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## Seasonal chemical composition of an unexplored essential oil of *Eugenia brevistyla*

[Composición química estacional de un aceite esencial no explorado de *Eugenia brevistyla*]

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**Abstract:** This study describes the qualitative and quantitative seasonal analysis of the essential oils from an unexplored plant *Eugenia brevistyla*, native from Brazilian Atlantic Rain Forest and Semidecidual Forest. Analysis by GC-FID and GC-MS allowed the identification of 28 compounds. The largest fraction corresponds to oxygenated sesquiterpenes in all seasons. The major compound was *E*-nerolidol in all seasons, being higher in winter (83.14%) and lower in spring (69.6%). The second major compound was bicyclogermacrene in the spring and in the summer essential oils. Alloaromadendrene and spathulenol were the second major compounds in autumn and winter, respectively. Sesquiterpenes hydrocarbons showed higher variation along the year (58%) than oxygenated sesquiterpenes (2%). No monoterpenes were found in the analyzed essential oils.

**Keywords:** *Eugenia brevistyla*; Myrtaceae; seasonal evaluation; essential oil composition; *E*-nerolidol

**Resumen:** Este estudio describe el análisis estacional cualitativo y cuantitativo del aceite esencial de la planta inexplorada *Eugenia brevistyla*, nativa de la Selva Tropical Atlántica y del Bosque Semidecidual de Brasil. El análisis por GC-FID y GC-MS permitió la identificación de 28 compuestos. La fracción más grande corresponde a sesquiterpenos oxigenados en todas las estaciones. El compuesto principal fue *E*-nerolidol en todas las estaciones, siendo más alto en invierno (83.14%) y más bajo en la primavera (69.6%). El segundo compuesto principal fue bicyclogermacrene en los aceites esenciales de la primavera y del verano. El aloaromadendreno y el espatulenol fueron los segundos compuestos principales en otoño e invierno, respectivamente. Los hidrocarburos sesquiterpénicos mostraron una mayor variación a lo largo del año (58%) que los sesquiterpenos oxigenados (2%). No se encontraron monoterpenos en los aceites esenciales analizados.

**Palabras clave:** *Eugenia brevistyla*; Myrtaceae; evaluación estacional; aceite esencial; *E*-nerolidol

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## INTRODUCTION

*Eugenia brevistyla* D. Legrand (sin. *Eugenia neoaustralis*), Myrtaceae family, well distributed in tropical and subtropical regions, mainly in South America (Fischer *et al.*, 2005, Zaki *et al.*, 2013). This species, popularly known as “cambuca-pitanga” or “guamirim-pitanga”, is a tree with 4 – 6 m height, occurs in the Semidecidual forest, over to 500 m of altitude and occasionally in the Atlantic rain forest, from the state of Rio Grande do Sul to São Paulo, Brazil. Its edible fruits are red, with 1.5 a 2.2 cm, and are popularly used to produce juices and jams (Flora do Brasil, 2018).

Essential oils of *Eugenia* genus have demonstrated many biological activities including antidepressant and antioxidant activity (Amorin *et al.*, 2009, Garmus *et al.*, 2014), antifungal (Chami *et al.*, 2005), anticarcinogenic (Zheng *et al.*, 1992), antiallergic (Kim *et al.*, 1998) and antimutagenic activity (Miyazawa & Hisama, 2001). Essential oils are potential sources of novel antimicrobial compounds especially against bacterial pathogens (Nuñez & D'Aquino, 2012). In this aspect some *Eugenia* species deserve to be highlighted, for example, *E. caryophyllata* (Chaieb *et al.*, 2007), *E. uniflora* (Pereira *et al.*, 2017) and *E. jambolana* (Pereira *et al.*, 2017). These biological activities are attributed to the complex mixture of low molecular weight molecules produced by the aromatic plants which comprise the essential oils.

The main compounds found in the essential oils of the genus *Eugenia* are oxygenated phenylpropanoids such as eugenol, already identified in the *E. caryophyllata* (Marchese *et al.*, 2007), *E. uniflora* (Pereira *et al.*, 2017) and *E. stigmatosa* (Apel *et al.*, 2002), and oxygenated sesquiterpenes as spathulenol from *E. brasiliensis* (Magina *et al.*, 2009), *E. cuprea* (Apel *et al.*, 2002), *E. uniflora* (Costa *et al.*, 2009) and *E. patrisii* (Silva *et al.*, 2017). In addition, there are non-oxygenated sesquiterpenes as  $\beta$ -caryophyllene, already identified in *E. puniceifolia* oils (Oliveira *et al.*, 2005) and *E. cuprea* (Apel *et al.*, 2002). Further terpenes are common to this genus, such as  $\alpha$ -pinene, limonene,  $\beta$ -selinene, among others have been already identified. However, the chemical composition of the oil, number of compounds identified and their concentration, may change according to the seasonality and characteristic of each season (Angioni *et al.*, 2003, Masotti *et al.*, 2003, Siebert *et al.*, 2015).

No previous study on the essential oil of *Eugenia brevistyla* was published in the literature.

Therefore, the objective of this study was to evaluate, for the first time, the qualitative and quantitative chemical composition of the essential oil of *E. brevistyla*, as well as to evaluate the seasonal variation of essential oil across the four seasons of the year.

## MATERIAL AND METHODS

### *Plant material*

Leaves of one *E. brevistyla* tree were collected in September and December of 2015, and March and June of 2016 for extraction of essential oil from all the four seasons. The collect was performed in Blumenau (27°36'13.65" S, 48°31'14.75" W), Santa Catarina state, Southern Brazil. The samples were identified by Professor Dr. André Luiz Gasper from the Botany Department of Universidade Regional de Blumenau, and a voucher specimen was deposited at the herbarium FLOR under number 47604.

### *Extraction procedure*

Essential oils of fresh leaves were obtained by hydrodistillation for 4 hours in a modified Clevenger-type apparatus, in the proportion of 1 g of leaves to 10 mL of distilled and deionized water. After extraction, the essential oils were dried with sodium sulfate and stored at low temperature.

### *GC-FID and GC-MS Analysis*

All reagents were of analytical scale. The qualitative analysis was performed by gas chromatography coupled with mass spectrometry (GC-MS) in a Shimadzu® GCMS-QP2010 Plus chromatograph (nonpolar capillary column RTX®-5MS: 30 m x 0.25 mm x 0.25  $\mu$ m), and the quantitative analysis by gas chromatography coupled with flame ionization detector (GC-FID) in a Shimadzu® GC-FID2010 chromatograph (nonpolar capillary column OV®-5: 30 m x 0.25 mm x 0.25  $\mu$ m), being the quantitative analysis performed in triplicate. The oven temperature program was 68° C for 4 minutes, with increase of 3° C each minute until 246° C remaining at this temperature for 2 minutes. Helium was used as gas carrier (Inlet pressure: 87 kpa; flow rate of 1 mL.minute<sup>-1</sup>), with the injector temperature of 250° C (1:20 split) and an ion source of 70 eV on the MS, with interface at 280° C. Identification of the essential oil components was based upon their retention indexes (in comparison with an homologous series of alkanes from C8 to C19), and by comparison of their spectral mass patterns with those reported in the literature (Adams, 2007, Silva *et al.*, 1999) and stored in the MS library NIST 2008

(National Institute for Standards and Technology) database. Since standards for the positive confirmation were not used in the identification of the compounds, it must be considered only as identified attempt. Compounds were considered identified when the similarity index in the NIST library and the arithmetic index were greater than 90%. The quantitative data of the compounds were obtained by electronic integration of the peak areas, resulting from three injections of the samples by the CG-FID technique. Results were expressed as mean  $\pm$  standard deviation. Data handling was carried out by GCsolution 2.3 (Shimadzu) software.

### Statistical analysis

The quantitative variation of the constituents from the essential oils in the seasons was analysed using descriptive statistics, where the coefficient of variation was calculated for each compound. The compounds of which the concentration could not be measured were considered as traces and for purposes of calculation equal to 0%. The coefficient of variation (CV) was calculated for the compounds present in at least two samples. As criterion of analysis, changes from 0 to 15% were considered low, 15 to 30% moderate and above 30% considered high (Löesch & Stein, 2011).

## RESULTS AND DISCUSSION

After extraction, a total of twenty-eight compounds were identified accounting 93.5, 98.6, 91.8, and 91.5% from the essential oils of spring, summer, autumn and winter, respectively. The yields obtained from the extraction of essential oils were different between seasons (0.07% ( $\text{ww}^{-1}$ ) in the spring, 0.05% ( $\text{ww}^{-1}$ ) in the summer, 0.05% ( $\text{ww}^{-1}$ ) in the winter, 0.06% ( $\text{ww}^{-1}$ ) in autumn). The variation between the yields (CV) was 16.65%. Table No. 1 shows the qualitative and quantitative chemical compositions of the studied samples.

In all analyzed oils, only sesquiterpenes were found, having the largest fraction corresponding to oxygenated sesquiterpenes in all seasons (spring: 87.5%; summer: 86.6% autumn: 89.4%; winter: 84.8%). The sesquiterpene hydrocarbons were found in lower concentration, ranging from 2.4 to 12.1% in all samples.

The major compound was *E*-nerolidol in all seasons, being higher in winter (83.14%) and lower in spring (69.6%). The variation of this compound through the year seasons was considered low (CV=8%). The second major compound was bicyclogermacrene in essential oils of spring and

summer, however, this compound was not detected in the samples collected in autumn and winter. Alloaromadendrene and spathulenol were the second major compounds in autumn and winter, respectively. Although the major compound has varied little over the seasons, minority sesquiterpenes varied greatly. Of the twenty-eight identified compounds, twenty presented great coefficient of variation along the seasons, with nineteen of them showing CV above 100%. Although their percentages in the samples are small, this cause a significant difference in the profiles of the analyzed essential oils.

The analyzed essential oils were also very different from each other in relation to the number of compounds. Samples collected in spring and summer were distinguished by the greater number of identified compounds (twenty-four and twenty-two, respectively), when compared to the essential oils extracted in autumn and winter (seven and nine, respectively).

The luminous intensity is a factor that influences the concentration as well as the composition of the essential oils. Hay & Svoboda (1993) claim that the greatest incidence of luminosity makes the higher production of glandular trichomes in vegetables, especially those that are rich in essential oils. The glandular trichomes are epidermal appendages that occur in several plant organs and are responsible for the synthesis and storage of terpenic compounds. Thus, the higher the concentration of these structures, the greater the production of essential oil. However, a greater insight of light, when coupled with high temperatures, could decrease the oil yield due to evaporation.

According to Taiz & Zeiger (2004), the higher production of secondary metabolites under high levels of solar radiation are explained because the biosynthetic reactions are dependent on carbon skeleton supplies, made by photosynthetic processes and energy compounds that participate in the regulation of these reactions. Further, the ultraviolet radiation increases the expression of several genes related with reducing oxidative stress. Monoterpenes and sesquiterpenes are highly lipophilic and possess good antioxidative capacity in the lipophilic test systems (Gil *et al.*, 2012). The synthesis of the terpenes is initiated from isopentenyl and dimethylallyl diphosphate precursors, being the steps of terpene production are catalyzed by terpene synthases (TPS), a very large family of enzymes with multiple representatives in all plant species studied so far. Previous works showed that the ultra-violet radiation regulate expression of genes encoding TPS,

increasing its activity (Pontin *et al.*, 2010).

**Table No. 1**  
**Compounds identified (%) in the essential oil from leaves of *E. brevistyla***  
**in the four seasons of the year**

| Compounds                    | Rt <sup>a</sup> | Relative concentration (%) |             |             |             | AI <sup>b</sup> |                | CV% <sup>e</sup> |
|------------------------------|-----------------|----------------------------|-------------|-------------|-------------|-----------------|----------------|------------------|
|                              |                 | Spring                     | Summer      | Autumn      | Winter      | E <sup>c</sup>  | L <sup>d</sup> |                  |
| δ-elemene                    | 24.477          | 1.7 ± 0.01                 | 1.0 ± 0.26  | t           | t           | 1331            | 1335           | 122              |
| β-elemene                    | 26.811          | 0.8 ± 0.02                 | 0.3 ± 0.10  | t           | t           | 1384            | 1389           | 138              |
| β-gurjunene                  | 27.533          | 0.4 ± 0.03                 | 0.5 ± 0.19  | t           | t           | 1401            | 1409           | 116              |
| β-caryophyllene              | 27.885          | 1.1 ± 0.01                 | 0.6 ± 0.15  | t           | t           | 1409            | 1417           | 123              |
| γ-elemene                    | 31.060          | t                          | t           | t           | 1.1 ± 0.06  | 1521            | 1434           | -                |
| Aromadendrene                | 28.706          | 1.1 ± 0.01                 | 0.9 ± 0.1   | t           | t           | 1429            | 1439           | 116              |
| α-humulene                   | 29.285          | 0.3 ± 0.03                 | t           | t           | t           | 1443            | 1452           | -                |
| Alloaromadendrene            | 29.589          | 1.4 ± 0.01                 | 0.8 ± 0.13  | 4.0 ± 0.01  | t           | 1450            | 1458           | 111              |
| γ-muurolole                  | 30.269          | 0.2 ± 0.01                 | 0.3 ± 0.10  | t           | t           | 1467            | 1478           | 117              |
| Germacrene D                 | 30.419          | 1.3 ± 0.01                 | 0.3 ± 0.03  | t           | t           | 1470            | 1484           | 152              |
| β-Seliene                    | 30.616          | 0.3 ± 0.02                 | 0.1 ± 0.06  | t           | t           | 1475            | 1489           | 143              |
| δ-selinene                   | 30.867          | 0.3 ± 0.01                 | 0.3 ± 0.07  | t           | t           | 1481            | 1492           | 116              |
| Bicyclgermacrene             | 31.061          | 7.0 ± 0.06                 | 4.2 ± 0.05  | t           | t           | 1486            | 1500           | 122              |
| α-muurolole                  | 31.263          | 0.2 ± 0.01                 | t           | t           | t           | 1491            | 1500           | -                |
| Z-α,-Bisabolene              | 31.395          | 0.3 ± 0.01                 | t           | t           | t           | 1494            | 1506           | -                |
| δ-amorphene                  | 31.510          | t                          | 1.0 ± 0.11  | t           | t           | 1496            | 1511           | -                |
| δ-cadinene                   | 32.168          | 0.8 ± 0.03                 | 0.4 ± 0.06  | 1.3 ± 0.03  | t           | 1513            | 1522           | 90               |
| α-calacorene                 | 32.839          | 0.3 ± 0.01                 | 0.3 ± 0.03  | t           | t           | 1531            | 1544           | 115              |
| E-nerolidol                  | 33.871          | 69.6 ± 0.05                | 81.3 ± 0.78 | 75.7 ± 0.74 | 83.1 ± 0.13 | 1557            | 1561           | 8                |
| Spathulenol                  | 34.209          | 0.9 ± 0.01                 | 1.3 ± 0.07  | 2.6 ± 0.13  | 3.0 ± 0.07  | 1566            | 1577           | 52               |
| Globulol                     | 34.492          | 1.9 ± 0.01                 | 1.7 ± 0.05  | 6.9 ± 0.09  | 1.2 ± 0.03  | 1573            | 1590           | 89               |
| Viridiflorol                 | 34.781          | 1.0 ± 0.01                 | 1.0 ± 0.07  | 0.8 ± 0.04  | 0.5 ± 0.02  | 1580            | 1592           | 28               |
| Cubeban-11-ol                | 34.869          | 0.5 ± 0.01                 | 0.5 ± 0.12  | 0.4 ± 0.03  | 0.2 ± 0.02  | 1583            | 1595           | 36               |
| Rosifoliol                   | 35.170          | 0.4 ± 0.15                 | 0.4 ± 0.07  | t           | 0.3 ± 0.04  | 1590            | 1600           | 68               |
| 1,10-di- <i>epi</i> -Cubenol | 35.547          | 0.3 ± 0.02                 | 0.5 ± 0.10  | t           | t           | 1600            | 1619           | 120              |
| 1- <i>epi</i> -Cubenol       | 36.036          | 0.4 ± 0.01                 | 0.2 ± 0.09  | t           | t           | 1613            | 1627           | 124              |
| Cadinol                      | 35.580          | t                          | t           | t           | 0.5 ± 0.01  | 1639            | 1638           | -                |
| α-cadinol                    | 37.099          | 0.6 ± 0.04                 | 0.5 ± 0.01  | t           | 0.5 ± 0.01  | 1642            | 1652           | 69               |
| Non-oxygenated compounds     |                 | 6.1 ± 0.01                 | 12.1 ± 0.10 | 2.4 ± 0.02  | 6.7 ± 0.06  |                 |                | 58               |
| Oxygenated compounds         |                 | 87.5 ± 0.03                | 86.6 ± 0.15 | 89.4 ± 0.20 | 84.8 ± 0.04 |                 |                | 2                |
| Total identified             |                 | 93.5 ± 0.02                | 98.6 ± 0.12 | 91.8 ± 0.15 | 91.5 ± 0.04 |                 |                |                  |

**Notes:** Identified compounds listed by order of elution in nonpolar column (RT<sub>x</sub><sup>®</sup>-5MS).

<sup>a</sup>Rt = Retention time in minutes.

<sup>b</sup>AI = Arithmetic index.

<sup>c</sup>E = Experimental data.

<sup>d</sup>L = Adams 2007.

<sup>e</sup>CV = Coefficient of variation in %.

t = Traces, not quantified.

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