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Eugenia neonitida Sobral and *Eugenia rotundifolia* Casar. (Myrtaceae) Essential Oils: Composition, Seasonality Influence, Antioxidant Activity and Leaf Histochemistry

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Investigou-se a influência da sazonalidade na composição do óleo essencial de folhas frescas de *Eugenia neonitida* Sobral e *Eugenia rotundifolia* Casar. (Myrtaceae) coletadas trimestralmente, assim como a atividade antioxidante do óleo essencial de folhas secas e a histoquímica foliar. Os componentes majoritários do óleo essencial de *E. neonitida* foram biciclogermacreno, germacreno D e β-cariofileno e os de *E. rotundifolia* foram α-pineno, β-pineno e β-cariofileno. Os óleos essenciais de ambas as espécies apresentaram principalmente hidrocarbonetos e álcoois cíclicos, sendo o óleo essencial de *E. neonitida* composto principalmente por sesquiterpenos e o de *E. rotundifolia* por monoterpenos. A precipitação parece influenciar na composição dos óleos essenciais de ambas as espécies. O óleo essencial de folhas secas apresentou baixa atividade antioxidante. Os testes histoquímicos evidenciaram que as cavidades secretoras armazenam soluções complexas e contêm, além dos óleos essenciais, outras classes de lipídeos.

The seasonality influence on the essential oil composition of trimestrially collected fresh leaves of *Eugenia neonitida* Sobral and *Eugenia rotundifolia* Casar. (Myrtaceae) was investigated, as well as the antioxidant activity of dried leaves essential oils, and the leaf histochemistry. *Eugenia neonitida* essential oil major compounds were bicyclogermacrene, germacrene D, and β-caryophyllene. *Eugenia rotundifolia* major compounds were α-pinene, β-pinene, and β-caryophyllene. The essential oils of both species were mainly composed by cyclic hydrocarbons and alcohols; sesquiterpenes in *E. neonitida* and monoterpenes in *E. rotundifolia*. Precipitation appears to influence both species essential oils composition. Essential oils from dried leaves exhibited weak antioxidant activities. Both species have presented secretory cavities filled with a complex solution of essential oils and other lipid classes, according to histochemical tests.

Keywords: *Eugenia neonitida*, *Eugenia rotundifolia*, essential oil, antioxidant activity, histochemistry

Introduction

Eugenia neonitida Sobral and *Eugenia rotundifolia* Casar. (Myrtaceae) are found only in Brazilian sandy coastal environments,[1] also referred to as "restingas", which are ecosystems constantly subjected to the impact of human occupation and to water and nutritional deficiencies due to sandy soil.[2]

Eugenia neonitida is commonly known as "pitangão", while the common name for *E. rotundifolia* is "araponga". Both species produce berry fruits, and *E. neonitida* ones are eatable, extremely aromatic, and used for beverage preparation.[3] They also possess numerous translucent dots in the whole plant defined as secretory cavities of the

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essential oils.[4] The composition of *Eugenia* leaf essential oils has been studied and many authors have reported the occurrence of α-pinene, α-copaene, β-elemene, allo-aromadendrene, δ-cadinene, spathulenol, globulol, epi-globulol, β-caryophyllene and α-humulene. These last two components are known for their anti-inflammatory properties and were identified in the essential oils of more than 30 *Eugenia* species.[5,6] However, neither *E. neonitida* nor *E. rotundifolia* essential oils composition was found in the literature.

This paper appraises the composition of fresh and dried leaves essential oils of *E. neonitida* and *E. rotundifolia*. In addition, variation in the amounts of fresh leaves essential oils major compounds was evaluated trimestrially seeking to correlate it with abiotic factors or with the plant's phenologic stage. The antioxidant activity of dried leaves essential oils and the leaves histochemistry were also reported.

Experimental

Abiotic factors

Data referring to the abiotic factors medium temperature (°C) and precipitation (mm) in Rio de Janeiro City during 2009 were obtained from Instituto Brasileiro de Meteorologia (INMET).[7] According to that, in Rio de Janeiro the medium temperature varied in the range of 22-29 °C and precipitation varied from 51 to 211 mm. Usually, tropical climate seasons are not well defined, occurring only periods of months colder and drier than others, as shown by Gaussen-Gagnouls ombrotermic diagram built with the climatological normals of medium temperature (°C) and precipitation (mm) during the period between 1961-1990.[7] However, analysis of the Gaussen-Gagnouls ombrotermic diagram built with the same abiotic factors during 2009 has shown a variation of this pattern, indicating the occurrence of a dry season from May to August in Rio de Janeiro.[7]

Plant material

Eugenia neonitida and *E. rotundifolia* leaves were obtained from georeferenced individuals in a sandy coastal environment located in Rio de Janeiro City called "Restinga de Grumari" (23º02'94''S and 43º31'98''W). Plant samples were collected trimestrially in a way that two collections occurred in drier and colder months (May and August) and both the other collections were carried out in wetter and hotter months (February and November). During the collection of the plant material the phenologic stages (presence of leaves, flowers and fruits) were observed.[8] Voucher specimens were deposited at Universidade Federal do Estado do Rio de Janeiro Herbarium (HUNI646 and HUNI650).

Essential oil extraction, seasonal variation and statistical analysis

Fresh leaves collected from four of both species georeferenced individuals during the months of analysis were subjected to hydrodistillation for 3 h using a Clevenger apparatus. Air dried leaves collected in November from five individuals of both species were likewise subjected to hydrodistillation. The essential oils obtained from dried leaves were weighted and the extraction yields expressed in relation to dry leaf mass were calculated.

A linear correlation test was used in order to detect any influence of medium temperature and precipitation on the percentages of the major compounds present in both species essential oils from the February, May, August and November 2009 collections. Likewise, major compounds of the essential oils were correlated with the phenologic stages of the collected georeferenced individuals. A *t* student test was also used to detect seasonal variation in the essential oils major compounds in May (cold and dry month) and in November (hot and wet month). In this case, the results presented here are the means of four replicate experiments. Statistical analyses were conducted using the software GraphPad Instat.

Gas chromatography-mass spectrometry (GC-MS)

The leaf essential oils were analyzed by high resolution gas chromatography with a flame ionization detector (GC-FID), and high resolution gas chromatography-mass spectrometry (GC-MS). For the GC-FID analysis a Varian Star 3350 apparatus equipped with a silica capillary column Quadrex Inc. 007-1 (50 m × 0.25 mm id; 0.5 μm film thickness) was used. For the GC-MS analysis a Varian Star CP3800 apparatus equipped with a triple quadrupole trap detector (Varian Star 12000L) and a fused silica capillary column Varian Inc. VF5-ms (30 m × 0.25 mm id; 0.25 μm film thickness) operating in electron impact mode (70 eV) were used. The carrier gas was H_2 in GC-FID and He in GC-MS. Similar chromatographic conditions were used in both analyses: injector and detector temperature was 250 °C; the column temperature was programmed to hold isothermal at 40 °C for 2 min, then heating rate of 6 °C min^{-1} to 250 °C, and at the end hold isothermal for 5 min. Injection volume was 0.5 μL of essential oil solution in CH_2Cl_2, in splitless mode.

Compounds identification was accomplished by comparing the obtained mass spectra with reference data

from National Institute of Standards and Technology database (NIST 05 2.0d), and also by comparing the experimental Kovat's retention index (IK_C) calculated from a C_7-C_{26} *n*-alkanes series with literature data.[9] The chemical compound amounts were expressed as percentage, calculated from the normalized area data.

Antioxidant activity

The radical scavenging activity was measured according to 2,2-diphenyl-1-picrylhydrazyl ($DPPH^•$) method.[10,11] The essential oils obtained from dried leaves of both species from November collection were diluted in ethanol to final concentrations of 1.000, 250, and 125 μg mL^{-1}. A 0.37 mL sample of 0.1 mmol L^{-1} $DPPH^•$ (Sigma, 90% purity) solution in ethanol was added to 0.93 mL aliquots of essential oil solutions. Ethanol (0.37 mL) plus essential oil solution (0.93 mL) was used as blank, while $DPPH^•$ solution (0.37 mL) plus ethanol (0.93 mL) was used as negative control. After 30 min of reaction in the dark and at room temperature the absorbance values (sample, blank and negative control) were measured at 517 nm. As positive control a 2,6-di-*tert*-butyl-4-methylphenol (BHT, Fluka, ≥ 99.0% purity) solution in the same concentration of the essential oil solutions was used.

The percentage of antioxidant activity (AA%) was calculated using the equation: $AA\% = 100 - \{[(Abs_{sample} - Abs_{blank}) \times 100] / Abs_{control}\}$. Each assay was repeated three times and results are means ± standard deviation. Efficient concentration values (EC_{50}) were calculated by linear regression of the data.

Histochemistry

Fresh leaves from both species were transversally hand sectioned and stained with 1% alcian blue and 1% basic fucsin in 50% ethanol. The following histochemical tests were also applied to transversal sections of fresh leaves: Sudan III and IV,[12] Sudan black B[13] and Sudan red B[14] for lipids; Nile blue sulphate for acid and neutral lipids;[15] 2,4-dinitrophenylhydrazine for terpenoids with carbonyl group[16] and Oil Red O for rubber.[17] The observations were captured in an Olympus BX41 light microscope.

Results and Discussion

Composition and seasonal variation of fresh leaves essential oils

Altogether, 41 compounds were identified in *E. neonitida* fresh leaves essential oil, and 40 compounds in *E. rotundifolia* (Tables 1 and 2). Essential oils major compounds were bicyclogermacrene, germacrene D and β-caryophyllene in *E. neonitida*, and α-pinene, β-pinene and β-caryophyllene in *E. rotundifolia*. These compounds are commonly identified in *Eugenia* leaves essential oils.[18]

The essential oils of both species are rich in cyclic compounds, especially hydrocarbons. A few alcohols and one oxide derivative were also identified. The essential oils compounds were grouped into four major classes: hydrocarbon monoterpenes, oxygenated monoterpenes, hydrocarbon sesquiterpenes, oxygenated sesquiterpenes and others, category in which non terpenoid compounds were included. *Eugenia neonitida* essential oil was rich in hydrocarbon sesquiterpenes throughout the analysis (Figure 1A), while hydrocarbon monoterpenes were the major compounds in the *E. rotundifolia* essential oil (Figure 1B).

The seasonal variation in the six major compounds of the essential oils of both species was addressed through the analysis of their relative abundance and is represented in Figures 1 and 2. In *E. neonitida* a decrease was observed in the amount of bicyclogermacrene, globulol, viridiflorol and epi-globulol during the coldest and driest month (August), while the amount of germacrene D increased remarkably (Figure 1A). These five mentioned compounds plus β-caryophyllene added up more than 50% of *E. neonitida* essential oil (Table 1). In *E. rotundifolia* a decrease was observed in the amount of β-caryophyllene, spathulenol and α-cadinol accompanied by an increase in the amount of α-pinene and β-pinene in August (Figure 1B). These last remarked components plus limonene added up more than 60% of *E. rotundifolia* leaves essential oil (Table 2).

Besides the mentioned quantitative variation, qualitative differences were also observed in both species essential oils. For instance, in *E. neonitida* essential oil β-copaene was not identified in February and May, while α-pinene, cyperene, valencene, and ledol were not identified in August (Table 1). In *E. rotundifolia* essential oil, α-cubebene, α-gurjunene, β-copaene and spathulenol were not identified in August (Table 2).

Since small differences in the amounts of the major compound of both species essential oils were detected, these values were statistically correlated to the phenologic stages of the collected individuals and the abiotic factors medium temperature (°C) and precipitation (mm). In relation to phenologic stages, leaves were observed in both species during the four months of analysis. Flowers and fruits were observed only in *E. neonitida* in February and November.

Statistically significant correlation between the amounts of major components of *E. neonitida* and *E. rotundifolia* essential oils with either the phenologic stage or the medium

Table 1. Seasonal variation in chemical composition of the essential oil of *Eugenia neonitida* fresh leaves, chemical composition of dried leaves essential oil, and calculated Kovat's retention index (KI_C). Entries are normalized area values

		Fresh leaves				Dried leaves
Compounds	KI_C	Feb	May	Aug	Nov	Nov
Ethyl acetate	824	0.8	0.6	-	1.0	-
(*E*)-3-Hexen-1-ol	837	0.5	0.2	-	0.6	-
α-Pinene	931	3.1	1.8	-	0.7	2.4
Terpinolene	1082	-	-	-	-	0.4
δ-Elemene	1337	0.8	0.9	0.9	1.2	1.6
α-Copaene	1379	1.8	2.8	3.6	3.4	5.2
β-Elemene	1390	1.3	1.3	1.2	1.2	1.5
Cyperene	1397	0.5	0.4	-	0.1	0.5
α-Gurjunene	1414	1.1	0.7	0.6	1.1	1.4
β-Caryophyllene	1423	6.7	9.2	9.9	11.0	12.5
β-Copaene	1433	-	-	0.7	0.1	-
Aromadendrene	1444	2.6	1.6	0.9	1.6	-
(*Z*)-β-Farnesene	1446	1.1	1.0	0.7	0.8	1.9
(*E*)-α-Bergamotene	1449	-	0.3	-	0.1	1.2
α-Himachalene	1456	3.4	3.2	4.5	3.0	3.5
(*E*)-9-epi-Caryophyllene	1460	-	0.2	-	-	-
α-Humulene	1464	2.8	2.8	2.5	3.0	3.9
β-Chamigrene	1474	1.3	1.2	1.7	0.9	1.1
Germacrene D	1481	8.2	5.7	18.7	6.0	6.5
Valencene	1491	1.4	1.8	-	0.6	0.9
Bicyclogermacrene	1496	18.7	16.3	15.2	21.0	24.3
β-Bisabolene	1501	2.5	2.0	2.4	2.1	2.7
(E,E)-α-Farnesene	1510	0.8	0.7	1.1	0.6	0.8
δ-Cadinene	1517	3.5	2.5	5.4	4.8	5.9
β-Sesquifelandrene	1521	-	0.9	-	-	-
(E)-γ-Bisabolene	1525	0.8	0.4	0.8	0.6	0.9
Germacrene B	1554	-	0.2	-	0.1	1.0
Ledol	1564	0.7	0.7	-	0.7	0.4
Spathulenol	1570	2.8	4.0	2.8	0.8	1.7
Caryophyllene oxide	1577	-	1.2	0.5	0.2	-
Globulol	1581	6.0	6.3	2.7	6.6	3.1
Viridiflorol	1589	9.1	7.5	3.5	7.7	4.6
Guaiol	1596	1.0	0.9	0.5	1.0	0.6
epi-Globulol	1599	4.9	5.2	2.8	4.4	3.2
5-epi-7-α-Eudesmol	1616	1.3	1.1	0.8	1.2	0.6
1,10-di-epi-Cubenol	1623	-	0.3	-	0.2	-
1-epi-Cubenol	1626	0.5	0.9	0.5	0.2	-
T-Muurolol	1631	2.8	2.6	3.1	0.7	-
(Z)-Cadin-4-en-7-ol	1633	0.5	1.2	0.5	1.9	1.4
α-Cadinol	1644	4.5	5.4	5.1	3.8	1.8
β-Bisabolol	1658	0.5	0.7	0.6	0.4	-
Total		98.5	96.8	94.3	95.3	97.3
Hydrocarbon monoterpenes		3.1	1.8	-	0.7	2.7
Oxygenated monoterpenes		-	-	-	-	-
Hydrocarbon sesquiterpenes		59.4	56.0	70.7	63.3	77.2
Oxygenated sesquiterpenes		34.7	38.2	23.6	29.7	17.4
Others		1.3	0.8	-	1.6	-
Oil yield / (%)		-	-	-	-	0.1

Table 2. Seasonal variation in chemical composition of the essential oil of *Eugenia rotundifolia* fresh leaves, chemical composition of dried leaves essential oil, and calculated Kovat's retention index (KI_C). Entries are normalized area values

		Fresh leaves				Dried leaves
Compounds	KI_C	Feb	May	Aug	Nov	Nov
(*E*)-3-Hexen-1-ol	841	-	-	0.3	-	-
α-Pinene	933	27.4	33.7	34.4	19.7	29.7
β-Pinene	973	31.9	29.8	34.1	20.6	25.5
Myrcene	982	0.8	0.7	0.7	0.3	0.5
Limonene	1021	4.2	2.9	2.3	1.9	2.0
Terpinolene	1079	0.5	0.4	0.4	0.1	-
α-Terpineol	1174	2.9	1.3	2.3	1.6	0.9
α-Cubebene	1351	0.5	0.2	-	0.3	0.5
α-Copaene	1379	0.4	-	-	0.4	-
Cyperene	1398	-	-	-	-	0.6
α-Gurjunene	1413	1.0	0.4	-	0.5	0.8
β-Caryophyllene	1423	8.0	7.1	3.6	11.7	8.5
β-Copaene	1434	0.5	0.1	-	0.3	-
β-Gurjunene	1437	0.5	0.3	-	0.5	-
Aromadendrene	1443	1.2	0.9	0.7	1.7	1.9
(*E*)-α-Bergamotene	1450	0.6	0.3	-	0.3	0.6
α-Himachalene	1455	0.7	0.7	0.4	1.1	0.9
(*E*)-9-epi-Caryophyllene	1463	0.7	0.4	-	0.7	0.8
(*E*)-Cadina-1(6).4-diene	1472	1.0	0.4	-	0.8	0.5
β-Chamigrene	1477	-	0.2	-	0.4	-
Valencene	1491	-	0.9	0.7	-	-
Bicyclogermacrene	1495	1.5	1.8	1.8	1.2	1.6
β-Bisabolene	1501	0.5	0.3	-	1.1	3.0
(*E*,*E*)-α-Farnesene	1513	0.7	0.6	-	0.5	0.5
δ-Cadinene	1517	1.9	1.3	0.8	1.8	1.7
β-Sesquifelandrene	1521	0.4	0.7	1.0	3.1	2.1
(*E*)-Cadina-1(2).4-diene	1528	1.3	0.8	0.6	1.3	1.7
Germacrene B	1557	0.5	0.4	0.4	0.5	2.2
Spathulenol	1570	1.5	1.9	-	2.3	1.6
Caryophyllene oxide	1576	0.6	0.9	2.1	2.7	-
Globulol	1581	2.2	1.4	1.7	2.7	0.8
Viridiflorol	1588	0.6	0.9	0.3	2.0	1.1
epi-Globulol	1599	0.4	0.4	0.5	0.9	0.9
5-epi-7-α-Eudesmol	1616	0.4	0.5	0.7	1.1	0.8
1.10-di-epi-Cubenol	1621	1.3	1.1	1.2	1.8	0.9
1-epi-Cubenol	1626	-	0.3	0.4	-	-
T-Muurolol	1631	1.0	0.7	0.9	1.2	0.6
(*Z*)-Cadin-4-en-7-ol	1637	0.8	0.6	0.3	1.3	0.5
α-Cadinol	1643	1.1	1.5	0.6	3.0	1.2
β-Bisabolol	1656	0.4	0.4	2.3	1.7	0.7
Total		100.0	97.1	95.4	93.1	95.4
Hydrocarbon monoterpenes		64.9	67.6	71.9	42.7	57.7
Oxygenated monoterpenes		3.0	1.3	2.3	1.7	0.9
Hydrocarbon sesquiterpenes		21.8	17.8	9.9	28.1	27.8
Oxygenated sesquiterpenes		10.4	10.4	11.1	20.7	9.0
Others		-	-	0.3	-	-
Oil yield / (%)		-	-	-	-	0.4

temperature was not detected. Statistically significant positive correlations were detected only between the precipitation and the compounds biclyclogermacrene (r = 0.987; P = 0.013*) in *E. neonitida* essential oil (Figure 2A), and β-caryophyllene (r = 0.973; P=0.057*) in *E. rotundifolia* (Figure 2B). Additionally, one statistically significant negative correlation was detected also between the precipitation and the α-pinene in *E. rotundifolia* essential oil (r = –0.984; P = 0.016*) (Figure 2B).

Thus, the phenologic stage and medium temperature do not seem to influence both species essential oils composition. Likewise, according to the *t* student test, statistically significant differences in the composition of essential oils of leaves collected during the coldest-driest and the hottest-wettest periods of the year were not detected. Similar observations were found by other researchers.[19]

Tropical climate seasons are not well defined. In Rio de Janeiro, for instance, 2009 monthly medium temperature and precipitation varied only from 22 to 29 °C and from 51 to 211 mm, respectively, as mentioned earlier.[7] This small variation does not establish the occurrence of spring, summer, autumn and fall, it only indicated the occurrence of a dry season from May to August. These abiotic factors are even more stable in sandy coastal environments such as "restinga de Grumari," characterized by high temperatures and low water availability,[2] which probably contributed for the small variation observed in the essential oils of both species.

However, statistically significant correlation was detected between precipitation and one out of six major compounds of *E. neonitida* essential oil and two out of six major compounds in the essential oil obtained from *E. rotundifolia* leaves. Even though only three statistically significant correlations were detected, a noteworthy decrease occurred in the percentages of viridiflorol, globulol and epi-globulol in *E. neonitida* essential oil and in the percentages of spathulenol and α-cadinol in *E. rotundifolia* essential oil in August, the month with the lowest precipitation level. In fact, many authors have linked the observed differences in the amount of essential oil compounds with season changes.[20-22]

Composition and antioxidant activity of dried leaves essential oils

The essential oils obtained from dried leaves collected from *E. neonitida* and *E. rotundifolia* in November are very similar to the essential oils obtained from fresh leaves. Most of their major compounds are also the major compounds in the essential oils obtained from fresh leaves (Tables 1 and 2). Bicyclogermacrene, β-caryophyllene and germacrene D are also the major compounds in *E. neonitida* dried leaves essential oil (Table 1). Likewise, α-pinene, β-pinene and β-caryophyllene are the major compounds in *E. rotundifolia* dried leaves essential oil (Table 2). *Eugenia neonitida* and *E. rotundifolia* dried leaves essential oils yields were 0.1% and 0.4%, respectively (Tables 1 and 2).

However, some qualitative and quantitative differences were observed when comparing both species fresh and dried leaves essential oils. In *E. neonitida*, for instance, aromadendrene and β-bisabolol were observed only in fresh leaves essential oil, while globulol, viridiflorol and α-cadinol percentages in dried leaves essential oil were inferior when compared to fresh leaves essential oil (Table 1). In *E. rotundifolia*, terpinolene and β-gurjunene were detected only in fresh leaves essential oil, while β-bisabolene and germacrene B percentages in dried leaves essential oil were superior when compared to fresh leaves essential oil (Table 2).

Another noteworthy comment is the occurrence of components such as terpinolene (*E. neonitida*) and cyperene (*E. rotundifolia*) only in the essential oils obtained from dried leaves, what may be explained by the superior number of individuals from which leaves were collected for essential oil extraction.

Eugenia neonitida and *E. rotundifolia* dried leaves essential oils exhibited weak antioxidant activities according to the DPPH$^{\bullet}$ assay. EC_{50} values were 8.6 mg mL^{-1} for *E. neonitida* and 5.8 mg mL^{-1} for *E. rotundifolia*. Because of the similarities in the composition of *E. neonitida*, *E. rotundifolia*, and other *Eugenia* species essential oils, it is possible to predict that the antioxidant activity of the last ones will also be weak. The exception is *E. caryophyllata*, commonly known as clove, whose essential oil exhibits strong antioxidant activity (EC_{50} = 0.2 μg mL^{-1}) due to the presence of eugenol.[5,6,23]

Histochemistry

The presence of lipids in secretory cavities and parenchyma cells of both species was detected by Sudan III, IV, black B, and red B. Nile blue sulphate indicated the presence of neutral lipids (such as hydrocarbons) also in both species parenchyma cells and secretory cavities. It also detected the presence of acid lipids (such as like fatty acids or phospholipids) only in *E. rotundifolia* secretory cavities. Rubber was detected by Oil Red O in both species secretory cavities and parenchyma cells, indicating once more the complexity of these structures content. Finally, 2,4-dinitrophenylhydrazine test used to detect the presence of terpenoids with carbonyl group did not react with the secretory cavities content, certifying the GC-FID and GC-MS analyses.

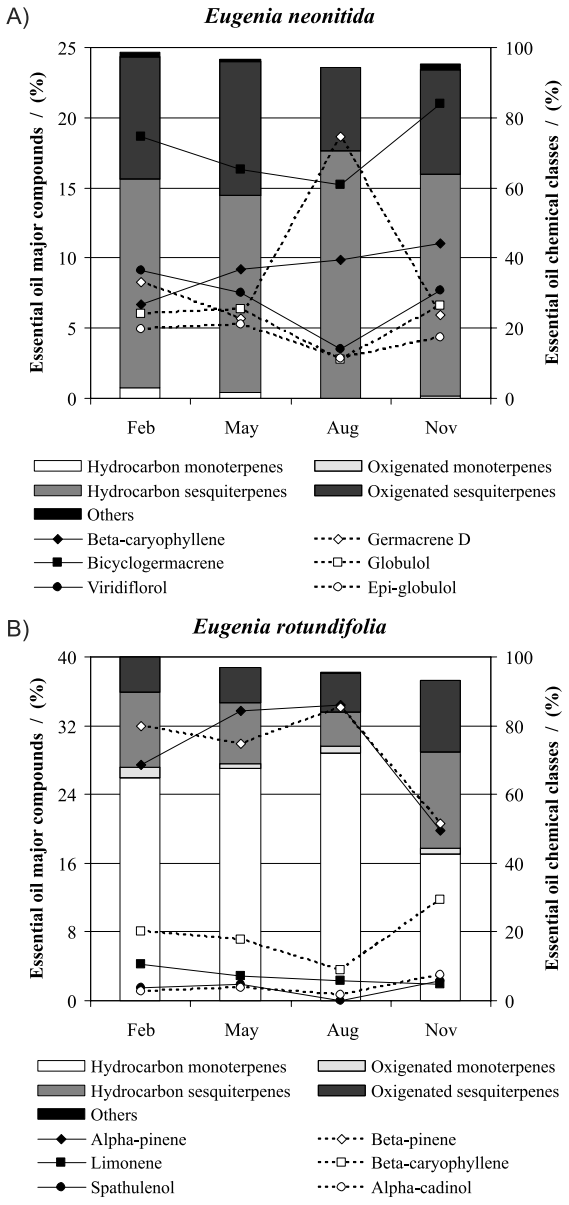

Figure 1. Seasonal variation in relative percentages of *Eugenia neonitida* (A) and *Eugenia rotundifolia* (B) essential oil major compounds and chemical classes during the analysis.

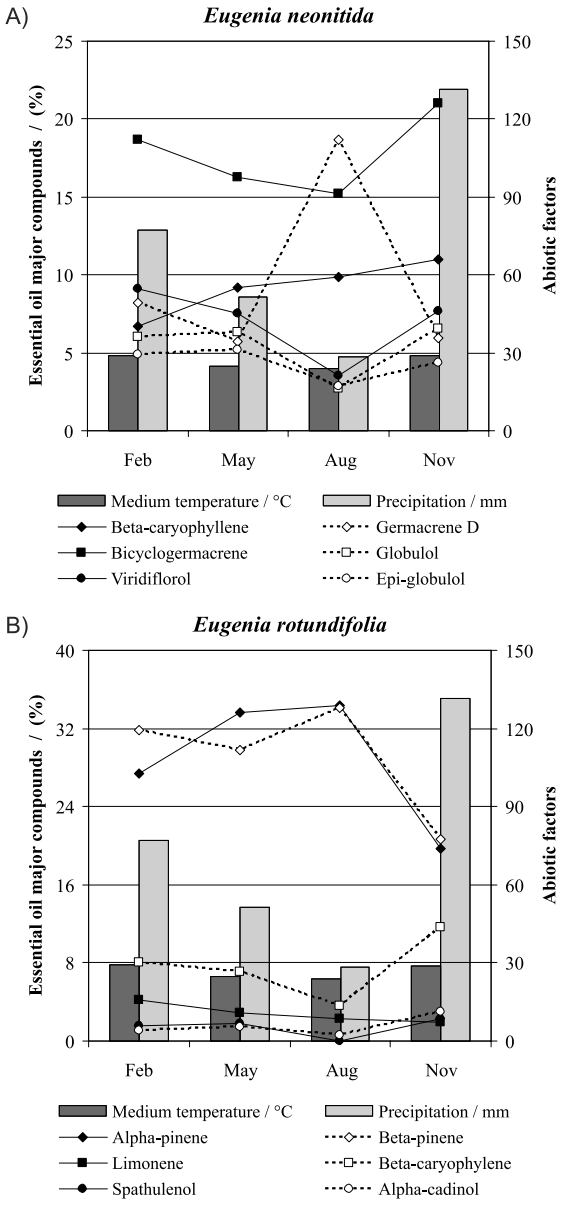

Figure 2. Seasonal variation in relative percentages of *Eugenia neonitida* (A) and *Eugenia rotundifolia* (B) essential oil major compounds during the analysis and the abiotic factors medium temperature and precipitation.[7]

Conclusions

The major compounds in the essential oils of fresh leaves were bicyclogermacrene, germacrene D and β-caryophyllene, in *E. neonitida*, and α-pinene, β-pinene and β-caryophyllene in *E. rotundifolia*. The essential oils of both species are mainly constituted by cyclic hydrocarbons and cyclic alcohols, but also possess cyclic oxides and non terpenoid aliphatic compounds. *Eugenia neonitida* essential oil is rich in sesquiterpenes, while *E. rotundifolia*'s is rich in monoterpenes. Despite the small quantitative and qualitative variations in both species essential oils, statistically significant correlation with either phenologic stages or medium temperature and essential oil major components was not detected. Statistically significant seasonal difference in these last was also not detected. Precipitation, however, seems to be related to the percentages of some essential oils major components. The essential oils of both species dried leaves are quite similar to the essential oils obtained from fresh leaves and exhibited weak antioxidant activity. The leaves' histochemistry certified the GC-FID and GC-MS analyses and also attested the complexity of the solutions stored in secretory cavities and parenchyma cells: besides hydrocarbon and alcohol lipids, they may possess fatty acids, phospholipids and rubber.

Supplementary Information

Gaussen-Gagnouls ombrotermic diagrams, GC-FID chromatograms, and histochemistry imaging are available free of charge at http://jbcs.sbq.org.br as a PDF file.

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Supplementary Information

Eugenia neonitida Sobral and *Eugenia rotundifolia* Casar. (Myrtaceae) Essential Oils: Composition, Seasonality Influence, Antioxidant Activity and Leaf Histochemistry

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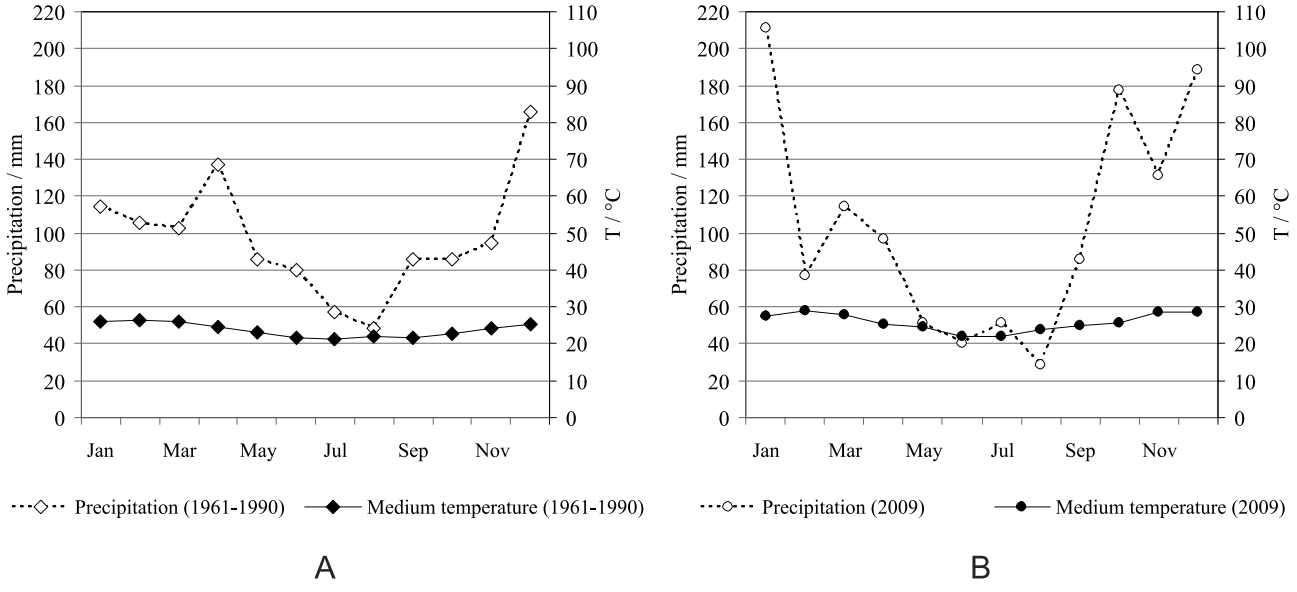

Figure S1. Gaussen-Gagnouls ombrotermic diagrams built using medium temperature (°C) and precipitation (mm) month means in Rio de Janeiro City during the period between 1961 and 1990 (climatological normal) (A) and also during 2009 (B). Data collected from reference 7.

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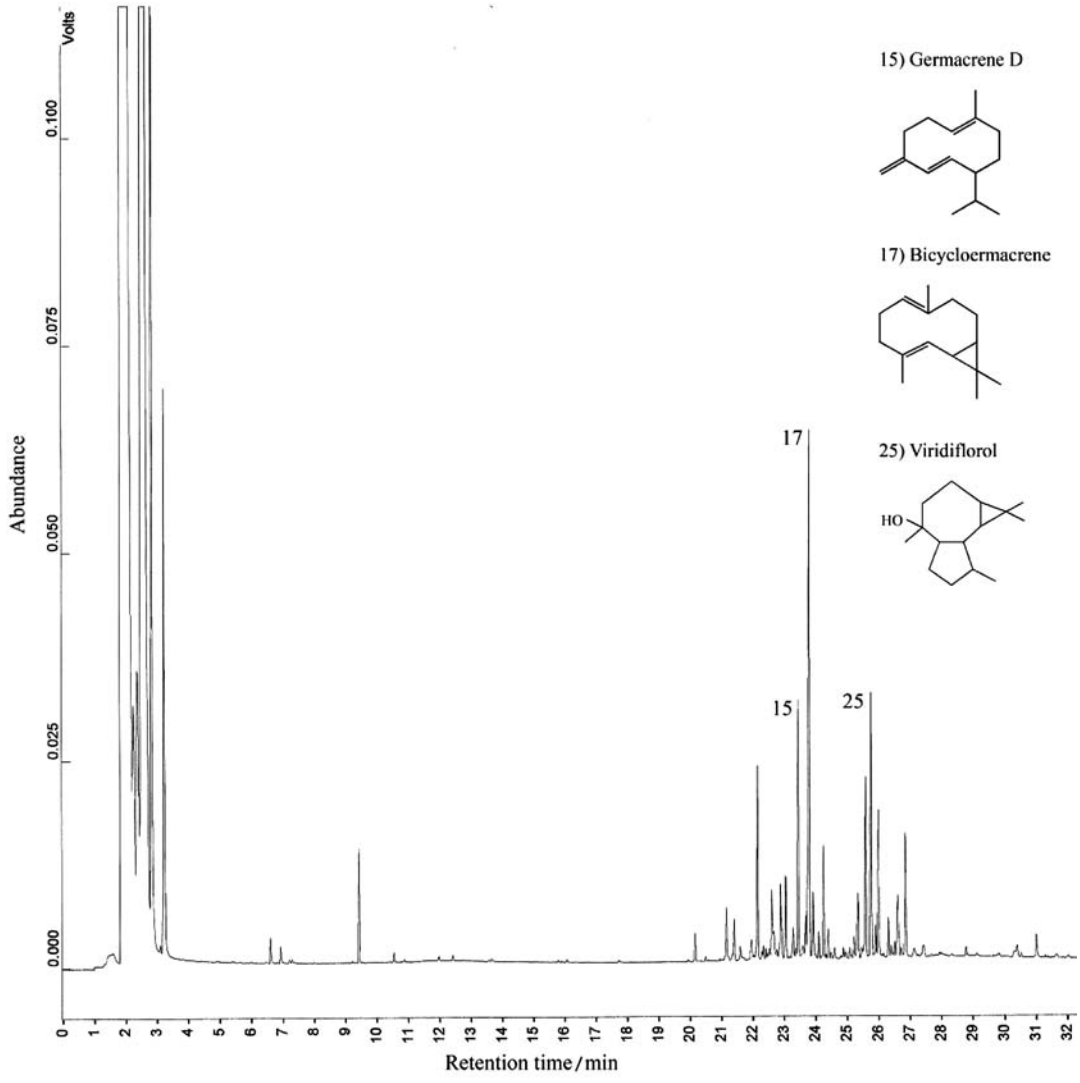

Figure S2. GC-FID of the essential oil obtained from *Eugenia neonitida* fresh leaves collected in February 2009 and the chemical structure of its major compounds.

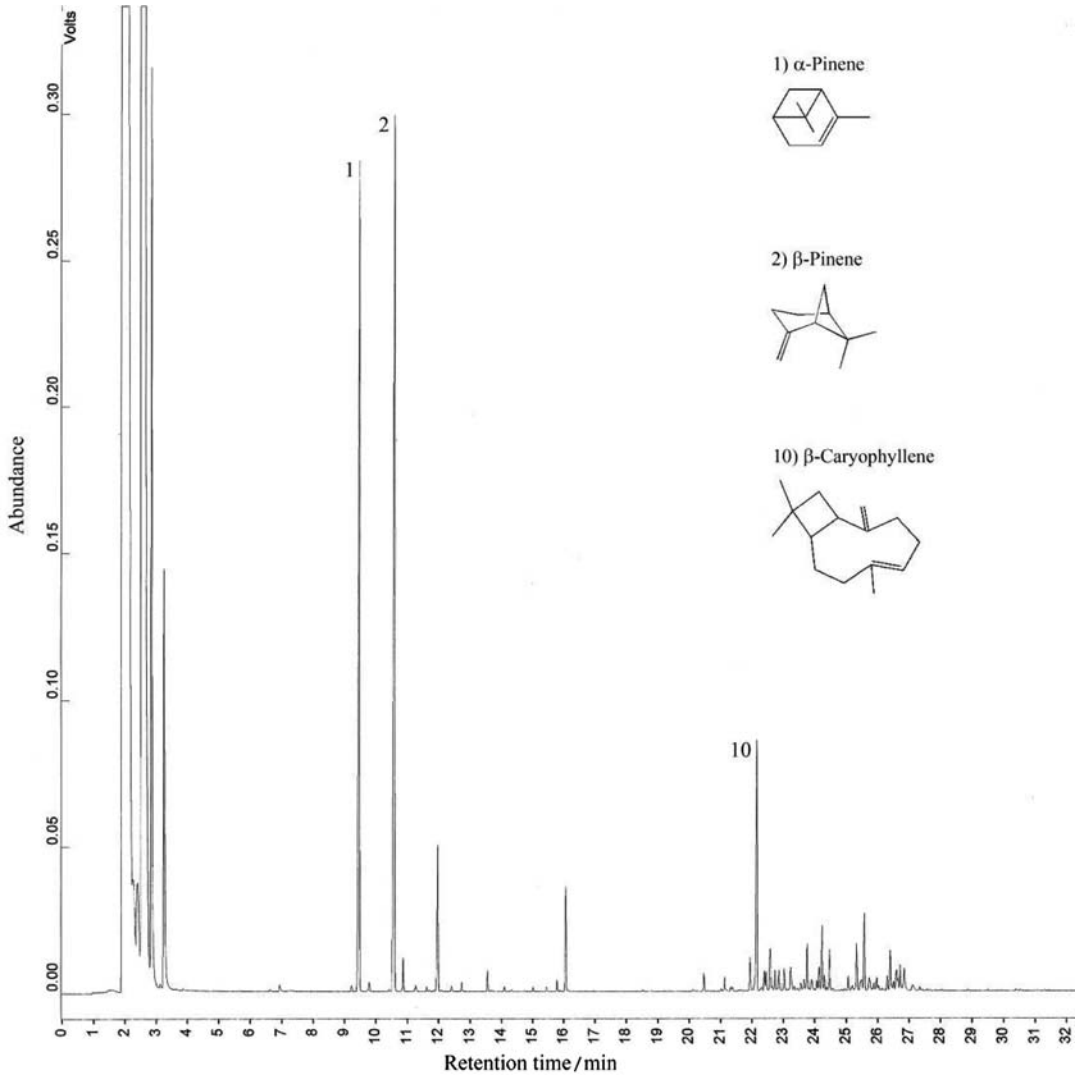

Figure S3. GC-FID of the essential oil obtained from *Eugenia rotundifolia* fresh leaves collected in February 2009 and the chemical structure of its major compounds.

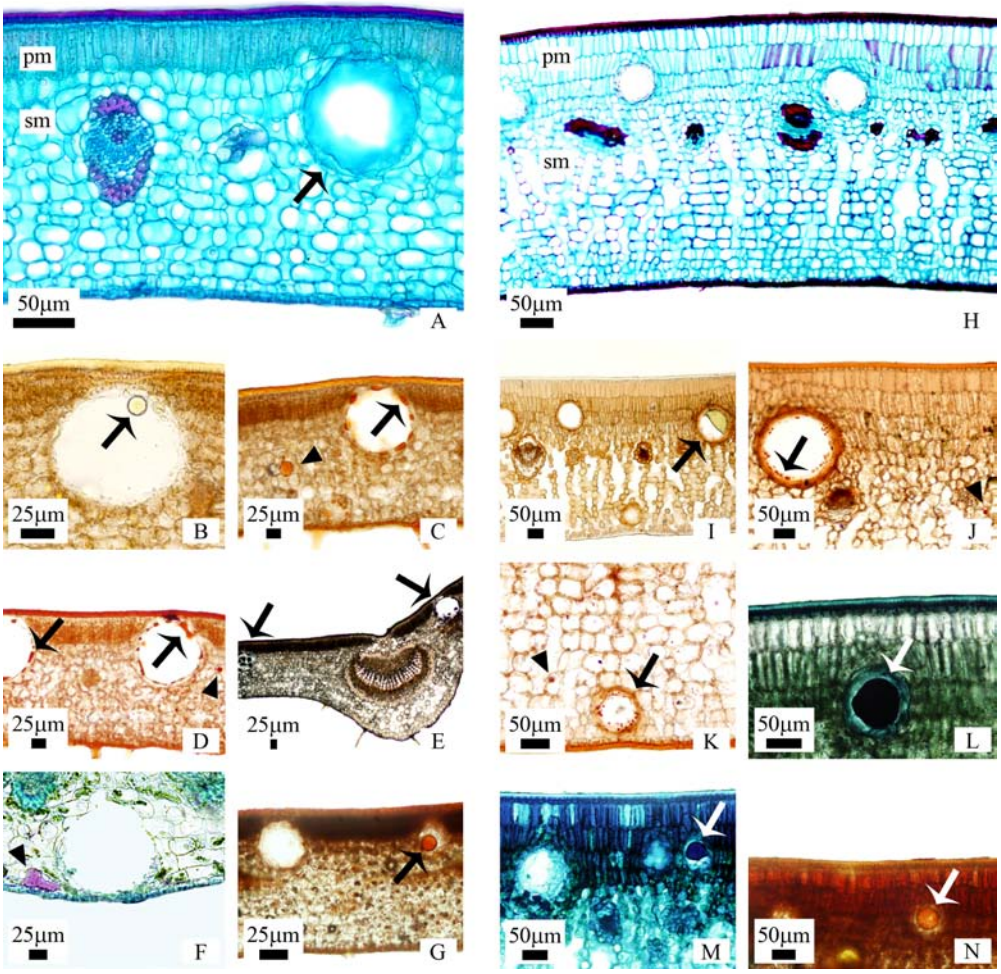

Figure S4. Transversal hand sections in *Eugenia neonitida* (A-G) and *Eugenia rotundifolia* (H-N) fresh leaves and its histochemistry, highlighting the response of secretory cavities (arrows) and parenchyma cells (arrowheads) to histochemical tests. A and H: transversal hand sections profiles stained with alcian blue and fucsin, showing the secretory cavities (arrows) overall distribution. B and I: arrows show the essential oil droplets in its natural color inside secretory cavities. C and J: lipids orange stained with Sudan III. D and K: lipids orange stained with Sudan IV. E and L: lipids dark stained with Sudan black B. F and M: pink neutral and blue acid lipids stained with Nile blue sulphate. G and N: rubber orange stained with Oil Red O. Legend: pm = palisade mesophyll, sm = spongy mesophyll, arrows = secretory cavities content, arrowheads = parenchyma cells content.