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Artigo

Seasonal Study of the Essential Oil from Aerial Parts of *Peperomia galioides* Kunth (Piperaceae)

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Estudo Sazonal do Óleo Essencial de Partes Aéreas de *Peperomia galioides* Kunth (Piperaceae)

Resumo: Objetivou-se realizar um estudo sobre a composição química e a variação sazonal do óleo essencial (OE) de partes aéreas de *Peperomia galioides* Kunth, coletadas no Parque Nacional da Serra dos Órgãos, Teresópolis, Rio de Janeiro, Brasil. Os componentes majoritários identificados foram: verão – α-pineno (22,75 ± 0,12 %) e mirceno (18,18 ± 0,03 %); outono – *trans*-cariofileno (22,37 ± 0,11 %) e *epi*-α-bisabolol (9,12 ± 0,12 %); inverno – *trans*-cariofileno (12,96 ± 1,01 %) e *epi*-α-bisabolol (11,11 ± 0,76 %); primavera – *trans*-cariofileno (16,25 ± 0,15 %) e *epi*-α-bisabolol (18,46 ± 0,32 %). Os resultados demonstram elevada correlação de Pearson diretamente proporcional com a temperatura ambiental *versus* a produção de monoterpenos e rendimentos dos OEs e inversamente proporcional com a temperatura e a produção de sesquiterpenos, principalmente, δ-cadinene, *E*-nerolidol e *epi*-α-bisabolol. Ainda, a correlação de Pearson demonstrou correlação entre monoterpenos oxigenados e índice de precipitação pluviométrica**.** Esse trabalho descreve pela primeira vez o perfil químico e sazonalidade do OE das partes aéreas de *P. galioides* coletadas na Mata Atlântica brasileira, destacando diferenças da composição química ao longo das estações do ano e da descrita na literatura.

Palavras-chave: *Trans*-cariofileno; α-pineno; monoterpeno; sesquiterpeno.

Abstract

The aim of this work is the study of the chemical composition and the seasonal variation of the essential oil from aerial parts of *Peperomia galioides* Kunth, collected at Serra dos Órgãos National Park, Teresópolis, Rio de Janeiro, Brazil. The major components identified were: summer - α-pinene (22.75 ± 0.12 %) and myrcene (18.18 ± 0.03 %); autumn - *trans*-caryophyllene (22.37 ± 0.11 %) and *epi*-α-bisabolol (9.12 ± 0.12 %); winter - *trans*-caryophyllene (12.96 ± 1.01 %) and *epi*-α-bisabolol (11.11 ± 0.76 %); spring- *epi*-α-bisabolol (18.46 ± 0.32 %) and *trans*-caryophyllene (16.25 ± 0.15 %). The results showed high Pearson's correlation directly between temperature and monoterpenes hydrocarbons biosynthesis as well as oils yielding and inversely between temperature and sesquiterpenes biosynthesis, mainly δ-cadinene, *E*-nerolidol and *epi*-α-bisabolol. Also Pearson's correlation showed direct correlation between oxygenated monoterpene and precipitation. This work describes for the first time the chemical profile and seasonality of the EO of the aerial parts of *P. gallioides* collected in the Brazilian Atlantic Forest, highlighting differences of the chemical composition between seasons and those described in the literature

Keywords: *Trans*-caryophyllene; α-pinene; monoterpene; sesquiterpene.

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Seasonal Study of the Essential Oil from Aerial Parts of *Peperomia galioides* Kunth (Piperaceae)

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1. Introduction

In the Plant Kingdom, the chemical diversity of volatile components can be observed in several botanical families, among then, Piperaceae, which has cosmopolitan distribution and is systematically classified in five genera: *Piper* L.; *Peperomia* Ruiz & Pav.; *Manekia* Trel.; *Verhuellia* Miq. and *Zippelia* Blume. The first two genera are the most important with the highest numbers of species.[1-3]

The genus *Peperomia* comprises about 1500 and 1700 species with pantropical distribution. The species are smaller and often have applications as ornamental.[3] *Peperomia galioides* Kunth, member of this genus, is an aromatic herb and popularly known in Brazil as “língua-de-sapo”.[4] In Peru it is used as a wound healing.[5] One scientific study evaluated the healing property of the lyophilized extracts and of an isolated compound of this extract, the (+)-*epi*-α-bisabolol. It has been shown that this sesquiterpene is responsible for the biological activity.[6] Extracts and fractions of *P. galioides* were also active against four species of *Leishmania,*[7,8] against *Trypanosoma cruzi*[7] and against two bacteria strains (*Staphylococcus aureus* and *S. epidermidis*).[9] Grifolic acid, grifolin, piperogalin, piperogalone, galopiperone, dihydroquinone and hydropiperone have already been isolated and identified from extracts of this plant.[10,11] To date, the chemical composition of essential oils (EOs) from leaves of *P. galiodes* has been studied in Bolivia[12] and from aerial parts, fresh or dried, in Peru.[13] However, there are no studies concerning about EOs for specimens of *P. galiodes* that occur in Brazil, nor is there any study evaluating the EO seasonal variation of this medicinal plant.

The importance of studying EOs in the field of chemical ecology lies in the fact that they exert important semiochemical roles mainly in the trophic level of plant-plant and plant-insect communication.[14] These interactions, as well as abiotic factors (edaphoclimatic conditions), provide changes in the chemical composition, which often promote changes in biological activity.[15] Thus, it is crucial to study this approach to increase the understanding of the influences of biotic and abiotic factors on the chemical composition of EOs.

Atlantic Forest microenvironments at altitudes, such as at Serra dos Órgãos National Park, Teresópolis, Rio de Janeiro, Brazil, show ecological pressure that in the evolutionary history of the species of these microenvironments, such as *P. gallioides*, may offer an interesting physiological profile and bioactive compounds different from other regions.[16] Therefore, the aim of this study was to evaluate the volatile chemical composition and the seasonal variation of the EO from aerial parts of *P. gallioides* collected at Serra dos Órgãos National Park. This unprecedented study can help in understanding ecological factors as well as reveal compounds of interest to the pharmaceutical and perfume industries.

2. Experimental

2.1. Plant material

Aerial parts of specimens of *Peperomia galiodes* Kunth were collected in an Atlantic Forest region at Serra dos Órgãos National Park, Teresópolis, Rio de Janeiro, Brazil (22°26'58''S, 42°59'16''W; Elevation: 1682m). Four collections were performed representing each season of the year at the end of the fourth week, in January 29th (summer), May 28th (autumn), August 27th (winter) and November 26th (spring) of 2017, at 9:00 a.m. in the morning, with five specimens for each sampling. Botanist specialist Dra. Elsie Franklin Guimarães identified the plant and herbal specimens were deposited in the Herbarium of the Botanic Garden of Rio de Janeiro, Rio de Janeiro, Brazil with voucher number RB730970. Collection register were done at SisGen under the number A6B1F29.

2.2. Essential oil obtaining

Fresh aerial parts from *P. galioides* (150g, triplicate) were submitted to hydrodistillation for two hours in a modified Clevenger-type apparatus.[17] The obtained EOs were separated and dried using anhydrous sodium sulfate, stored in sealed amber glass vials and kept under refrigeration at -20 °C until analysis by gas chromatography. Total EOs yielding were expressed as a percentage value (g/ 100 g of fresh plant material).

2.3 Essential oil analysis

The EOs from the aerial parts were initially diluted in dichloromethane (1 mg/mL) to be analyzed by gas chromatography coupled to flame ionization detector (GC-FID) and by gas chromatography coupled to mass spectrometry (GC-MS).

GC-FID analyzes were performed using gas chromatograph HP-Agilent 6890 GC-FID, equipped with capilar column HP-5MS (30 m x 0.25 mm i.d. x 0.25 µm film thickness), temperature programing from 60 °C to 240 °C, with increasing of 3 °C/min, using hydrogen as carrier gas at a flow rate of 1.0 mL/min. EOs solution was injected at 1 µL, with injector temperature at 270 °C, splitless. Retention times (tR) were measured in minutes. The relative percentage content of each compound in the mixture was obtained directly from the GC-FID.[18]

GC-MS analyzes were performed using gas chromatograph HP Agilent GC 6890 coupled to a mass spectrometer Agilent MS 5973N, with 70 eV of ionization energy, capilar column HP-5MS (30 m x 0.25 mm i.d. x 0.25 µm) film thickness, temperature programing from 60 °C to 240 °C, with increasing of 3 °C/min, using helium as carrier gas at a flow rate of 1.0 mL/min, and mass range *m/z* 40 – 600 atomic mass unit (u). EOs solution was injected at 1 µL, with injector temperature at 270 °C, splitless[18].

Retention indices (RI) were calculated using data obtained from the gas chromatography of a homologous series of saturated aliphatic hydrocarbons (C_8-C_{28}, Sigma-Aldrich) carried out in the same column and conditions used in GC-FID analysis for EOs[19].

The compounds identification was done by comparing the mass spectra obtained with the spectra from the equipment databases (WILEY7n; NIST), and with findings in the literature records.[20] The comparison of the calculated retention indices with the literature was used to assist on identification. All analyzes were performed in the Analytical Platform of Farmanguinhos, Fiocruz, Rio de Janeiro, Brazil. All experiments were carried out in triplicate of each essential oil.

2.4. Abiotic factors

Data on abiotic factors such as mean temperature (°C), humidity (%) and precipitation (mm) were obtained at the meteorological station at 100 m from the collection site (A618 - Code OMM: 86888) by the average collection months. The data obtained and used in this study are shown in Table 1.

Table 1. Climatic variables of the Serra dos Órgãos National Park in the collection period of *P. galioides*

Climatic Variables	January/2017	May/2017	August/2017	November/2017
Temperature (°C)	20.86	16.13	14.75	18.24
Humidity (%)	80.90	89.79	83.48	83.82
Precipitation (mm)	263.2	106.6	130.0	373.8

2.5. Statistical analysis

The areas related to the signs of the compounds in the chromatogram were shown in percentage (%) by the mean of the triplicate of the essential oil samples ± standard deviation (SD). The relationships between yielding, variations in chemical composition of compounds and classes of EOs and abiotic factors were investigated by Pearson's correlation using Statistica 10 software (StartSoft Inc., Tulsa, USA).

3. Results and Discussion

Retention indices, identified compounds, total yields of quantification, and percentages of compositions of essential oils per seasonal period of aerial parts from *P. galiodes* are shown in Table 2. Figure 1 shows the structures of the main compounds identified in the EOs. The Pearson´s correlation coefficients of the chemical composition and yields of the essential oils between the abiotic factors are presented in Table 3.

The EOs showed yellowish coloration and citrus odors during the summer and autumn period. In the winter and spring seasons they were shown to be colorless and odor characteristic. Lira *et al*[13] found these same characteristics for a sample of EO from aerial parts of *P. galiodes* analyzed in the month of January in the district of Lares, Calca province, Peru. The yielding values ranged from 0.32 % to 2.80 % (g/ 100g of fresh plant material), with the highest recorded during the summer (2.80 %) and autumn (1.02 %). These values are higher than those reported in the literature for this species.[12,13]

It was possible to observe values of significant Pearson´s correlations directly for temperature and yielding (r = 0.9120, Tables 3). For Botrel *et al*[21] the reduction of EO yielding is considered a natural mechanism in the period of lower temperatures, since plant species tend to concentrate their energy reserves for production and maintenance of basal or primary metabolism.

It was possible to identify 74 different compounds. A number of 52 compounds were registered in the summer period, which compared to the other seasons, is the largest number of identified compounds. The total percentage of compounds identified ranged from 98.01 % to 99.63 %, being the largest in the spring (Table 2).

The chemical compositions during the seasons of the year had significant variations and showed predominance of compounds produced by acetate-mevalonate or methylerythriol-4-phosphate metabolic pathways (Table 2). The EO of the summer period had a higher concentration of monoterpenes (68.93 %), with predominance of monoterpenes hydrocarbons (59.25 %), having as main cyclic monoterpene α-pinene (**1**, 21.71 %) and acyclic myrcene (**2**, 17.18 %) (Figure 1, Table 2). There are no reports in the literature of composition of *P. galioides* EO rich in monoterpenes. However, several EOs of Piperaceae are rich in this class of compounds.[22]

The percentages of monoterpenes in the analyzed samples decreased considerably in the colder seasons autumn (average temperature 16.13 °C) and winter (average temperature 14.75 °C). When analyzing the results obtained by the Pearson´s correlation coefficient it was verified a directly proportional strong value for monoterpenes hydrocarbons for environmental variable (r = 0.8371, $p < 0.05$ %) (Figures 2 and 3). It is also observed that when the pluviometric increase occurs, the percentage content of oxygenated monoterpenes (r = 0.7874, $p < 0.05$ %) tends to increase significantly (Table 3, Figure 3). Lima *et al*[23] describe that EOs tend to show an increase in monoterpene content when producing plants are in high temperature environments, however, excessive temperature increase may provide the opposite.

According to Table 3 it is possible to confirm that there is a correlation to increase the content of sesquiterpene hydrocarbons with humidity and reduction with increasing temperature. Sesquiterpene percentages are high during the autumn to spring period (Table 2), ranging from 86.08 % to 88.26 %, respectively. Similar results were found in Peru[13] and Colombia.[12] Compounds that show high levels during these periods were sesquiterpenes *trans*-caryophyllene (**4**, 16.25 % - 23.23 %) and caryophyllene oxide (**6**, 5.70 % – 11.38 %), and *epi*-α-bisabolol (**3**, 8.12 % - 18.46 %). These compounds have already been found in aerial parts from *P. galiodes* (dry and fresh) samples in Peru[13] *trans*-caryophyllene (13.1 % - 16.0 %) and *epi*-α-bisabolol (15.1 % - 21.3 %). High levels of compounds from shikimate pathway were found in samples from Colombia (safrole, 42.3 %) besides terpenes compounds, such as *epi*-α-bisabolol (29.2 %).[12] Several biological activities in EOs are described in the literature for sesquiterpene *epi*-α-bisabolol, such as anti-inflammatory, healing, antiplasmodic and anti-infective.[24]

Table 2. Chemical constituents and seasonal variation of the essential oil from *Peperomia galioides* fresh aerial parts

Compounds[1]	**RI^{lit}**[2]	**RI^{c}**[3]	**Relative peak area (%) ± SD**			
			Summer	**Autumn**	**Winter**	**Spring**
2*E*-hexenal	846	847	0.09±0.00			
santolina triene	906	910	2.06±0.00			
α-pinene	932	936-938	21.75±0.12	1.44±0.01	0.26±0.00	0.21±0.01
camphene	946	949	1.51±0.03			
β-pinene	982	978	6.11±0.04			
trans-isolimonene	980	983	0.14±0.00			
myrcene	988	990-992	17.18±0.03	1.51±0.02	0.40±0.00	0.28±0.02
3-octanol	988	994	0.23±0.00			
α-phellandrene	1002	1005	0.11±0.00			
α-terpinene	1014	1017	0.35±0.00			
p-cymene	1020	1023	0.30±0.01			
limonene	1029	1028	3.26±0.02			
1,8-cineole	1026	1030				0.48±0.02
β-phellandrene	1025	1028	1.28±0.01			
Z-β-ocimene	1032	1035	3.34±0.01			
γ-terpinene	1054	1058	1.14±0.02			
terpinolene	1088	1087	0.43±0.02			
linalool	1086	1096-1100	4.05±0.03	0.92±0.01	1.66±0.02	2.61±0.02
allo-ocimene	1128	1130-1131	0.30±0.00		0.14±0.01	
borneol	1165	1168	0.16±0.00		0.15±0.00	
terpinen-4-ol	1174	1175-1178	0.30±0.01	0.37±0.01	0.29±0.00	0.41±0.01
α-terpineol	1186	1187-1190	0.33±0.00	0.35±0.01	0.53±0.02	0.45±0.02
Z-ocimenone	1226	1227-1229	0.91±0.01			1.53±0.02
geranial	1264	1265-1267	0.65±0.02	4.33±0.02	7.93±0.02	7.29±0.01
α-cubebene	1345	1345-1348			1.65±0.03	0.79±0.00
neryl acetate	1359	1360	3.01±0.01			
α-copaene	1374	1375-1378	0.62±0.00	1.02±0.00	1.04±0.01	0.29±0.01
β-elemene	1389	1389-1392			2.95±0.03	0.35±0.01
β-longipinene	1400	1399				0.19±0.02
α-gurjunene	1409	1408				0.15±0.00
***trans*-caryophyllene**	**1417**	**1417-1420**	**9.21±0.12**	**22.37±0.11**	**16.25±0.15**	**23.23±0.87**
aromadendrene	1439	1439-1442	1.07±0.02	1.34±0.00		0.15±0.01
2E,6Z-dodecadienal	1445	1447-1448	0.26±0.01	0.89±0.01		
α-humulene	1454	1453-1454	0.80±0.00	3.84±0.03		
allo-aromadendrene	1458	1459-1460	0.25±0.01	0.56±0.01		
trans-cadina-1(6),4-diene	1475	1475-1478	0.19±0.00	0.76±0.00	1.08±0.01	1.69±0.02
α-amorphene	1483	1482-1486	0.93±0.02	2.83±0.02	2.03±0.01	
β-selinene	1489	1489-1490	0.18±0.00	0.37±0.01	0.58±0.00	0.30±0.01
trans-muurola-4(14),5-diene	1493	1493	0.43±0.01			
γ-amorphene	1495	1495	0.38±0.00	1.26±0.02		
α-muurolene	1500	1499-1501	0.32±0.01	0.89±0.00	0.29±0.00	
epizonarene	1501	1501		0.85±0.00	0.53±0.00	
trans-β-guaiene	1502	1502		0.80±0.01	0.26±0.00	

Compounds[1]	**RI^{lit}**[2]	**RI^{c}**[3]	**Relative peak area (%) ± SD**			
			Summer	**Autumn**	**Winter**	**Spring**
E,E-α-farnesene	1505	1504	0.30±0.00			
γ-cadinene	1513	1512-1513	1.23±0.03	3.29±0.08	2.91±0.02	
δ-cadinene	1522	1521-1524	2.04±0.01	6.13±0.04	7.90±0.12	4.30±0.02
trans-calamenene	1522	1527-1530		0.65±0.01	0.18±0.00	0.50±0.01
cis-calamenene	1528	1530-1531		0.44±0.00	0.60±0.00	0.54±0.01
Z-nerolidol	1531	1532				0.16±0.00
trans-cadina-1,4-diene	1533	1534-1536	0.21±0.01	0.61±0.00	0.44±0.02	0.43±0.01
α-cadinene	1537	1537-1538	0.20±0.01	0.55±0.00	0.26±0.00	0.49±0.01
10-*epi*-*cis*-dracunculifoliol	1540	1541				0.34±0.01
cis-sesquisabinene hydrate	1542	1543				0.19±0.00
α-calacorene	1544	1546				0.14±0.00
E-nerolidol	1561	1561-1564	1.82±0.02	8.53±0.06	6.86±0.21	4.35±0.06
caryophyllenyl alcohol	1570	1570-1572	0.14±0.00	0.46±0.01	0.47±0.01	0.60±0.03
spathulenol	1577	1576				0.38±0.01
caryophyllene oxide	**1582**	**1580-1584**	**2.41±0.04**	**5.70±0.04**	**8.79±0.03**	**11.32±0.42**
viridiflorol	1592	1592		0.18±0.00	0.26±0.00	0.33±0.01
carotol	1594	1594		0.27±0.01	0.15±0.00	0.88±0.02
longiborneol	1599	1597-1599		1.26±0.01	1.27±0.02	
guaiol	1600	1602		0.39±0.00	0.31±0.00	
geranyl isovalerate	1606	1606		0.34±0.00	0.19±0.00	
humulene epoxide II	1608	1607-1609	0.30±0.02	0.38±0.00	0.45±0.02	
cis-isolongifolanone	1612	1612		0.34±0.01	0.15±0.00	
isolongifolan-7-α-ol	1618	1618		1.00±0.00	0.39±0.02	
1,10-di-*epi*-cubenol	1618	1618-1620	0.28±0.00	0.41±0.00	0.24±0.01	
citronellyl pentanoate	1624	1623-1626	1.64±0.03	5.15±0.02	7.96±0.04	6.95±0.02
epi-α-cadinol	1638	1639-1642	0.57±0.01	1.94±0.01	1.78±0.09	2.15±0.03
epi-α-muurolol	1640	1640-1644	0.83±0.02	2.90±0.03	2.66±0.02	4.36±0.05
14-hydroxy-9-*epi*-*E*-caryophyllene	1668	1669-1672	0.35±0.00	0.95±0.02	0.40±0.01	1.81±0.02
Z-nerolidyl acetate	1676	1676-1679		0.41±0.01	0.15±0.00	0.56±0.00
***epi*-α-bisabolol**	**1683**	**1684-1686**	**1.57±0.03**	**8.12±0.12**	**13.93±0.07**	**18.46±0.32**
Monoterpene hydrocarbons			59.25	2.95	0.80	0.49
Oxygenated Monoterpenes			9.68	6.85	10.55	12.76
Sesquiterpene hydrocarbons			18.84	49.50	39.64	33.54
Oxygenated sesquiterpenes			9.91	38.76	46.44	52.84
Other compounds			0.32			
Total compounds quantified in essential oil (%)			98.01	98.05	97.43	99.63
Oil yielding (%)			2.80±0.55	1.02±0.02	0.32±0.08	0.89±0.29

[1]Compound listed in order of elution. [2]Retention index from Adams;[20] [3]Retention index calculated; Highlight for the most important identified compounds

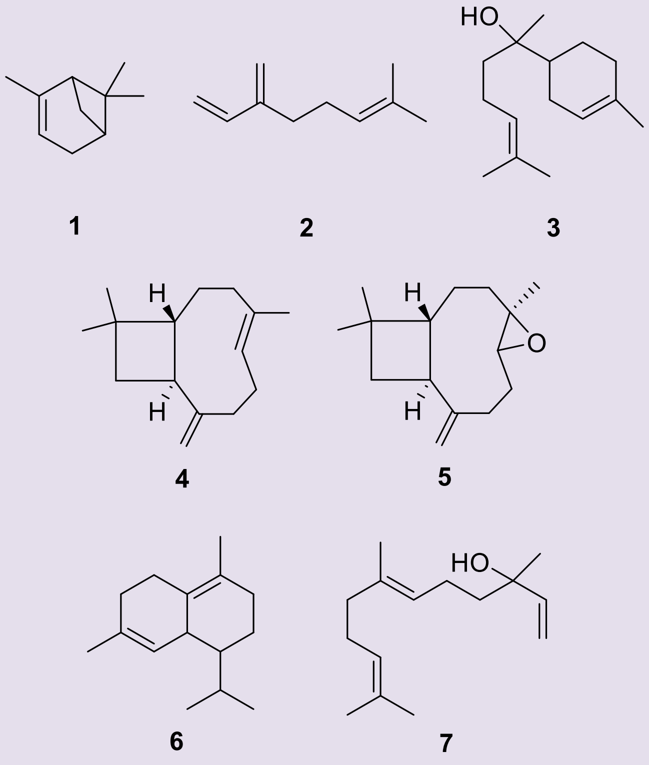

Figure 1. Major compounds identified in essential oil of *Peperomia galioides.* (1) α-pinene; (2) myrcene; (3) *epi*-α-bisabolol; (4) *trans*-caryophyllene; (5) caryophyllene oxide; (6) δ-cadinene; (7) *E*-nerolidol

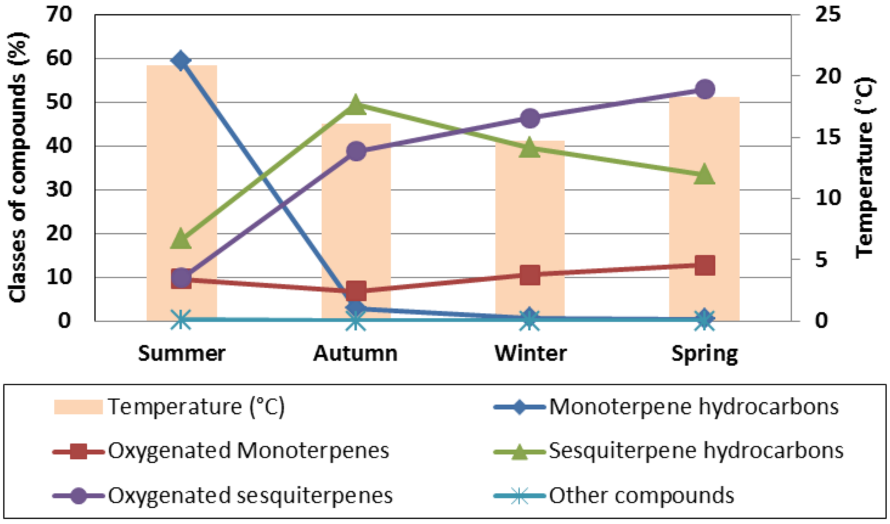

Figure 2. Correlation between temperature and essential oil compound classes

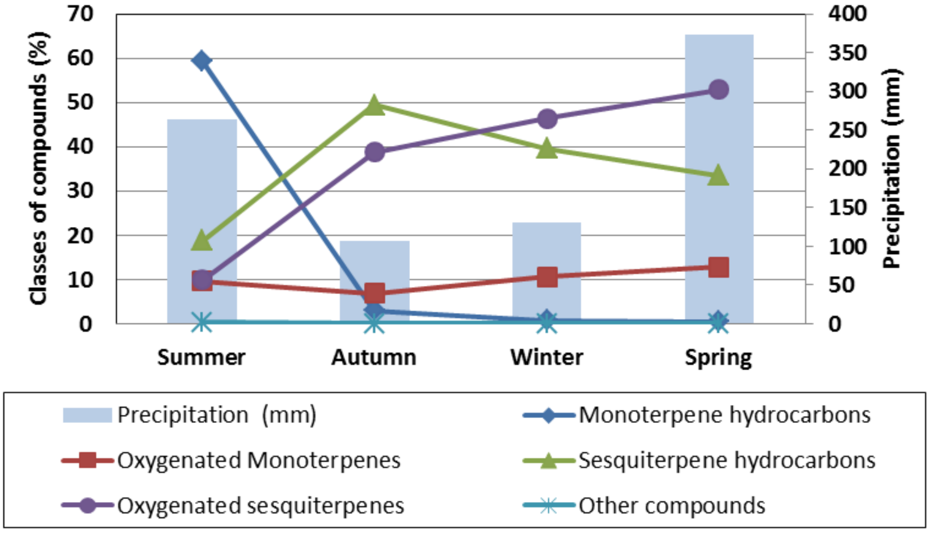

Figure 3. Correlation between precipitation and essential oil compound classes

Table 3. *Pearson's* correlation coefficients between essential oil chemical classes, compounds, yielding and abiotic factors

	Analyzed variables	**Pearson's correlation coefficient (*r*)**		
		Precipitation (mm)	**Humidity (%)**	**Temperature (°C)**
Compound higher concentration > 5 %	α-pinene	0.2100	-0.5975	0.8368
	myrcene	0.2003	-0.5880	0.8335
	geranial	-0.0175	0.2016	-0.7541
	trans-caryophyllene	-0.0107	0.7656	-0.5235
	δ-cadinene	-0.6657	0.4978	-0.9963***
	E-nerolidol	-0.7321	0.8657	-0.9001*
	caryophyllene oxide	0.3145	0.1125	-0.4986
	citronellyl pentanoate	-0.1223	0.2729	-0.8196
	epi-α-bisabolol	0.2551	0.1957	-0.5484*
Classes of compounds (%)	Monoterpene hydrocarbons	0.2165	-0.6095	0.8371*
	Oxygenated monoterpenes	0.7874*	-0.6505	0.1941
	Sesquiterpene hydrocarbons	-0.6274	0.9153*	-0.8468*
	Oxygenated sesquiterpenes	0.0148	0.4281	-0.7187
	Oil yielding (%)	0.3033	-0.4630	0.9120*

Significance: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

Other compounds with significant relative percentage greater than 5 % were found in the analyzed EOs over the four seasons, such as sesquitepenes *E*-nerolidol (**7**) and δ-cadinene (**6**). Both compounds showed values of Pearson´s correlation coefficients high and inversely proportional to temperature (*E*-nerolidol, $r = -0.9001$, $p < 0.05$ %; δ-cadinene, $r = -0.9963$, $p < 0.001$). The compound *epi*-α-bisabolol showed a significant correlation with temperature ($r = -0.5484$, $p < 0.05$ %). In other words, our findings show that higher temperature; the lower it will be the biosynthetic production of these sesquiterpenes, which confirms the inversely proportional relationship between the sesquiterpenes production and the temperature in the EO of *P. galiodes*. The EO of leaves of *Lychnophora ericoides* Mart. (Asteraceae) showed similar pattern related to the influence of abiotic factor temperature and variation of *E*-nerolidol contente.[25]

4. Conclusions

The results demonstrate a composition rich in monoterpenes in the summer and sesquiterpenos during the other seasons of the year. This work describes for the first time the chemical profile and seasonality of the EO from aerial parts of *P. gallioides* collected in the Brazilian Atlantic Forest, highlighting differences in chemical composition throughout the seasons and in the literature.

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