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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*]

Adrieli L. de Souza[1], José L. de Souza-Junior[1], Adrielli Tenfen[1], Dilamara R. Scharf[2], Edésio L. Simionatto[2] & Michele D. Alberton[1]

[1]*Departamento de Ciências Farmacêuticas, Universidade Regional de Blumenau, Brazil Blumenau-SC, Brazil.*
[2]*Instituto de Pesquisas Tecnológicas, Universidade Regional de Blumenau, Brazil*
Contactos | Contacts: **Michele D. ALBERTON** - ***E-mail address:*** michele@furb.br

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 byciclogermacrene 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 dey 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 biciclogermacreno 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 β-caryophyllene, already identified in *E. punicifolia* oils (Oliveira *et al*., 2005) and *E. cuprea* (Apel *et al*., 2002). Further terpenes are common to this genus, such as α-pinene, limonene, β-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 perfomed 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 μm), 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 μm), 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 $mL.minute^{-1}$), 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 ± 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% (ww^{-1}) in the spring, 0.05% (ww^{-1}) in the summer, 0.05% (ww^{-1}) in the winter, 0.06% (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 byciclogermacrene 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

		Relative concentration (%)						
						AI[b]		CV%[e]
Compounds	Rt[a]	Spring	Summer	Autumn	Winter	E[c]	L[d]	
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-muurolene	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
Bicyclogermacrene	31.061	7.0 ± 0.06	4.2 ± 0.05	t	t	1486	1500	122
α-muurolene	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-*epi*- Cubenol	35.547	0.3 ± 0.02	0.5 ± 0.10	t	t	1600	1619	120
1-*epi*-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-oxigenated compounds		6.1 ± 0.01	12.1 ± 0.10	2.4 ± 0.02	6.7 ± 0.06			58
Oxigenated 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 (RTx®-5MS).
[a]Rt = Retention time in minutes.
[b]AI = Arithmetic index.
[c]E = Experimental data.
[d]L = Adams 2007.
[e]CV = Coefficient of variation in %.
t = Traces, not quantified.

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REFERENCES

Adams RP; 2007. Identification of essential oil components by gas chromatography/mass spectroscopy, 4th edn., Carol Stream, Allured.

Amorin ACL, Lima CKF, Hovell AMC, Miranda ALP. 2009. Antinociceptive and hypothermic evaluation of the leaf essential oil and isolated terpenoids from *Eugenia uniflora* L. (Brazilian Pitanga). **Phytomedicine** 16: 923 - 928.

Angioni A, Barra A, Coroneo V, Dessi S, Cabras P. 2003. Chemical composition, seasonal variability, and antifungal activity of *Lavandula stoechas* L. ssp. *stoechas* essential oils from stem/leaves and flowers. **J Agric Food Chem** 54: 4364 - 4370.

Apel M, Sobral M, Shapoval EES, Menut ATHC, Bessiere JM. 2002. Essential oils from *Eugenia* Species—Part VII: sections phyllocalyx and stenocalyx. **J Essent Oil Res** 16: 135 - 138.

Chaieb K, Hajlaou H, Zmantar T, Kahla-Nakbi A, Rouabhia M, Mahdouani K, Bakhrouf A. 2007. The chemical composition and biological activity of clove essential oil, *Eugenia caryophyllata* (*Syzigium aromaticum* L. Myrtaceae): A short review. **Phytotherapy Res** 21:501–506.

Chami F, Chami N, Bennis S, Bouchikhi T, Remmal A. 2005. Oregano and clove essential oils induce surface alteration of *Saccharomyces cerevisiae*. **Phytotherapy Res** 19: 405 - 408.

Costa DP, Santos SC, Seraphin JC, Ferri PH. 2009. Seasonal variability of essential oils of *Eugenia uniflora* leaves. **J Braz Chem Soc** 20: 1287 - 1293.

Fischer DC, Limberger RP, Henriques AT, Moreno PR. 2005. Essential oils from leaves of two *Eugenia brasiliensis* specimens from southeastern Brazil. **J Essent Oil Res** 17: 499 - 500.

Flora do Brasil. 2018. **Http://floradobrasil.jbrj.gov.br/reflora/herbarioVirtual/ConsultaPublicoHVUC/BemVindoConsultaPublicaHVConsultar.do?modoConsulta=LISTAGEM&quantidadeResultado=20&nomeCientifico=eugenia+brevistyla**

Garmus TT, Paviani LC, Queiroga CL. 2014. Extraction of phenolic compounds from pitanga (*Eugenia uniflora* L.) leaves by sequential extraction in fixed bed extractor using supercritical CO_2, ethanol and water as solvents. **J Supercritic Fluids** 86: 4 - 14.

Gil M, Pontin M, Berli F, Bottini R, Piccoli P. 2012. Metabolism of terpenes in the response of grape (*Vitis vinifera* L.) leaf tissues to UV-B radiation. **Phytochemistry** 77: 89 - 98.

Hay RKM, Svoboda KP. 1993. **Botany.** In: Hay RKM, Waterman PG, Volatile oil crops: their biology, biochemistry and production, Longman Group, Essex, UK.

Kim HM, Lee EH, Hong SH. 1998. Effect of *Syzygium aromaticum* extract on immediate hypersensitivity in rats. **J Ethnopharmacol** 60: 125 - 131.

Löesch C, Stein CE. 2011. **Estatística descritiva e teoria das probabilidades** Edifurb, Blumenau, Brasil.

Magina MDA, Dalmarco EM, Wisniewski A, Simionatto EL, Dalmarco J, Pizzolatti MG, Brighente IB. 2009. Chemical composition and antibacterial activity of essential oils of *Eugenia species.* **J Nat Med** 63: 345 - 350.

Marchese A, Barbieri R, Coppo E, Orhan IE, Daglia M, Nabvi SF, Izadi M, Abdollahi M, Nabvai SM, Ajami M. 2007.Antimicrobial activity of eugenol and essential oils containing eugenol: A mechanistic viewpoint. **Crit Rev Microbiol** 43: 668 - 689.

Masotti V, Juteau F, Bessiere JM, Viano J. 2003. Seasonal and phenological variations of the essential oil from the narrow endemic species *Artemisia molinieri* and its biological activities. **J Agric Food Chem** 51: 7115 - 7121.

Miyazawa M, Hisama M. 2001. Suppression of chemical mutagen induced SOS response by alkylphenols from clove (*Syzygium aromaticum*) in *Salmonella typhymurium* TA1535/pSK1002 umu test. **J Agric Food Chem** 49: 4019 - 4025.

Nuñez L, D´Aquino M. 2012. Microbicide activity of clove essential oil (*Eugenia caryophyllata*). **Braz J Microbiol** 43: 1255 - 1260.

Oliveira RN, Dias IJM, Câmara CAG. 2005. Estudo comparativo do óleo essencial de *Eugenia*

punicifolia (HBK) DC. de diferentes localidades de Pernambuco. **Rev Bras Farmacog** 15: 39 - 43.

Pereira NLF, Aquino EA, Júnior JGAS, Cristo JS, Vieira-Filho MA, Najla FFM, Ferreira MN, Silva MKN, Nascimento EM, Correia FMA, Cunha FAB, Boligon AA, Coutinho HDM, Ribeiro-Filho J, Matia EFF, Guedes MIF. 2017. Antibacterial activity and antibiotic modulating potential of the essential oil obtained from *Eugenia jambolana* in association with led lights. **J Photochem Photobiol** 174: 144 - 149.

Pontin MA, Piccoli PN, Francisco R, Bottini R, Martínez-Zapater JM, Lijavetzky D. 2010. Transcriptome changes in grapevine (*Vitis vinifera* L.) cv. Malbec leaves induced by ultraviolet-B radiation. **BMC Plant Biol** 10: 224 - 237.

Siebert DA, Tenfen A, Yamanaka CN, Cordova CMM, Scharf DR, Simionatto EL, Alberton MD. 2015. Evaluation of seasonal chemical composition, antibacterial, antioxidant and anticholinesterase activity of essential oil from *Eugenia brasiliensis* Lam. **Nat Prod Res** 29: 289 - 292.

Silva JKR, Andrade EHA, Barreto LH, Silva NCF, Ribeiro AF, Montenegro RC, Maia JG. 2017. Chemical composition of four essential oils of *Eugenia* from the Brazilian amazon and their cytotoxic and antioxidant activity. **Medicines** (Basel) 4: 51 - 55.

Silva MHL, Andrade EHA, Zoghbi MGB, Luz AIR, Silva JD, Maia JGS. 1999. The essential oils of *Lantana camara* L. occurring in North Brazil. **Flav Frag J** 14: 208 - 210.

Taiz L, Zeiger E. 2004. **Fisiologia vegetal**. Artmed, Rio de Janeiro, Brasil.

Zaki MA, Balachandran P, Khan S, Wang M, Mohammed R, Hetta MH, Pasco DS, Muhammad I. 2013. Cytotoxicity and modulation of cancer-related signaling by (Z)- and (E)-3,4,3',5'-tetramethoxystilbene isolated from *Eugenia rigida*. **J Nat Prod** 76: 679 - 684.

Zheng GQ, Kenney PM, Lam LKT. 1992. Sesquiterpenes from clove (*Eugenia caryophyllata*). **J Nat Prod** 55: 999 - 1003.