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# Chemical variability of essential oils of *Eugenia uniflora* L. genotypes and their antioxidant activity.

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**Abstract:** *Eugenia uniflora*, known as the “Brazilian cherry”, is an economically important neotropical Myrtaceae in the cosmetics and pharmaceutical industries due the production of essential oils with antioxidant activity. On account of its significant genetic variability, genotype evaluations are needed in order to identify genetic features related to the essential oil production that meet the industry requirements. The main objective of the present study was to evaluate the yield, composition, and antioxidant activity of essential oils isolated from the leaves of 36 genotypes of *E. uniflora*. Essential oil samples were obtained by hydrodistillation, and their composition was determined by gas chromatography coupled with mass spectrometry. A variation of 0.22% to 1.68% in the essential oil yield was observed, in which 78 compounds, namely oxygenated sesquiterpenes, were identified. According to the cluster analysis of the major compounds, six groups were revealed. The observed diversity demonstrates the genetic variability of the species. Also, the antioxidant activity was affected by the composition of the essential oils, ranging from 176.66 to 867.57  $\mu\text{M}$  TEAC.

**Key words:** DPPH, genetic diversity, Myrtaceae, sesquiterpenes.

## INTRODUCTION

*Eugenia uniflora* L., popularly known as the “Brazilian cherry” or “Pitangueira”, is one of the neotropical species of the Myrtaceae family employed in the food and cosmetics industries.

Until recently, the species had been utilized in fruit production on account of its exotic flavors and aromas, as well as its high pulp yield and nutritional value. The fruit’s pulp is used in the production of juice, ice cream, jellies, and liqueurs, or commercialized *in natura* or frozen. According to Oliveira et al. (2006), its juice is rich in vitamins and antioxidant compounds.

*E. uniflora* presents ducts and secretory cavities in its mesophyll (Amstrong et al. 2012, Retamales & Scharaschkin 2015, Pacheco-Silva & Donato 2016), where oil resins and terpene

compounds are synthesized (Thadeo et al. 2009, Stesevic et al. 2016, Bomboa et al. 2017). The plant’s essential oil, composed primarily of terpenes, is utilized by pharmaceutical and cosmetics industries because of its antioxidant activity (Victoria et al. 2012), which can be used against human tumor cells, as demonstrated by Ogunwande et al. (2005), while working with *Leishmania amazonensis* (Rodrigues et al. 2013, Silva et al. 2018).

The chemical constitution of the aromatic species’ essential oils exhibits considerable variability due to environmental factors. *E. uniflora* leaves produce essential oils with distinct chemical compositions according to the different seasons of the year, altering the percentage of sesquiterpenes between the humid and dry periods (Costa et al. 2009). Such

composition variability in the species was also verified in genotypes collected in different regions of Brazil. The major compounds present in the plants collected in the Brazilian *cerrado* (Silva et al. 2018) were germacrone (8.52%) and  $\alpha$ -selinene (7.50%). Meanwhile, the oil from plants collected in the state of Ceará was composed primarily of Selina-1,3,7(11)-trien-8-one (36.37%) and Selina-1,3,7(11)-trien-8-one epoxide (27.32%) (Santos et al. 2018), whereas in Maranhão, the predominant constituent was curzerene (47.30%) (Rodrigues et al. 2013).

Another reason for the variation in the chemical composition of the species' essential oils is genetic. According to Santos et al. (2015), essential oil from young leaves presented higher percentages of curzerene (22.37%) than mature leaves (16.60%), and furanodiene was not found in mature leaves, nor was germacrone in young leaves. When evaluating the correlation between the chemical composition of essential oils from different colored fruits, Costa et al. (2010) identified three different chemotypes. The compound curzerene was absent in plants whose fruits were orange, although present in 20.50% in plants with yellow fruits and 42.60% in plants with red fruits.

Thus, the present study aimed at evaluating the yield and chemical composition of essential oil from leaves of *E. uniflora* L. genotypes and their antioxidant activity.

## MATERIALS AND METHODS

### Plant material

The plant material was collected from 36 genotypes, aged 11 years old, obtained from seedlings of four plants cultivated in the Experimental Farm of the Federal University of Paraná, Pinhais - PR, Brazil, located at approximately 25°23'30" S, 49°07'30" W, in March 2017.

### Essential oil extractions and analysis

A total of 400 g of leaves were dried at room temperature during 48 hours for essential oil isolation (Assis et al. 2020). The oil samples were obtained by hydrodistillation in a Clevenger apparatus for 4 hours in three repetitions, each one containing 100 g of leaves with 1000 mL of distilled water. The essential oil yield was determined based on dry mass and expressed as a percentage (% m.m<sup>-1</sup>).

The extracted oil was stored in a freezer at -20°C until analysis. In order for the identification and quantification of the essential oil components, the samples were diluted in hexane until an oil concentration of 1%. A 1.0  $\mu$ L aliquot of such solution was injected into a gas chromatograph coupled with a mass spectrometer (GC/MS) (Shimadzu 2010 Plus) – UFPR Chemistry Department, using an injector maintained at 250°C. Separation of the constituents was performed using an HP-5MS capillary column (30 m x 0.25 mm x 0.25  $\mu$ m) with helium gas as a carrier (1.0 mL.min<sup>-1</sup>). The oven temperature was programmed to gradually increase from 60°C to 240°C at a rate of 3°C.min<sup>-1</sup>. Chemical constituent identification was conducted by comparing their mass spectra with a database, and also their linear retention indices, calculated by the injection of a homologous series of *n*-alkanes (Van Den Dool & Kratz 1963), which were compared with data available in the literature (Adams 2007). Meanwhile, for compound quantification, a GC coupled with a flame ionization detector (FID) was used, in the same conditions described above, with the exception of the carrier gas (hydrogen, at 1.5 mL.min<sup>-1</sup>). The percentage composition, in turn, was obtained by the electronic integration of the FID signal, by dividing the area of each component by the total area (%).

## Antioxidant activity (AA)

The antioxidant activity of the essential oils was determined by measuring the amount of the free radical DPPH (2,2-diphenyl-1-picrylhydrazyl), according to the procedure described by Brand-Williams et al. (1995). The oils were diluted in methanol 1:5 (20%) to a final volume of 100  $\mu\text{L}$  and added to 3.9 mL of DPPH solution in methanol (23.66  $\mu\text{g}\cdot\text{mL}^{-1}$ ). The reaction was performed in the dark and at room temperature for 30 minutes, and the absorbances were read using a UV/VIS Shimadzu®-1800 spectrophotometer, at 515 nm. Trolox (6-hydroxy-2,5,7-tetramethylchroman-2-carboxylic acid) was used as a synthetic reference, and the analyses were conducted in triplicate. The results were expressed as Trolox Equivalent Antioxidant Capacity (TEAC), in  $\mu\text{M}$ .

## Statistical analysis

In order to measure the essential oil yield and antioxidant activity, the variances were tested for homogeneity by the Bartlett test, and the means were compared using the Scott-Knott test ( $p < 0.05$ ). The statistical analysis was carried

out with the ASSISTAT program version 7.7 (Silva & Azevedo 2016).

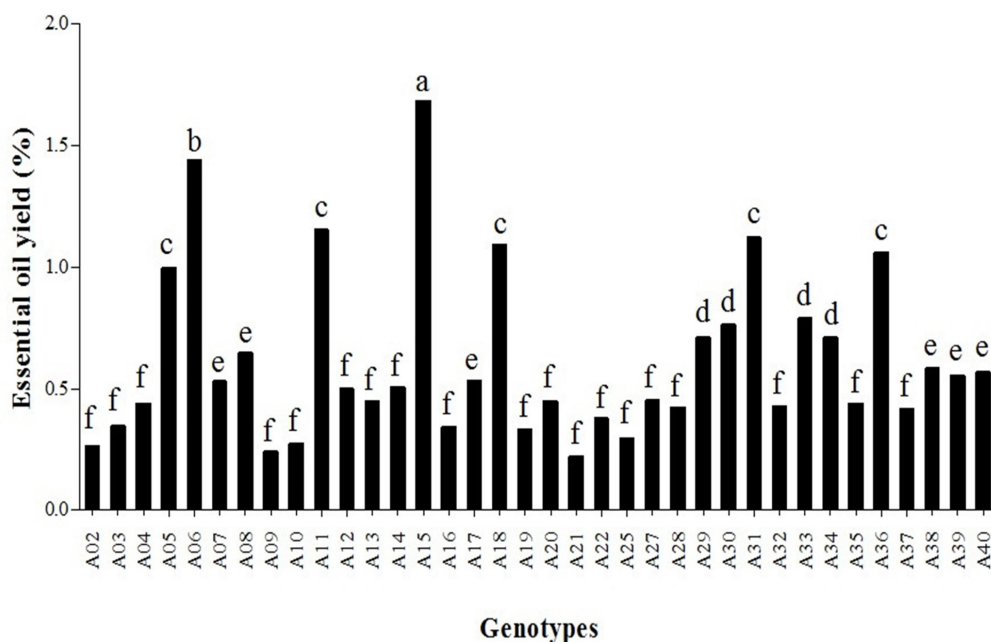
The hierarchical cluster analysis method was performed using the R software, version 3.4.1 (R Core Team 2014), to determine the chemical similarity of the genotypes. In this analysis, 15 major compounds were included, with a percentage greater than 5% in at least one of the genotypes (Table II).

## RESULTS AND DISCUSSION

### Essential oil yield and composition

All of the analyzed *E. uniflora* genotypes produced essential oil, with yields ranging from 0.22 to 1.68% (average of 0.62%) (Figure 1; Table I). The genotypes A05, A06, A11, A15, A18, A31, and A36 presented essential oil yields above 1% on average.

The average essential oil yield was higher than those reported in other studies performed with the species. Galhiane et al. (2006) obtained a yield equivalent to 0.55% from dried leaves. Rodrigues et al. (2013), in turn, reported a 0.30%



**Figure 1. Essential oil yields from the leaves of 36 *E. uniflora* genotypes. Coefficient of variation (CV) = 21.76%. Columns with the same letters do not differ statistically by the Scott-Knott test at 5% probability.**

**Table I. Essential oil yield, identified compounds, hydrocarbon and oxygenated monoterpenes, and hydrocarbon and oxygenated sesquiterpenes, observed in the analyzed *E. uniflora* genotypes.**

	Means (± SD)
Essential Oil yield (%)	0.62 (± 0.34)
Identified compounds (%)	67.26 (± 15.67)
Monoterpene hydrocarbons (%)	0.31 (± 0.76)
Oxygenated monoterpenes (%)	0.00 (± 0.00)
Sesquiterpene hydrocarbons (%)	26.05 (± 9.17)
Oxygenated sesquiterpenes (%)	40.90 (± 20.17)

SD = Standard deviation.

yield, whereas Silva et al. (2018) found an average essential oil yield of 0.51% in *E. uniflora*.

The activation of biosynthetic routes of terpene compounds is influenced by changes in environmental conditions (Verna & Shukla 2015). Plants subjected to the same conditions, as in the present study, vary due to genetic characteristics.

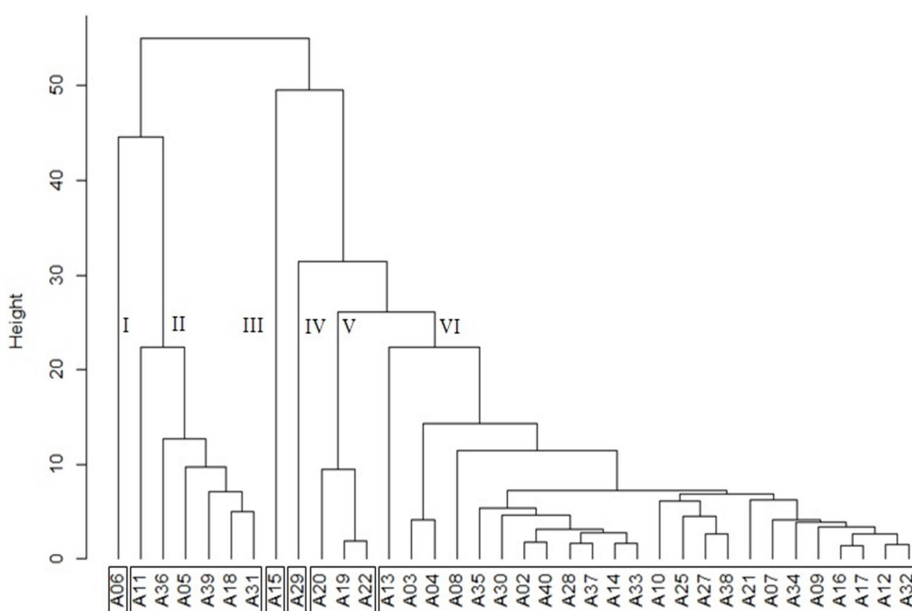
A total of 78 compounds were identified in the essential oil samples (Table SIV - Supplementary Material), with an average of

67.26% of the identified compounds present in the genotypes. Most of the constituents were oxygenated sesquiterpenes (Table I), corroborating with findings described by other authors (Costa et al. 2009, Gallucci et al. 2010, Chang et al. 2011, Rodrigues et al. 2013).

According to Stefanello et al. (2011), sesquiterpene compounds, predominant in most species of the Myrtaceae family, are responsible for their biological properties. Essential oils from these species containing such compounds have demonstrated potent antioxidant activity (Theanphong et al. 2015), antimicrobial and antiparasitic properties (Lago et al. 2011, Sousa et al. 2015, Araújo et al. 2017, Ghazouani et al. 2017), inhibitory action on tumor cell growth (Liu et al. 2013, Zhong et al. 2016, Pereira et al. 2017), and antihypertensive effects (Kumar et al. 2017).

The essential oil analysis of the 36 genotypes using the cluster analysis method enabled the identification of six groups associated with different chemical compositions (Figure 2; Figure 3).

Groups I, III, and IV were composed of only one access each, the first of which presented germacrene B (9.27%), Selina-1,3,7(11)-trien-8-one



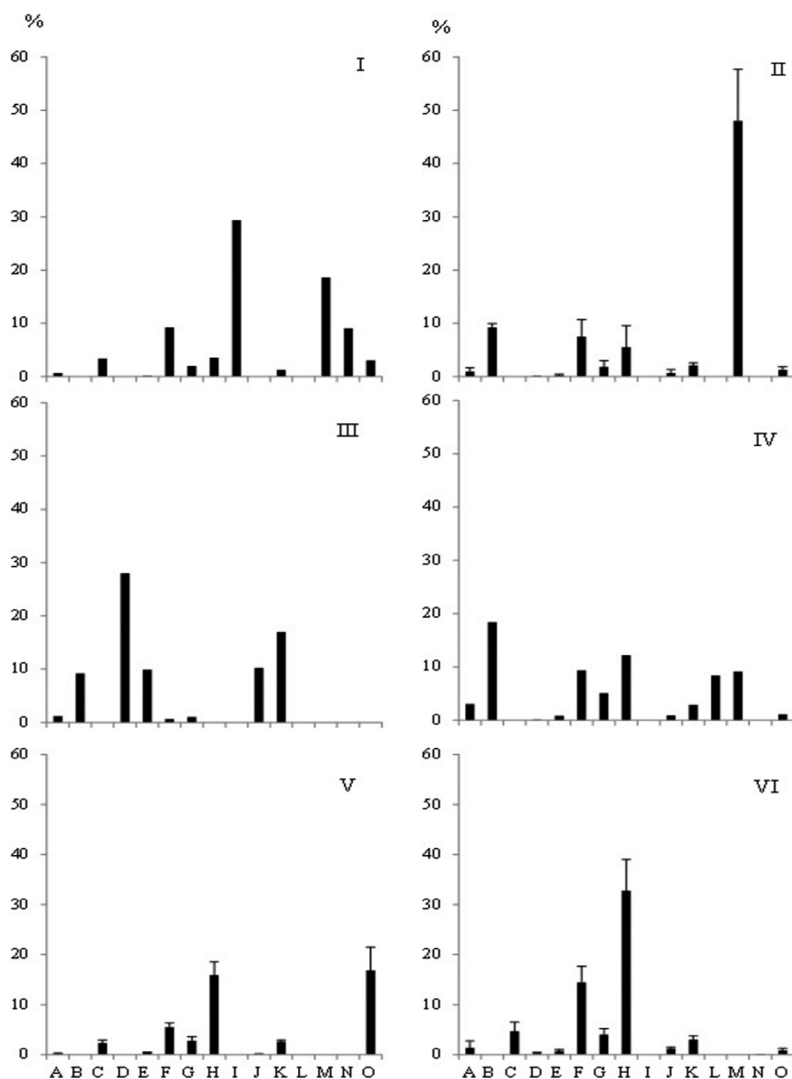
**Figure 2. Cluster analysis based on the essential oil main constituents of 36 genotypes of *E. uniflora*, obtained by the UPGMA method. Cophenetic correlation = 95.88%.**

(29.41%), germacrone (18.58%), and Selina-1,3,7(11)-trien-8-one epoxide (9.07%) as primary compounds. The essential oil of group III was composed mainly of curzerene (9.18%), 7-*epi*- $\alpha$ -selinene (28.00%),  $\delta$ -cadinene (9.90%), *epi*- $\alpha$ -muurolol (10.25%), and  $\alpha$ -cadinol (16.98%). The major compounds found in group IV, in turn, were curzerene (18.45%), germacrone B (9.40%), C<sub>15</sub>H<sub>24</sub>O (12.22%), germacrone (9.15%), and furanodiene (8.36%).

Group II, on the other hand, consisted of six genotypes, composed mainly of curzerene (7.95 – 13.01%), germacrone B (2.18 – 11.36%), C<sub>15</sub>H<sub>24</sub>O (1.46 – 11.39), and germacrone (35.26 – 65.03%). Group

V was represented by three major genotypes: germacrone B (4.77 – 6.36%), C<sub>15</sub>H<sub>24</sub>O (12.78 – 17.57%), and 7,14-anhydro-amorpha-4,9-diene (13.65 – 22.16%). A total of 24 genotypes were included in group VI, whose primary compounds comprised bicyclogermacrene (1.39 – 8.39%), germacrone B (8.43 – 19.75%), and C<sub>15</sub>H<sub>24</sub>O (13.60 – 46.13%).

The chemical diversity of an essential oil can be due to environmental conditions, such as the collection site (Zoghbia et al. 2011, Victoria et al. 2012, Rezende et al. 2013), season of the year (Costa et al. 2009, Ferreira et al. 2017), and age of the leaves and plants (Santos et al. 2015).



**Figure 3. Primary essential oil constituents of the six groups of *E. uniflora* genotypes. Values are expressed as mean  $\pm$  SD (standard deviation). A germacrone D; B curzerene; C bicyclogermacrene; D 7-*epi*- $\alpha$ -selinene; E  $\delta$ -cadinene; F germacrone B; G thusopsan-2 $\alpha$ -ol; H C<sub>15</sub>H<sub>24</sub>O; I selina-1,3,7(11)-trien-8-one; J *epi*- $\alpha$ -muurolol; K  $\alpha$ -cadinol; L furanodiene; M germacrone; N selina-1,3,7(11)-trien-8-one epoxide; O 7,14-anhydro-amorpha-4,9-diene.**

**Table II. Mean values ( $\pm$ SD) of the compounds (%) used as markers in the clusters analysis.**

Genotypes	Compounds															
	A	B	C	D	E	F	G	H*	I	J	K	L**	M	N	O	
A02	<b>0.58</b> ( $\pm$ 0.11)		<b>2.80</b> ( $\pm$ 0.39)		<b>0.53</b> ( $\pm$ 0.01)	<b>13.67</b> ( $\pm$ 1.41)	<b>3.16</b> ( $\pm$ 0.31)	<b>36.08</b> ( $\pm$ 3.29)			<b>3.84</b> ( $\pm$ 0.13)				<b>0.94</b> ( $\pm$ 0.14)	
A03	<b>0.33</b> ( $\pm$ 0.03)		<b>1.39</b> ( $\pm$ 0.11)		<b>0.48</b> ( $\pm$ 0.01)	<b>8.43</b> ( $\pm$ 0.63)	<b>2.43</b> ( $\pm$ 0.13)	<b>46.13</b> ( $\pm$ 1.54)			<b>3.48</b> ( $\pm$ 0.10)				<b>0.37</b> ( $\pm$ 0.03)	
A04	<b>0.71</b> ( $\pm$ 0.06)		<b>3.70</b> ( $\pm$ 0.30)	<b>0.35</b> ( $\pm$ 0.01)	<b>0.67</b> ( $\pm$ 0.01)	<b>8.94</b> ( $\pm$ 0.62)	<b>3.41</b> ( $\pm$ 0.06)	<b>43.07</b> ( $\pm$ 1.41)			2.40 ( $\pm$ 0.12)				<b>0.56</b> ( $\pm$ 0.01)	
A05	<b>0.37</b> ( $\pm$ 0.04)	<b>7.95</b> ( $\pm$ 0.20)			<b>0.19</b> ( $\pm$ 0.01)	<b>5.44</b> ( $\pm$ 0.61)	<b>0.72</b> ( $\pm$ 0.02)	<b>11.39</b> ( $\pm$ 0.12)			1.97 ( $\pm$ 0.10)		<b>46.52</b> ( $\pm$ 1.55)		<b>0.60</b> ( $\pm$ 0.02)	
A06	<b>0.70</b> ( $\pm$ 0.02)		<b>3.39</b> ( $\pm$ 0.13)		<b>0.20</b> ( $\pm$ 0.03)	<b>9.27</b> ( $\pm$ 0.94)	<b>2.01</b> ( $\pm$ 0.32)	<b>3.57</b> ( $\pm$ 1.34)	<b>29.41</b> ( $\pm$ 3.15)		<b>1.28</b> ( $\pm$ 0.25)		<b>18.58</b> ( $\pm$ 0.75)	<b>9.07</b> ( $\pm$ 0.44)	<b>3.05</b> ( $\pm$ 0.43)	
A07	<b>3.37</b> ( $\pm$ 0.18)		<b>5.59</b> ( $\pm$ 0.24)	<b>0.33</b> ( $\pm$ 0.04)	<b>1.26</b> ( $\pm$ 0.01)	<b>13.59</b> ( $\pm$ 0.25)	<b>4.42</b> ( $\pm$ 0.11)	<b>29.09</b> ( $\pm$ 0.74)		<b>1.06</b> ( $\pm$ 0.04)	<b>3.55</b> ( $\pm$ 0.08)				<b>0.91</b> ( $\pm$ 0.06)	
A08	<b>5.65</b> ( $\pm$ 1.65)		<b>8.39</b> ( $\pm$ 2.14)	<b>0.27</b> ( $\pm$ 0.03)	<b>1.59</b> ( $\pm$ 0.18)	<b>12.88</b> ( $\pm$ 2.94)	<b>4.27</b> ( $\pm$ 0.90)	<b>23.94</b> ( $\pm$ 4.29)		<b>1.06</b> ( $\pm$ 0.10)	<b>3.27</b> ( $\pm$ 0.38)				<b>0.75</b> ( $\pm$ 0.09)	
A09	<b>0.82</b> ( $\pm$ 0.10)		<b>5.80</b> ( $\pm$ 0.59)	<b>0.41</b> ( $\pm$ 0.02)	<b>0.56</b> ( $\pm$ 0.02)	<b>15.69</b> ( $\pm$ 0.89)	<b>5.98</b> ( $\pm$ 0.23)	<b>29.26</b> ( $\pm$ 0.32)			2.60 ( $\pm$ 0.17)					
A10	<b>0.98</b> ( $\pm$ 0.14)		<b>4.93</b> ( $\pm$ 0.60)	<b>0.25</b> ( $\pm$ 0.05)	<b>0.61</b> ( $\pm$ 0.04)	<b>19.75</b> ( $\pm$ 1.77)	<b>4.40</b> ( $\pm$ 0.29)	<b>27.85</b> ( $\pm$ 1.86)			2.80 ( $\pm$ 0.17)				<b>0.74</b> ( $\pm$ 0.14)	
A11	<b>0.50</b> ( $\pm$ 0.07)	<b>9.21</b> ( $\pm$ 0.25)			<b>0.16</b> ( $\pm$ 0.03)	<b>2.18</b> ( $\pm$ 0.27)	<b>0.89</b> ( $\pm$ 0.08)	<b>1.46</b> ( $\pm$ 0.13)		0.35 ( $\pm$ 0.03)	<b>2.14</b> ( $\pm$ 0.14)		<b>65.03</b> ( $\pm$ 0.20)		<b>1.33</b> ( $\pm$ 0.41)	
A12	<b>0.90</b> ( $\pm$ 0.02)		<b>6.25</b> ( $\pm$ 0.14)	<b>0.29</b> ( $\pm$ 0.01)	<b>0.63</b> ( $\pm$ 0.01)	<b>12.74</b> ( $\pm$ 0.45)	<b>5.79</b> ( $\pm$ 0.11)	<b>30.42</b> ( $\pm$ 0.96)			<b>2.85</b> ( $\pm$ 0.09)				<b>0.68</b> ( $\pm$ 0.09)	
A13	<b>4.41</b> ( $\pm$ 0.12)		<b>7.17</b> ( $\pm$ 0.08)		<b>1.58</b> ( $\pm$ 0.02)	<b>22.71</b> ( $\pm$ 0.75)	<b>4.93</b> ( $\pm$ 0.18)	<b>13.60</b> ( $\pm$ 0.66)		<b>1.59</b> ( $\pm$ 0.08)	<b>3.47</b> ( $\pm$ 0.17)			<b>0.12</b> ( $\pm$ 0.01)	<b>0.69</b> ( $\pm$ 0.04)	
A14	<b>0.78</b> ( $\pm$ 0.09)		<b>4.41</b> ( $\pm$ 0.50)		<b>0.55</b> ( $\pm$ 0.04)	<b>15.16</b> ( $\pm$ 1.11)	<b>4.28</b> ( $\pm$ 0.14)	<b>36.38</b> ( $\pm$ 1.24)			<b>2.99</b> ( $\pm$ 0.25)					
A15	<b>1.19</b> ( $\pm$ 0.31)	<b>9.18</b> ( $\pm$ 0.18)		<b>28.00</b> ( $\pm$ 0.85)	<b>9.90</b> ( $\pm$ 1.56)	<b>0.64</b> ( $\pm$ 0.01)	<b>1.00</b> ( $\pm$ 0.05)			<b>10.25</b> ( $\pm$ 0.28)	<b>16.98</b> ( $\pm$ 0.54)					
A16	<b>0.79</b> ( $\pm$ 0.03)		5.40 ( $\pm$ 0.38)		<b>0.56</b> ( $\pm$ 0.02)	<b>14.28</b> ( $\pm$ 0.69)	<b>4.56</b> ( $\pm$ 0.02)	<b>31.30</b> ( $\pm$ 0.19)			<b>2.93</b> ( $\pm$ 0.13)				<b>1.05</b> ( $\pm$ 0.12)	

**Table II. Continuation**

A17	<b>0.75</b> (± 0.07)		<b>5.25</b> (± 0.46)	<b>0.51</b> (± 0.03)	<b>0.51</b> (± 0.02)	<b>13.57</b> (± 0.81)	<b>4.43</b> (± 0.18)	<b>30.76</b> (± 0.84)			<b>2.61</b> (± 0.12)			<b>1.97</b> (± 0.21)
A18	<b>0.87</b> (± 0.05)	<b>9.63</b> (± 0.63)			<b>0.26</b> (± 0.03)	<b>9.01</b> (± 0.82)	<b>2.19</b> (± 0.13)	<b>3.83</b> (± 0.50)	<b>0.28</b> (± 0.02)		<b>1.30</b> (± 0.10)		<b>51.01</b> (± 1.89)	<b>1.60</b> (± 0.13)
A19	<b>0.38</b> (± 0.08)		<b>2.10</b> (± 0.57)		<b>0.41</b> (± 0.09)	<b>6.36</b> (± 1.18)	<b>2.65</b> (± 0.10)	<b>17.57</b> (± 0.67)			2.94 (± 0.04)			<b>13.65</b> (± 3.78)
A20	<b>0.50</b> (± 0.12)		<b>1.81</b> (± 0.42)		<b>0.32</b> (± 0.07)	<b>4.77</b> (± 1.22)	<b>2.19</b> (± 0.22)	<b>12.78</b> (± 0.45)	<b>0.28</b> (± 0.08)		<b>2.36</b> (± 0.16)			<b>22.16</b> (± 5.12)
A21	<b>2.67</b> (± 0.18)		<b>2.44</b> (± 0.10)		<b>1.31</b> (± 0.06)	<b>11.79</b> (± 0.47)	<b>3.21</b> (± 0.17)	<b>28.77</b> (± 0.24)			<b>5.05</b> (± 0.25)			<b>0.89</b> (± 0.25)
A22	<b>0.57</b> (± 0.05)		<b>3.02</b> (± 0.17)		0.51 (± 0.03)	<b>5.51</b> (± 0.26)	<b>3.63</b> (± 0.03)	<b>17.25</b> (± 0.68)			<b>2.57</b> (± 0.08)			<b>14.62</b> (± 1.15)
A25	<b>2.81</b> (± 0.28)		<b>2.10</b> (± 0.11)		<b>1.22</b> (± 0.05)	<b>17.80</b> (± 0.35)	<b>1.90</b> (± 0.01)	<b>30.34</b> (± 1.20)	<b>1.19</b> (± 0.05)		<b>4.64</b> (± 0.23)			<b>0.62</b> (± 0.04)
A27	<b>0.84</b> (± 0.08)		<b>3.84</b> (± 0.26)		<b>0.61</b> (± 0.03)	<b>18.44</b> (± 0.85)	<b>2.55</b> (± 0.11)	<b>33.93</b> (± 1.22)			<b>2.79</b> (± 0.15)			<b>0.35</b> (± 0.01)
A28	<b>0.73</b> (± 0.11)		<b>4.88</b> (± 0.60)		<b>0.56</b> (± 0.04)	<b>13.61</b> (± 0.76)	<b>4.76</b> (± 0.14)	<b>33.84</b> (± 0.17)			<b>2.81</b> (± 0.18)			0.82 (± 0.07)
A29	<b>3.04</b> (± 0.04)	<b>18.45</b> (± 0.35)			<b>0.80</b> (± 0.02)	<b>9.40</b> (± 0.65)	<b>5.10</b> (± 0.34)	<b>12.22</b> (± 0.81)	<b>0.87</b> (± 0.06)		<b>2.89</b> (± 0.12)		<b>8.36</b> (± 0.55)	<b>1.13</b> (± 0.02)
A30	<b>0.54</b> (± 0.03)		<b>2.90</b> (± 0.17)		<b>0.48</b> (± 0.01)	<b>16.89</b> (± 1.12)	<b>2.83</b> (± 0.06)	<b>38.13</b> (± 1.72)			<b>2.69</b> (± 0.04)			<b>1.30</b> (± 0.05)
A31	<b>0.93</b> (± 0.07)	<b>10.05</b> (± 0.27)			<b>0.27</b> (± 0.03)	<b>8.54</b> (± 0.40)	<b>1.98</b> (± 0.09)	<b>3.04</b> (± 0.03)	<b>0.31</b> (± 0.03)				<b>46.27</b> (± 0.45)	<b>2.25</b> (± 0.25)
A32	<b>1.06</b> (± 0.17)		<b>7.36</b> (± 1.03)		<b>0.63</b> (± 0.03)	<b>13.24</b> (± 1.07)	<b>5.97</b> (± 0.42)	<b>31.08</b> (± 1.02)			<b>2.32</b> (± 0.19)			<b>0.80</b> (± 0.06)

**Table II. Continuation**

A33	<b>1.07</b> (± 0.08)		<b>5.57</b> (± 0.23)	<b>0.27</b> (± 0.01)	<b>0.63</b> (± 0.03)	<b>15.09</b> (± 0.22)	<b>3.61</b> (± 0.14)	<b>36.50</b> (± 0.41)			<b>2.40</b> (± 0.06)			<b>0.73</b> (± 0.08)
A34	<b>1.15</b> (± 0.04)		<b>7.37</b> (± 0.25)	<b>0.34</b> (± 0.01)	<b>0.67</b> (± 0.02)	<b>15.86</b> (± 0.10)	<b>4.57</b> (± 0.08)	<b>32.77</b> (± 0.14)			<b>0.72</b> (± 0.02)			<b>0.83</b> (± 0.01)
A35	<b>0.70</b> (± 0.02)		<b>4.85</b> (± 0.39)	<b>0.34</b> (± 0.01)	<b>0.60</b> (± 0.01)	<b>10.90</b> (± 0.38)	<b>5.78</b> (± 0.05)	<b>38.49</b> (± 2.45)			2.52 (± 0.09)			<b>1.18</b> (± 0.08)
A36	<b>0.93</b> (± 0.06)	<b>9.84</b> (± 0.23)		<b>0.17</b> (± 0.02)	<b>0.32</b> (± 0.05)	<b>11.36</b> (± 0.43)	<b>1.62</b> (± 0.19)	<b>9.79</b> (± 1.11)			<b>2.32</b> (± 0.09)		<b>36.28</b> (± 1.23)	<b>1.10</b> (± 0.43)
A37	<b>0.76</b> (± 0.15)		<b>4.44</b> (± 0.79)	<b>0.55</b> (± 0.06)	<b>0.68</b> (± 0.10)	<b>14.07</b> (± 2.88)	<b>3.77</b> (± 0.35)	<b>34.97</b> (± 3.79)			<b>2.65</b> (± 0.11)			<b>0.83</b> (± 0.06)
A38	<b>0.52</b> (± 0.05)		<b>2.81</b> (± 0.23)	<b>0.15</b> (± 0.01)	<b>0.44</b> (± 0.02)	<b>16.99</b> (± 0.73)	<b>2.61</b> (± 0.08)	<b>32.50</b> (± 0.61)			<b>3.24</b> (± 0.14)			<b>1.55</b> (± 0.04)
A39	<b>2.26</b> (± 0.60)	<b>8.87</b> (± 2.02)			0.58 (± 0.16)	<b>8.59</b> (± 2.37)	<b>3.88</b> (± 0.54)	<b>3.83</b> (± 0.25)			<b>1.67</b> (± 0.14)		<b>42.90</b> (± 8.32)	<b>0.66</b> (± 0.13)
A40	<b>0.66</b> (± 0.24)		<b>3.49</b> (± 1.17)		<b>0.61</b> (± 0.10)	<b>12.33</b> (± 3.41)	<b>3.07</b> (± 0.45)	<b>35.32</b> (± 4.21)			<b>3.24</b> (± 0.39)			<b>0.75</b> (± 0.05)

A germacrene D; B curzerene; C bicyclogermacrene; D 7-*epi*- $\alpha$ -selinene; E  $\delta$ -cadinene; F germacrene B; G thusopsan-2 $\alpha$ -ol; H C<sub>15</sub>H<sub>26</sub>O; I selina-1,3,7(11)-trien-8-one; J *epi*- $\alpha$ -muurolol; K  $\alpha$ -cadinol; L furanodiene; M germacrene; N selina-1,3,7(11)-trien-8-one epoxide; O 7,14-anhydro-amorpha-4,9-diene. \* Unidentified Sesquiterpene (Retention index = 1594; molecular weight = 216); \*\* Identification according to Santos et al. 2015.



**Table III. Antioxidant activity of essential oils extracted from leaves of different genotypes of *E. uniflora* by the DPPH method.**

Genotypes	TEAC ± SD (µM)	Genotypes	TEAC ± SD (µM)
A02	679.69 ± 54.55 <sup>d</sup>	A20	464.54 ± 60.53 <sup>f</sup>
A03	516.05 ± 72.73 <sup>f</sup>	A21	655.45 ± 41.99 <sup>d</sup>
A04	461.51 ± 54.55 <sup>f</sup>	A22	552.42 ± 48.10 <sup>e</sup>
A05	867.57 ± 20.99 <sup>a</sup>	A25	509.99 ± 20.99 <sup>f</sup>
A06	231.21 ± 27.77 <sup>g</sup>	A27	491.81 ± 20.99 <sup>f</sup>
A07	582.72 ± 20.99 <sup>e</sup>	A28	643.33 ± 72.73 <sup>d</sup>
A08	576.66 ± 45.76 <sup>e</sup>	A29	843.33 ± 26.76 <sup>a</sup>
A09	606.96 ± 65.66 <sup>e</sup>	A30	503.93 ± 63.85 <sup>f</sup>
A10	576.66 ± 41.99 <sup>e</sup>	A31	546.36 ± 75.70 <sup>e</sup>
A11	861.51 ± 26.96 <sup>a</sup>	A32	716.05 ± 62.98 <sup>c</sup>
A12	764.54 ± 27.77 <sup>b</sup>	A33	697.87 ± 18.18 <sup>c</sup>
A13	643.33 ± 65.56 <sup>d</sup>	A34	570.60 ± 36.36 <sup>e</sup>
A14	497.87 ± 5.33 <sup>f</sup>	A35	655.45 ± 63.85 <sup>d</sup>
A15	176.66 ± 41.99 <sup>g</sup>	A36	588.78 ± 72.73 <sup>e</sup>
A16	643.33 ± 48.10 <sup>d</sup>	A37	740.30 ± 55.55 <sup>c</sup>
A17	734.24 ± 18.18 <sup>c</sup>	A38	516.05 ± 48.10 <sup>f</sup>
A18	522.12 ± 10.50 <sup>f</sup>	A39	782.72 ± 27.77 <sup>b</sup>
A19	709.99 ± 45.76 <sup>c</sup>	A40	464.54 ± 60.53 <sup>f</sup>

Values are expressed as mean TEAC (Trolox equivalent antioxidant capacity) ± SD (standard deviation) in µM. Same letters do not differ statistically by the Scott-Knott test at 5% probability. Coefficient of variation (CV) = 8.27%.

Costa et al. (2010) verified variability in the essential oil composition of “Brazilian cherry” leaves regarding the fruit color of the genotype. According to Griffis & Manners (2005), the inheritance concerning the fruit color of *E. uniflora* is determined by a single gene. Therefore, the correlation observed by Costa et al. (2010) may indicate that, in addition to environmental conditions, the essential oil composition can be determined genetically.

The genetic variability of the yield and composition of the essential oils obtained from the analyzed genotypes may be related to the preferred form of reproduction of the species. According to Silva & Pinheiro (2007), *E. uniflora* presents intermediate sexual reproduction; that

is, both self-fertilization and cross-fertilization may occur. Nonetheless, allogamy is its preferred mode of reproduction, which is facilitated by its main pollinating agent, *Apis mellifera* (Diniz & Buschini 2016), thus promoting the genetic variability of the species.

### Antioxidant activity

The essential oils isolated from all genotypes of *E. uniflora* were positive for antioxidant activity by the DPPH inhibition method (Table III), which ranged from 176.66 to 867.57 µM TEAC. Genotypes A05, A11, and A29 presented more than 800 µM TEAC, statistically higher than the other genotypes, and were composed primarily of curzerene, furanodiene, and germacrone.

Studies with species of the genus *Curcuma* have demonstrated that such compounds isolated from their essential oils exhibited strong antioxidant activity (Zhao et al. 2010, Hamdi et al. 2015) and, therefore, may be responsible for the high sequestration rate of DPPH.

The genotypes whose main essential oil compounds were germacrone and curzerene (A18, A31, and A39) did not present the same DPPH free radical inhibition performance. Those composed only of curzerene (A15) or germacrone (A06) also showed little effect on the free radical. Other compounds, which were not observed and/or were in lower percentages in the essential oils that showed higher antioxidant activity, were also found in the three genotypes, including Selina-1,3,7(11)-trien-8-one (A06) and 7-*epi*- $\alpha$ -selinene (A15). According to Wang et al. (2008), it is difficult to attribute an antioxidant effect to one or a few compounds present in essential oils since they are considered a complex mixture of chemical components and, therefore, the interaction between them, including minority compounds, can influence such effects (Botelho et al. 2007). Thus, the association between all of the compounds present in the oil are fundamental for the sequestration of this free radical.

## CONCLUSIONS

The genotypes of the “Brazilian cherry” showed significant genetic variability regarding essential oil yield and composition. Six groups were identified according to chemical constitution, in which Selina-1,3,7(11)-trien-8-one, Selina-1,3,7(11)-trien-8-one epoxide, curzerene, germacrone, 7-*epi*- $\alpha$ -selinene, furanodiene, 7,14-anhydro-amorpha-4,9-diene, and C<sub>15</sub>H<sub>24</sub>O were the main compounds that differentiated the groups. Most of the *E. uniflora* essential oil

constituents found in the present study were sesquiterpenes (94.45%), a fact that may have influenced in the antioxidant activity of the analyzed oils. The variability of the essential oil composition and the amplitude in the antioxidant effect reinforce the need for quality control of the oils used in the pharmaceutical and cosmetics industries.

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## SUPPLEMENTARY MATERIAL

### Table SIV.

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