. The Sp3 essential oil presented 52.1% of oxygenated sesquiterpenes and 4.1% of sesquiterpene hydrocarbons being that germacra-3,7 (11), 9-trien-6-one (27.0%) was the major chemical compound. The sp4 oil consisted mainly of 36.5% curzerene, followed by α -guaiene (7.7%) and δ -maaliene (2.7%). The sesquiterpene hydrocarbons (49.8%) were the major components in the su1 essential oil composition and 32.0% of oxygenated sesquiterpenes, mainly as β -elemenone (35.6%), spathulenol (16.3%), germacra-3,7 (11),9-trien-6-one (15.7%). In the Su2 oil only two components were identified: eudesma-4(14),11-diene (8.4%), and α -selinene (3.6%). Oxygenated sesquiterpenes (51.6%) were the major components in the Su3 oil and sesquiterpene hydrocarbons (36.1%).

Curzerene (26.2%) was the major constituent in this oil, followed by germacra-3,7 (11),9-trien-6-one (23.4%) and β -elemenone (21.8%). In Su4 oil, oxygenated sesquiterpenes (50.5%) and sesquiterpene hydrocarbons (22.5%) were observed. The predominant component in this oil was curzerene (46.6%), followed by caryophyllene (8.4%) and β -elemene (8.2%). Sesquiterpene hydrocarbons (22.4%) were the major components in the Au1 oil composition, followed by 12.2% by oxygenated sesquiterpenes. This oil composition was marked by the presence of germacra-3,7 (11),9-trien-6-one (21.7%), β -elemenone (16.3%), spathulenol (4.9%), viridiflorol (4.6%), β -elemene (4.1%) and caryophyllene (2.0%). The chemical composition of the au2 oil showed 53.1% of oxygenated sesquiterpenes and 9.9% of sesquiterpene hydrocarbons. The oxygenated sesquiterpene curzerene was the major component in this oil (46.2%). In the Au3 was identified only the bis(2-ethylhexyl) phthalate (58.2%). Only two oxygenated sesquiterpenes, caryophyllene oxide (9.7%) and spathulenol (6.3%) were identified in the Au4 oil. The chemical composition of Wi1 oil showed 38.8% of oxygenated sesquiterpenes and 7.7% of sesquiterpene hydrocarbons. The oxygenated sesquiterpene curzerene was the major component (20.5%). The curzerene is the main component (53.4%) identified in the Wi2 oil. Atypical chemical composition was observed to the Wi3 oil which showed mainly the presence of fatty acid derivatives (29.7%), phthalate derivatives (29.0%), hydrocarbons (26.2%), and only one oxygenated sesquiterpene (1.9%). The literature reports the importance of fatty acids as an important source of the reserve of energy and the essentiality on the membrane lipids composition of all living organisms, like the plants. Specifically, the role presence of fatty acids in plants involves metabolic pathways to pathogen defence or as a biosynthetic precursor for cuticular components (Kachroo and Kachroo, 2009). Thus, in this case, individuals 2 and 3 can undergo a possible attack from a predator and or the specimens(s) need to store energy during the drought in the winter period.

Another atypical chemical profile was observed in the Wi4 oil, since only phthalate and fatty acids derivatives were identified. Phthalate derivatives were also detected in Eu3 during fall and winter. Phthalate derivatives have been recognized as contaminants to be utilized as plasticizers and environmental hazards, particularly those with reduced molecular weight, and can be observed in the essential oil constitution (Manayi et al., 2014; Roy, 2020). According to Chen (2004), the difference between the natural and synthetic phthalate derivatives is the abundance of ¹⁴C. Phthalic acid dioctyl ester (30) was isolated from *Nigella glandulifera* and *Eichhornia crassipes* (Nguyen et al., 2007; Shanab et al., 2010); while bis(2-ethylhexyl) phthalate (29) was isolated from *N. glandulifera* (Nguyen et al., 2007). There are various bioactivities of these natural phthalates derivatives such as antimicrobial, antioxidant, antitumor, larvicidal, and others (Roy, 2020).

Table 1. Individual compounds and main chemical classes identified in essential oil from *E. uniflora* in different seasons by CG-MS analysis and their percentage (% relative area).

Compounds' coding	Name of the identified compound	RI (library)	RI (experimental)	Individuals*																
				Sp1	Sp2	Sp3	Sp4	Su1	Su2	Su3	Su4	Au1	Au2	Au3	Au4	Wi1	Wi2	Wi3	Wi4	
1	2-Butanone	313	NC	0.0	0.0	0.0	0.0	1.3	0.0	0.0	1.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
2	δ-Maaliene	1380	1377	0.0	0.0	0.0	2.7	0.0	0.0	0.0	0.0	0.0	1.8	0.0	0.0	0.0	0.0	0.0	0.0	
3	b-Elementene	1387	1391	5.8	4.8	2.5	0.0	0.0	0.0	5.0	8.2	4.1	6.3	0.0	0.0	5.5	0.0	0.0	0.0	
4	Caryophyllene	1420	1415	2	0.0	0.0	0.0	0.0	0.0	0.0	8.4	2.0	1.8	0.0	0.0	0.0	0.0	0.0	0.0	
5	α-Guaiene	1439	1440	0.0	0.0	1.6	7.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
6	g-Elementene	1445	1431	3.9	2.8	0.0	0.0	0.0	0.0	5.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
7	7-Isopropenyl-1-methyl-4-methylene decahydroazulene	1456	1495	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
8	Eudesma-4(14),11-diene	1476	1480	0.0	3	0.0	0.0	5.4	8.4	0.0	2.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
9	Germacrene D	1482	1476	0.0	0.0	0.0	0.0	0.0	0.0	1.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
10	α-Selinene	1494	1489	0.0	2.8	0.0	0.0	0.0	3.6	0.0	2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
11	Curzerene	1495	1498	34.2	37.7	10.5	36.5	0.0	0.0	26.2	46.6	0	46.2	0.0	0.0	20.5	53.4	0.0	0.0	
12	Guaia-1(10),11-diene	1500	1508	4.5	0.0	0.0	0.0	3.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.2	0.0	0.0	0.0	
13	Selina-3,7(11)-diene	1538	1541	0.0	0.0	0.0	0.0	3.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.0	
14	Germacrene B	1557	1551	9.5	0.0	0.0	0.0	0.0	0.0	1.9	1.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
15	<u>Spathuleno</u>	1578	1579	0.0	3.4	9.3	0.0	16.3	0.0	1.0	2.0	4.9	3.5	0.0	6.3	4.9	0.0	0.0	0.0	
16	Caryophyllene oxide	1581	1579	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.8	0.0	9.7	0.0	0.0	0.0	0.0	
17	Viridiflorol	1595	1593	2.6	2.4	5.3	0.0	0.0	0.0	1.0	0.0	4.6	0.0	0.0	0.0	3.6	0.0	0.0	0.0	
18	β-Elementene	1597	1600	14.4	0.0	0.0	0.0	35.6	0.0	21.8	0.0	16.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
19	Atractylon	1652	1657	3.3	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.6	0.0	0.0	0.0	0.0	0.0	0.0	
20	Germacra-3,7 (11),9-trien-6-one	1693	1695	9.6	4.8	27.0	0.0	15.7	0.0	23.4	1.9	21.7	0.0	0.0	0.0	9.8	0.0	0.0	0.0	
21	Myristic acid	1768	1770	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.9	2.2	
22	Palmitic methyl ester	1934	1931	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.0	
23	Palmitic acid	1970	1973	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	16.9	13.4	
24	Margaric acid methyl ester	2008	2011	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	16.0	
25	phthalic acid, 2,4-Dimethylpent-3-yl isobutyl ester	2055	2050	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	15.8	0.0	
26	Heneicosane	2100	2108	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	12.7	0.0	
27	Stearic acid	2158	2156		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	10.9	10.9	
28	n-Pentacosane	2500	2486	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	13.5	0.0	
29	Bis(2-ethylhexyl) phthalate	2552	2555	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	58.2	0.0	0.0	0.0	0.0	0.0	0.0	
30	Phthalic acid dioctyl ester	2741	NC	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	13.2	13.2	
Total identified compounds (%)				89.8	64.7	56.2	46.9	81.5	12.0	87.7	74.2	53.6	63.0	58.2	16.0	46.5	53.4	84.9	61.7	
Class of compounds																				
Sesquiterpene hydrocarbons (Sr. N° 2-10, 12-14)				25.7	13.4	4.1	10.4	12.6	12.0	14.3	22.5	6.1	9.9	-	-	7.7	-	-	3.0	

Table 1. Contd.

Oxygenated sesquiterpenes (Sr. Nº 11, 15-20)	64.1	51.3	52.1	36.5	67.6	-	73.4	50.5	47.5	53.1	-	16.0	38.8	53.4	-	-
Phthalate derivatives (Sr. Nº 25, 29-30)	-	-	-	-	-	-	-	-	-	-	58.2	-	-	-	29.0	13.2
Hydrocarbons (Sr. Nº 26, 28)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	26.2	-
Fatty acids and methyl esters (Sr. Nº 21-24, 27)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	29.7	45.5
Aliphatic ketone (Sr. Nº 1)	-	-	-	-	1.3	-	-	1.2	-	-	-	-	-	-	-	-

*Individuals' coding: Sp, Su, Au, Wi stand for spring, summer, autumn, and winter seasons, respectively. 1,2,3 and 4 refer to the four different locations of vegetable material harvest, Eu1, Eu2, Eu3, and Eu4, respectively RI: Retention Index; NC: Uncalculated, because the elution of the compound was before the last hydrocarbon of the standard or after de first one.

In our case, the phthalate derivatives in some samples exhibited a high percentage, even higher than the sesquiterpene present in the essential oil. According to Table 1, these compounds' class were the majority in the Wi3 and Wi4 samples. The results obtained from the essential oil analysis of four different individuals, indicate the absence of a biomarker compound, even in individuals from the same habitat. According to Costa et al. (2009), the seasonal study of the essential oils of *E. uniflora* leaves showed the presence of spathulenol and caryophyllene oxide in dry seasons and seline 1,3,7 (11)-trine-8-one epoxide as the major compound in rainy seasons. However, our data showed the occurrence curzerene in all individuals during spring, but not in other seasons. Moreover, it was the major metabolite or one of it in the composition of the Sp1, Sp2, Sp3 Sp4, Su3, Su4, Au2, Wi1, and Wi2 oils (9.7- 53.4%). The individual Eu3 showed a considerable increase in curzerene content from spring to summer (10.5-26.2%) and this was not observed in the other seasons. The highest content was observed during the winter (53.4%) for specimen 2.

The data presented in Table 1, it confirmed the predominance of several sesquiterpenes in different seasons for the studied individuals, except in winter for specimens 3 and 4, and autumn for 3. In the winter it was observed the

presence of fatty acid derivatives. This is the first report of phthalate derivatives occurrence in the essential oil of *E. uniflora* leaf. The literature reports the presence of these compounds in the essential oil of other plants such as *Silybum marianum* seeds, *Rizophora flowers* (Saranya et al., 2015), and *Calycotome villosa subsp. intermedia* (Chikhi et al., 2014). There was no similarity between the chemical compositions of specimens from the same habitat. According to De Morais et al. (1996), the chemical composition of essential oils may differ depending on the necessity of the plant to adapt to its habitat and due to chemotypes.

Principal components analysis (PCA) and hierarchical cluster analysis (HCA) for identified chemical compounds in essentials oils

The five more atypical samples were Wi3 (Group 1), Wi4 (Group 1), Su1 (Group 2), Sp1 (Group 3), and Su3(Group 3). The remaining samples showed a similar chemical profile (Figure 1). This finding is also confirmed by the scores plot (Figure 2A). Overlapping score and loading plots (Figure 2A and B) with support of biplot graph for the first two principal components (Figure 3) is possible to identify that the Group 1 samples, which are

from different locations in winter, were relatively plenty of 4 common compounds myristic acid (21), palmitic acid (23), stearic acid (27) and phthalic acid dioctyl ester (30). However, the Wi3 sample was also abundant in phthalic acid 2,4-dimethylpent-3-yl isobutyl ester (25), heneicosane (26), and *n*-pentacosane (28), while the Wi4 sample was relatively rich in palmitic methyl ester (22) and margaric acid methyl ester (24). Su1 (Group 2) was comprised mainly by 2-butanone (1), guaia-1(10),11-diene (12), spathulenol (15), β -elemenone (18), and germacra-3,7 (11),9-trien-6-one (20). Group 3 samples from the same location and different seasons (spring and summer) were rich in γ -elemene (6), germacrene B (14) and viridiflorol (17). A significant number of samples had a high amount of curzerene (Table 1). This volatile oxygenated sesquiterpene has been identified as one of the major constituents in the essential oils of *E. uniflora* leaves, especially, when are derived from bright red fruit specimens (Costa et al., 2010).

According to the literature, plants can exhibit changes on their chemical composition by the high CO₂ levels, such as the increase monoterpene concentrations (Idso and Idso, 2000). However, the essential oils from individuals 1 and 3, located in area with high concentration of CO₂ by vehicles emission, were not affected the secondary metabolites production. The PCA and

Figure 1. Dendrogram corresponding to HCA for the identified compounds by relative area percentage (%) of CG-MS analysis.

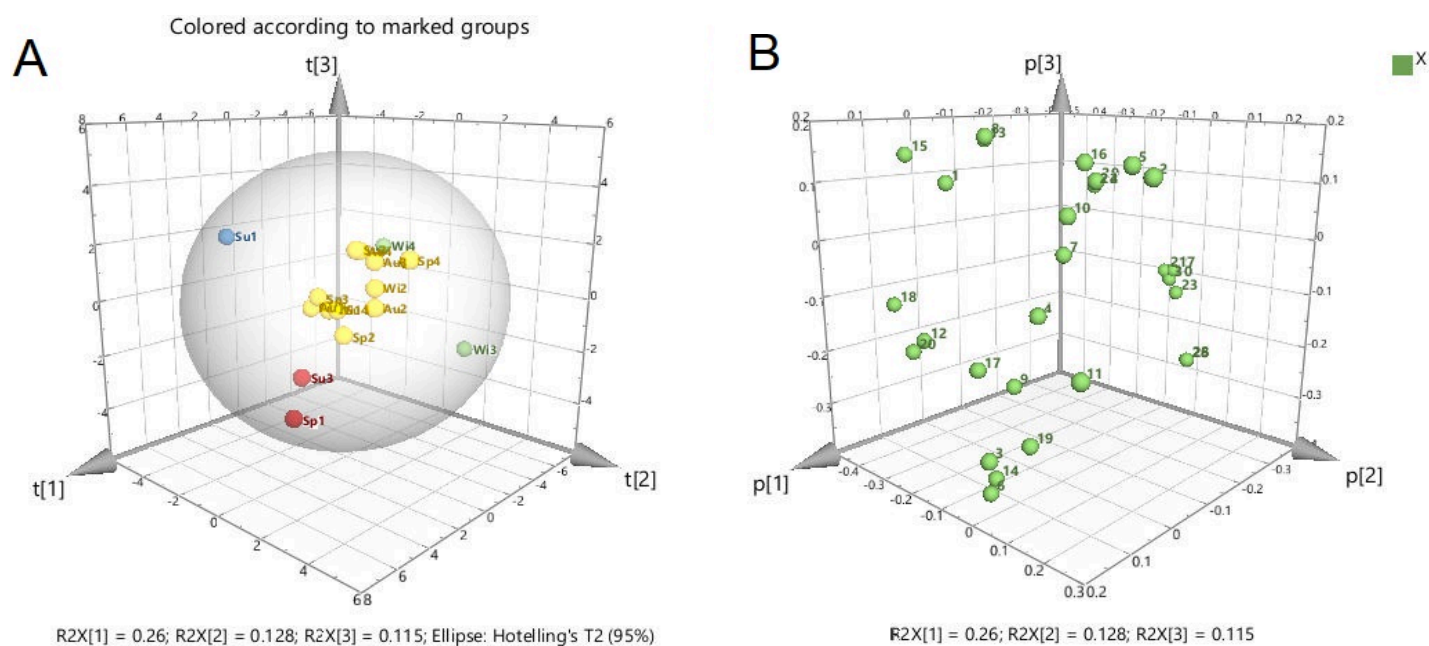


Figure 2. Score scatter (A) and loading (B) 3D plots corresponding to PCA for the identified compounds in the essential oil samples using relative peak area (%). The four main groups categorized by HCA were also identified using the same color scale (Figure 1).

HCA data showed the season and location are not the major factors to influence the chemical variability of essential oil composition from *E. uniflora* leaves. This chemical variability can be associated to the ecosystem differences, not only by the biotic and abiotic factors. The secondary metabolites production can depend on the

climate changes, which can influence on the soil microflora, all pollinisers and other insects affecting the plant ontogeny, adaptation, and including phytochemicals productivities (Thakur et al., 2019).

This should be taken into consideration for commercial applications, specifically in perfumery, where the

Figure 3. Biplot graph for the two first principal components of the chemical profile in “mass percentage (% w/w)” of identified compounds in essential oils. Individuals and chemicals are represented by hexagons and circles, respectively. The coding of figure elements is described in Table 1 and the groups' color in Figure 1

chemical composition of this feedstock has a paramount impact on final product quality attributes (Gallucci et al., 2010). Also, the essential oil of *E. uniflora* leaves is strongly recommended because of its powerful industrial or pharmaceutical properties, the high variability of essential oils chemistry from different specimens, should be considered during the chemical synthesis or biotechnological products manufacturing (Ochoa-Villarreal et al., 2016). Besides, none of the groups found by the multivariate techniques matched the chemotypes reported for this species (Costa et al., 2009).

Conclusion

In all essential oil samples from *E. uniflora* leaves, sesquiterpene hydrocarbons and oxygenated sesquiterpenes were prevalent, except for two specimens during the winter and autumn seasons. The curzerene, an oxygenated sesquiterpene, was the most abundant compound in samples with sesquiterpenes. The chemical compositions of specimens from the same habitat are different and without correlations, and there are no direct influence of metabolites production according to the type habitat. Considering the chemical profile variability of essential oils among specimens by principal components analysis, commercial applications should be manufactured using other approaches.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

- Amorim ACL, Lima CKF, Hovell AMC, Miranda ALP, Rezende CM (2009). Antinociceptive and hypothermic evaluation of the leaf essential oil and isolated terpenoids from *Eugenia uniflora* L. (Brazilian pitanga). *Phytomedicine* 16(10):923-928. Available at <https://doi.org/10.1016/j.phymed.2009.03.009>.
- Ascari J, Pereira MFM, Schaffka VM, Nunes DS, Magalhães CG, Santos JS, Granato D, do Carmo MAV, Luciana Azevedo, Archilha MVL, Scharf DR (2021). Selina-1,3,7(11)-trien-8-one and Oxidoselina-1,3,7(11)-trien-8-one from *Eugenia uniflora* leaf essential oil and their cytotoxic effects on human cell lines. *Molecules* 26(3):740. Available at <https://doi.org/10.3390/molecules26030740>.
- Chen CY (2004). Biosynthesis of di-(2-ethylhexyl) phthalate (DEHP) and di-n-butyl phthalate (DBP) from red alga—*Bangia atropurpurea*. *Water Research* 38(4):1014-1018. Available at <https://doi.org/10.1016/j.watres.2003.11.029>.
- Chikhi I, Allali H, Bechlaghem K, Fekih N, Muselli A, Djabou N, Dib MEA, Tabti B, Halla N, Costa J (2014). Assessment of in vitro antimicrobial potency and free radical scavenging capacity of the essential oil and ethanol extract of *Calycotome villosa* subsp.

- intermedia growing in Algeria. *Asian Pacific Journal of Tropical Disease* 4(5):356-362. Available at [https://doi.org/10.1016/S2222-1808\(14\)60587-9](https://doi.org/10.1016/S2222-1808(14)60587-9).
- COPPETEC-UFRJ (2020). Flora do Brasil 2020 [WWW Document]. Flora do Brasil 2020-Algas, Fungos e Plantas. URL <http://floradobrasil.jbrj.gov.br/reflora/listaBrasil/ConsultaPublicaUC/ConsultaPublicaUC.do#CondicaoTaxonCP> (accessed 6.6.20).
- Costa DP, Alves-Filho EG, Silva LMA, Santos SC, Passos XS, Silva M do RR, Seraphin JC, Ferri PH (2010). Influence of fruit biotypes on the chemical composition and antifungal activity of the essential oils of *Eugenia uniflora* leaves. *Journal of the Brazilian Chemical Society*, 21(5):851-858. Available at <https://doi.org/10.1590/S0103-50532010000500012>.
- Costa DP, Santos SC, Seraphin JC, Ferri PH (2009). Seasonal variability of essential oils of *Eugenia uniflora* leaves. *Journal of the Brazilian Chemical Society* 20(7):1287-1293. Available at <https://doi.org/10.1590/S0103-50532009000700013>.
- da Silva VP, Alves CCF, Miranda MLD, Bretanha LC, Balleste MP, Micke GA, Silveira EV, Martins CHG, Ambrosio MALV, de Souza Silva T, Tavares DC, Magalhães LG, Silva FG, Egea MB (2018). Chemical composition and in vitro leishmanicidal, antibacterial and cytotoxic activities of essential oils of the Myrtaceae family occurring in the Cerrado biome. *Industrial Crops and Products* 123:638-645. Available at <https://doi.org/10.1016/j.indcrop.2018.07.033>.
- de Morais SM, Craveiro AA, Machado MIL, Alencar JW, Matos FJA (1996). Volatile constituents of *Eugenia uniflora* leaf oil from Northeastern Brazil. *Journal of Essential Oil Research* 8(4):449-451. Available <https://doi.org/10.1080/10412905.1996.9700664>.
- dos Santos JFS, Rocha JE, Bezerra CF, Silva MKN, de Matos YMLS, de Freitas TS, dos Santos ATL, da Cruz RP, Machado AJT, Rodrigues THS, de Brito ES, Sales DL, Almeida WO, da Costa JGM, Coutinho, HDM, Morais-Braga MFB (2018). Chemical composition, antifungal activity and potential anti-virulence evaluation of the *Eugenia uniflora* essential oil against *Candida* spp., *Food Chemistry* 261:233-239.
- Falcão TR, de Araújo AA, Soares LAL, de Moraes Ramos RT, Bezerra ICF, Ferreira MRA, de Souza Neto MA, Melo MCN, de Araújo RF Jr, de Aguiar Guerra ACV, de Medeiros JS, Guerra GCB (2018). Crude extract and fractions from *Eugenia uniflora* Linn leaves showed anti-inflammatory, antioxidant, and antibacterial activities. *BMC Complementary Alternative Medicine* 18:84. Available at <https://doi.org/10.1186/s12906-018-2144-6>.
- Figueiredo PLB, Pinto LC, da Costa JS, da Silva ARC, Mourão RHV, Montenegro RC, da Silva JKR, Maia JGS (2019). Composition, antioxidant capacity and cytotoxic activity of *Eugenia uniflora* L. chemotype-oils from the Amazon. *Journal of Ethnopharmacology* 232:30-38. Available at <https://doi.org/10.1016/j.jep.2018.12.011>.
- Gallucci S, Neto AP, Porto C, Barbizan D, Costa I, Marques K, Benevides P, Figueiredo R (2010). Essential oil of *Eugenia uniflora* L.: an industrial perfumery approach. *Journal of Essential Oil Research* 22(2):176-179. Available at <https://doi.org/10.1080/10412905.2010.9700296>.
- Idso CD, Idso KE (2000). Forecasting world food supplies: The impact of rising atmospheric CO2 concentration. *Technology* 7:33-55.
- Gomes DB, Zanchet B, Serpa PZ, Locateli G, Miorando D, Steffler AM, Zanatta ME de C, Predebon AJ, Carteri CS, Lutinski J, Ruiz ALTG, Santos M de F da C, Barison A, Junior WA R (2020). Chemical analysis and in vitro antiproliferative potential of *Eugenia uniflora* L. (Myrtaceae). *European Journal of Medicinal Plants* 31(6):34-44. Available at <https://doi.org/10.9734/ejmp/2020/v31i630246>.
- Kachroo A, Kachroo P (2009). Fatty Acid-Derived Signals in Plant Defense. *Annual Review of Phytopathology* 47:153-176. Available at <https://doi.org/10.1146/annurev-phyto-080508-081820>.
- Leite AM, de O Lima E, de Souza EL, de Diniz MFFM, Leite SP, Xavier AL, de Medeiros IA (2009). Preliminary study of the molluscicidal and larvicidal properties of some essential oils and phytochemicals from medicinal plants. *Revista Brasileira de Farmacognosia* 19(4):842-846. Available at <https://doi.org/10.1590/S0102-695X2009000600008>.
- Lobo AP, da Camara CAG, de Melo JPR, de Moraes MM (2019). Chemical composition and repellent activity of essential oils from the leaves of *Cinnamomum zeylanicum* and *Eugenia uniflora* against *Diaphania hyalinata* L. (Lepidoptera: Crambidae). *Journal Plant Disease Protection* 126:79-87. Available at <https://doi.org/10.1007/s41348-018-0190-4>.
- Maia JGS, Andrade EHA, da Silva MHL, Zoghbi MGB (1999). A New Chemotype of *Eugenia uniflora* L. from North Brazil. *Journal of Essential Oil Research* 11(6):727-729. Available at <https://doi.org/10.1080/10412905.1999.9712006>.
- Manayi A, Kurepaz-mahmoodabadi M, Gohari AR, Ajani Y, Saeidnia S (2014). Presence of phthalate derivatives in the essential oils of a medicinal plant *Achillea tenuifolia*. *DARU Journal of Pharmaceutical Sciences* 22(1):78. Available at <https://doi.org/10.1186/s40199-014-0078-1>.
- Marques AM, Aquino VHC, Correia VG, Siani AC, Tappin MRR, Kaplan MAC, Figueiredo MR, (2018). Isolation of two major sesquiterpenes from the leaf essential oil of *Eugenia uniflora* by preparative-scale high-speed countercurrent chromatography. *Separation Science Plus* 1:785-792. Available at <https://doi.org/10.1002/sscp.201800104>.
- Morais SM, Cossolosso DS, Silva AAS, de Moraes Filho MO, Teixeira MJ, Campello CC, Bonilla OH, de Paula Júnior VF, Vila-Nova NS (2019). Essential oils from *Croton* species: Chemical composition, in vitro and in silico antileishmanial evaluation, antioxidant and cytotoxicity activities. *Journal of Brazilian Chemical Society* 30(11):2404-2412. Available at <http://dx.doi.org/10.21577/0103-5053.20190155>.
- Nguyen DTM, Nguyen DH, Hwa-La L, Lee H-B, Lee H-B, Kim E-K (2007). Inhibition of melanogenesis by dioctyl phthalate isolated from *Nigella glandulifera* Freyn. *Journal of Microbiology and Biotechnology* 17(10):1585-1590.
- Ochoa-Villarreal M, Howat S, Hong S, Jang MO, Jin Y-W, Lee E-K, Loake GJ (2016). Plant cell culture strategies for the production of natural products. *BMB Reports* 49(3):149-158. Available at <https://doi.org/10.5483/BMBRep.2016.49.3.264>.
- Pinheiro PF, Gonçalves LV, Cricco KB, Pinheiro CA, Tuler A, da Cunha JB, Ignacchiti MDC (2017). Chemical characterization and molluscicidal activity of essential oil from leaves of *Eugenia uniflora* L. on *Lymnaea columella* (Say, 1817) and *Biomphalaria tenagophila* (D'Orbigny, 1835). *Journal of Essential Oil Bearing Plants* 20(6):1482-1491. Available at <https://doi.org/10.1080/0972060X.2017.1410075>.
- Roy RN (2020). Bioactive natural derivatives of phthalate ester. *Critical Reviews in Biotechnology* 40(7):913-929. Available at <https://doi.org/10.1080/07388551.2020.1789838>.
- Saranya J, Pious BR, Eganathan P, Ramasubramanian R, Selvam V (2015). Essential oil Composition of Whole Flowers of *Rhizophora apiculata*, *Rhizophora mucronata* and *Rhizophora x annamalayana* from Pichavaram, India. *Journal of Essential Oil Bearing Plants* 18(3):728-733. Available at <https://doi.org/10.1080/0972060X.2014.909748>.
- Savegnago L, Lenardão EJ, Alves da S, Victória FN, Jacob RG, Perin G (2015). Fitoterápicos sesquiterpênicos para o tratamento da dor. *BR Patent* 10 2013 023341-2 A2.
- Shanab SMM, Shalaby EA, Lightfoot DA, El-Shemy HA (2010). Allelopathic Effects of Water Hyacinth [*Eichhornia crassipes*]. *PLoS ONE* 5(10):e13200. Available at <https://doi.org/10.1371/journal.pone.0013200>.
- Siega T de C (2018). Essential oils in the control of fungus *Sclerotinia sclerotiorum* (LIB.) de bary in vitro. 95f. Dissertação (mestrado em Agronomia) Universidade Tecnológica Federal do Paraná.
- Sobeh M, Braun MS, Krstin S, Youssef FS, Ashour ML, Wink M (2016). Chemical profiling of the essential oils of *Syzygium aqueum*, *Syzygium samarangense* and *Eugenia uniflora* and their discrimination using chemometric analysis. *Chemistry and Biodiversity* 13:1537-1550. Available at <https://doi.org/10.1002/cbdv.201600089>.
- Sobeh M, El-Raey M, Rezaq S, Abdelfattah MAO, Petruk G, Osman S, El-Shazly AM, El-Beshbishy HA, Mahmoud MF, Wink M (2019). Chemical profiling of secondary metabolites of *Eugenia uniflora* and their antioxidant, anti-inflammatory, pain killing and anti-diabetic activities: A comprehensive approach. *Journal of Ethnopharmacology* 240:111939. Available at <https://doi.org/10.1016/j.jep.2019.111939>.
- Souza L, Silva-Rocha W, Ferreira M, Soares L, Svidzinski T, Milan E, Pires R, Fusco Almeida A, Mendes-Giannini M, Maranhão Chaves G

- (2018). Influence of *Eugenia uniflora* extract on adhesion to human buccal epithelial cells, biofilm formation, and cell surface hydrophobicity of *Candida* spp. from the oral cavity of kidney transplant recipients. *Molecules* 23(10):2418. Available at <https://doi.org/10.3390/molecules23102418>.
- Stashenko EE, Martínez JR, Ruíz CA, Arias G, Durán C, Salgar W, Cala M (2010). *Lippia origanoides* chemotype differentiation based on essential oil GC-MS and principal component analysis. *Journal of Separation Science* 33(1):93-103. Available at <https://doi.org/10.1002/jssc.200900452>.
- Thakur M, Bhattacharya S, Khosla PK, Puri S (2019). Improving production of plant secondary metabolites through biotic and abiotic elicitation, *Journal of Applied Research on Medicinal and Aromatic Plants* 12:1-12.
- The Plant List (2013a). The Plant List, Angiosperms, Myrtaceae [WWW Document]. The Plant List (version 1.1). URL <http://www.theplantlist.org/browse/A/Myrtaceae/> (accessed 6.12.20).
- The Plant List (2013b). The Plant List, Angiosperms, *Myrtaceae*, *Eugenia* [WWW Document]. The Plant List (version 1.1). URL <http://www.theplantlist.org/browse/A/Myrtaceae//Eugenia/> (accessed 6.12.20).
- van Den Dool H, Dec Kratz P (1963). A generalization of the retention index system including linear temperature programmed gas—liquid partition chromatography. *Journal of Chromatography A* 11:463-471. Available at [https://doi.org/10.1016/S0021-9673\(01\)80947-X](https://doi.org/10.1016/S0021-9673(01)80947-X).
- Victoria, FN, Lenardão, EJ, Savegnago L, Perin G, Jacob RG, Alves D, da Silva WP, da Motta A de S, Nascente P da S (2012). Essential oil of the leaves of *Eugenia uniflora* L.: Antioxidant and antimicrobial properties. *Food and Chemical Toxicology* 50(8):2668-2674. Available at <https://doi.org/10.1016/j.fct.2012.05.002>.
- Weyerstahl P, Marschall-Weyerstahl H, Christiansen C, Oguntimein B, Adeoye A (1988). Volatile constituents of *Eugenia uniflora* leaf oil. *Planta Medica* 54(6):546–549. Available at <https://doi.org/10.1055/s-2006-962544>.