Revista Virtual de Química

Article

https://dx.doi.org/10.21577/1984-6835.20210067

Chemodiversity of Essential Oils in *Piper* L. (Piperaceae) Species from Marambaia Island, Rio de Janeiro-RJ, Brazil

Quimodiversidade de Óleos Essenciais em Espécies de Piper L. (Piperaceae) da Restinga da Ilha da Marambaia, Rio de Janeiro-RJ, Brasil

Rudá Antas Pereira,[a] Ygor Jessé Ramos,[a,b,c] George Azevedo de Queiroz,[c] Elsie Franklin Guimarães,[d] Anna Carina Antunes e Defaveri,[c] Davyson de Lima Moreira[a,b,*]

[a] Universidade do Estado do Rio de Janeiro, Instituto de Biologia, Pós-graduação em Biologia Vegetal, Maracanã, CEP 20550-013. Rio de Janeiro-RJ, Brazil.
[b] Departamento de Produtos Naturais, Farmanguinhos, Fundação Oswaldo Cruz, Manguinhos, CEP 21041-250, Rio de Janeiro-RJ, Brazil.
[c] Centro de Responsabilidade Socioambiental do Instituto de Pesquisas Jardim Botânico do Rio de Janeiro, Jardim Botânico, CEP 22460-030, Rio de Janeiro-RJ, Brazil
[d] Diretoria de Pesquisa do Instituto de Pesquisas Jardim Botânico do Rio de Janeiro, Jardim Botânico, CEP 22460-030, Rio de Janeiro-RJ, Brazil

***E-mail:** davysonmoreira@hotmail.com

Recebido: 22 de Março de 2021

Aceito: 22 de Março de 2021

Publicado online: 6 de Outubro de 2021

This study aimed to determine the pattern of chemodiversity from the chemical composition of the leaf essential oil (EO) of ten species and two varieties of *Piper* (*Piper amalago* L.; *P. amplum* Kunth; *P. anisum* (Spreng.) Angely; *P. arboreum* Aubl. var. *arboreum*; *P. arboreum* var. *hirtelum* Yunck.; *P. diospyrifolium* Kunth; *P. divaricatum* G. Mey.; *P. gaudichaudianum* Kunth; *P. lepturum* (Kunth) C.DC. var. *lepturum*; *P. lepturum* var. *angustifolium* (C.DC.) Yunck.; *P. mollicomum* Kunth and *P. vicosanum* Yunck.) collected in the Marambaia Island, Rio de Janeiro (Brazil). The main compounds identified in the EO were Linalool, Guaiol, 1-Butyl-3,4-methylenedioxybenzene, *iso*-Leptospermone, α-Eudesmol, *trans*-Nerolidol, Germacrene D, β-Elemene, 7-*epi*-α-Selinene, 1,8-Cineole, α-Eudesmol, α-Copaene, *trans*-γ-Bisabolene, Bicyclogermacrene and β-Bisabolol. In the chemometric analysis performed, it was possible to find correlations between the taxonomic characteristics of the genus *Piper* and the chemical composition of EO. In addition to registering *trans*-Caryophyllene as chemodiversity standard for this genus in this region. It is also the first study to compare the chemical composition of *Piper arboreum* Aubl. var. *arboreum* and *Piper arboreum* var. *hirtelum* Yunck.

Keywords: Piper, Essential Oil, monoterpenes, sesquiterpenes, Chemodiversity

1. Introduction

The Marambaia Island located in Southern of the Rio de Janeiro State is one of the few Atlantic Forest reserves in Southeastern Brazil. This region was very important for trade in the Brazilian colonial period, and due to the extensive degradation, nowadays it has restricted access and is an environmental protection area. [1,2] In this region there is a large restinga area, and the vegetation has suffered deforestation and shows different ecological pressure compared to seasonal and ombrophilous forests. So, the conservation and study of the current plant remnants are of great importance. [1-3]

The genus *Piper* L. (Piperaceae) has a wide occurrence in Brazil. About 180 species are registered for the State of Rio de Janeiro. In the restinga vegetation of this State, there are 16 species registered as *Piper*. Due to the number of *Piper* species in restinga areas, it is permissible to propose possible indications that they may present new physiological and morphological adaptation strategies for survival in this environment, making it interesting for a chemical study.[4-12]

Piper species are recognized for showing ritualistic and medicinal usages, as well as aromatic plants rich in essential oils (EOs). Some of the species are medicinal recognized and of commercial importance, such as *Piper nigrum* L. (Black pepper), *Piper methysticum* Forst (Kava-kava) and *P. hispidinervium* C. DC. (Long pepper), while *P. amalago* L. (Aperta-ruão), *P. mollicomum* Kunth (Jaborandi-manso) and *P. umbellatum* L. (Capeba) are used in folk medicine. [13-14] EOs from *Piper* species are recognized for their pharmacological properties, such as antimicrobial, antioxidant, antiparasitic, diuretic and insecticide activities.[15-18] These are mainly composed of monoterpenes, sesquiterpenes, arylpropanoids and benzoic acids derivatives [16-20]

©2021 Sociedade Brasileira de Química

This is an open-access article distributed under the terms of the Creative Commons Attribution License.

Evaluating the chemodiversity patterns of EOs derived from the chemical phenotypic plasticity of plants in different environments can significantly support the morphological analysis, understand different stimuli to environmental responses and their ecological functions.[21-23] This type of approach based on the chemical compositions of the *Piper* EOs from the Marambaia Island has never been described before. Thus, the present study aimed to determine the chemodiversity pattern from the chemical composition of the leaf EOs of ten (10) species and two (2) varieties of *Piper* (*Piper amalago* L.; *P. amplum* Kunth; *P. anisum* (Spreng.) Angely; *P. arboreum* Aubl. var. *arboreum; P. arboreum* var. *hirtelum* Yunck.; *P. diospyrifolium* Kunth; *P. divaricatum* G. Mey.; *P. gaudichaudianum* Kunth; P. lepturum (Kunth) C.DC. var. *lepturum*; *P. lepturum* var. *angustifolium* (C.DC.) Yunck.; *P. mollicomum* Kunth and *P. vicosanum* Yunck.) collected in the Marambaia Island. This proposal is also based in be able to assist in a better understanding of the chemical phenotypic plasticity of the genus *Piper* L.

2. Experimental

2.1. Plant material

Leaves from ten species and two varieties of *Piper* were collected in different areas of Marambaia Island, Mangaratiba, Rio de Janeiro, Brazil. They were collected during Spring, on November 3rd, 2019, from 9 a.m. to 12 p.m. Botanist specialist Elsie Franklin Guimarães identified the plants and herbal specimens were deposited in the Herbarium at Botanic Garden of Rio de Janeiro, Rio de Janeiro, Brazil. The location on the island and the specimen vouchers are shown in table 1.

2.2. Essential oil obtaining

Fresh leaves (150 g) of each specimen were submitted to hydrodistillation for two hours in a modified Clevenger-type apparatus.[24] The obtained EOs were separated from water and dried using anhydrous sodium sulfate (Sigma-Aldrich, Brazil), stored in sealed amber glass vials and kept under refrigeration at -20 °C until analysis by gas chromatography (GC). Total EOs yielding were expressed as a percentage value (g/ 100 g of fresh plant material).

2.3 Essential oil analysis

The EOs from fresh leaves were initially diluted in dichloromethane (1 mg/ mL) and then submitted to analysis by GC coupled to flame ionization detector (GC-FID) and by GC coupled to mass spectrometry (GC-MS).

GC-FID analysis was performed using gas chromatograph HP-Agilent 6890 GC-FID, equipped with capillary 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 without any correction. The relative percentage content of each compound in the mixture was obtained directly from the GC-FID analysis.[15]

GC-MS analysis was performed using gas chromatograph HP Agilent GC 6890 coupled to a mass spectrometer Agilent MS 5973N, with 70 eV of ionization energy, in positive mode, capillary 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.[15]

Retention indices (RI) were calculated using data obtained from the GC-FID of a homologous series of saturated aliphatic hydrocarbons (C_8-C_{28}, Sigma-Aldrich, Brazil) carried out in the same column and conditions used in GC-FID analysis for EOs.[25] 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.[26] The comparison of the calculated retention indices with the literature was used to assist on identification. All experiments were carried out in triplicate of each essential oil.

Table 1. Location and voucher for ten species and two varieties of *Piper* species collected in Marambaia Island, Mangaratiba, Rio de Janeiro, Brazil

Code	Species	Latitude and Longitude	Voucher
PAL	*Piper amalago* L.	23°03'37.8"S 43°58'49.4"W	RB00275754
PAP	*Piper amplum* Kunth	23°03'34.2"S 43°58'52.0"W	RB00556846
PAN	*Piper anisum* (Spreng.) Angely	23°03'47.3"S 43°59'00.1"W	RB01319732
PAA	*Piper arboreum* Aubl. var. *arboreum*	23°03'43.0"S 43°58'57.3"W	RB00298180
PAH	*Piper arboreum* var. *hirtelum Yunck*	23°03'42.9"S 43°58'57.2"W	RB00302890
PDP	*Piper diospyrifolium* Kunth	23°03'45.4"S 43°59'03.0"W	RB01319735
PDV	*Piper divaricatum* G. Mey.	23°03'39.4"S43°58'45.7"W,	RB01319736
PG	*Piper gaudichaudianum* Kunth	23°03'36.5"S 43°58'40.3"W	RB00299683
PLA	*Piper lepturum* var. *angustifolium* (C.DC.) Yunck.	23°03'36.4"S 43°58'40.8"W	RB01319712
PLL	*Piper lepturum* (Kunth) C.DC. var. *lepturum*	23°03'34.9"S 43°58'51.4"W	RB01319740
PM	*Piper mollicomum* Kunth	23°03'36.6"S 43°58'52.2"W	RB01319730
PV	*Piper vicosanum* Yunck.	23°03'23.9"S 43°58'35.2"W	RB00564792

2.4. Chemometric analysis

The data were analyzed using variance. Principal component analysis (PCA) and hierarchical analysis (HCA) were performed to assess the variance between the different operational taxonomic units (OTU) from chemical compositions of the EOs of each species.[20] The results were processed using STATISTICA software version 10 (StartSoft Inc., Tulsa, USA).

3. Results

The identified compounds, retention indices, EO yields, and percentages from the ten species and two varieties of *Piper* are shown in Table 2. Figure 1 shows the chemical structures of the main identified compounds.

Table 2. Chemical constituents of the essential oils from leaves of 10 *Piper* sp. and 2 varieties obtained from Marambaia Island, Mangaratiba, Rio de Janeiro, Brazil.

Compounds[a]	RIcalc	RIlit	Relative peak area (%)											
			PAA	PAH	PAL	PAN	PAP	PDP	PDV	PG	PLA	PLL	PM	PV
Nonane	900	900				0.13								
α-Thujene	930	930				0.23								
α-Pinene	938	939	0.29	1.02		1.30	0.46	3.56	0.39	0.42			**7.67**	
Camphene	956	954			0.14									
β-Pinene	982	979		0.38		0.17		0.29	0.34				6.38	
Myrcene	990	990		0.15		0.53			0.31		3.04	3.27	0.94	
α-Phellandrene	1006	1002				**7.53**								
3-Carene	1013	1011	4.57	0.19			0.52			0.53				4.52
α-Terpinene	1013	1017				**8.32**								
o-Cymene	1028	1026		0.16		0.35				0.64				3.78
Limonene	1033	1029	0.30	0.18		2.62		1.47	1.50				1.78	
β-*cis*-Ocimene	1035	1037	5.54	6.85	2.80		1.07	0.46	1.12	0.82				
β-Phellandrene	1036	1029				1.28								
1,8-Cineole	1037	1031											**15.00**	1.43
β-*trans*-Ocimene	1049	1050	2.25	2.76	0.86						0.68		0.41	
γ-Terpinene	1062	1059				0.14							0.32	
Terpinolene	1088	1088	0.66	1.19		0.24							0.14	
Linalool	1100	1096		0.43	**17.79**	0.33	5.43	0.23		7.43				
cis-4,8-Dimethyl-1,3,7-nonatriene	1115	1113	1.75	1.58										
neo-allo-Ocimene	1131	1132	0.73	0.96	0.30									
α-Terpineol	1197	1188											1.78	
3,5-Dimethoxytoluene*	1269	1264			0.32									
2-Undecanone	1291	1294											0.87	
δ-Elemene	1335	1338	0.72		1.15	0.14		1.32	0.53					
Bicycloelemene[b]	1339		5.28	1.30	2.29	1.40	0.74	0.32	2.69	1.99	6.96		0.67	
α-Cubebene	1350	1351		0.22	1.29	0.28			0.30		0.79			
Cyclosativene	1372	1371						0.42						
α-Ylangene	1372	1375							0.48					
α-Copaene	1378	1376	1.11	1.74	0.73	2.18	4.30	5.43	6.86		0.91			**12.34**
β-Bourbonene	1386	1388			0.77	0.18			0.94					
β-Cubebene	1389	1388				0.15								
β-Elemene	1390	1390	4.80	4.37	1.49	0.22	0.65	**9.51**	1.18	1.28	0.43		2.48	
Sesquithujene	1403	1405										0.33		
5-Isobutyl-1,3-benzodioxole[c]	1403			0.38			1.37			1.40				
1-Butyl-3,4-methylenedioxybenzene[d]	1408					**58.36**								
α-Gurjunene	1409	1409											0.36	
α-*cis*-Bergamotene	1415	1412										0.82		
β-Ylangene	1416	1420									0.28			
trans-Caryophyllene	1421	1419	**13.71**	**13.22**	**9.88**	1.20	**11.87**	**7.64**	**5.10**	**11.73**	**22.45**	**8.77**	**6.25**	**7.60**

Continua...

Continuação...

γ-Elemene	1432	1436	1.05		0.68			1.70	1.83	6.99			1.42	
β-Gurjunene	1433	1433		1.01		0.37								
α-*trans*-Bergamotene	1435	1434				0.18	1.38	5.52			1.70	0.76		6.18
Aromandendrene	1441	1441	0.52	0.18	0.16	0.15			1.00	0.90	0.95			
trans-Muurola-3,5-diene	1451	1453		0.25	0.18				0.23	0.98				
β-*trans*-Farnesene	1454	1456				0.77		0.71			3.60	**7.73**		
α-Humulene	1458	1454	1.86	2.06	0.79	1.08	1.37	1.19	0.85	2.50	1.54		2.68	
Cabreuva oxide B	1462	1464						0.82						
*allo*Aromadendrene	1463	1460							0.50	0.66	0.24		0.70	
trans-Cadina-1(6),4-diene	1474	1476		0.49					0.43				0.54	
γ-Muurolene	1477	1479	0.82	1.25	0.20	0.16		1.39	3.12					
cis-4,10-epoxy-Amorphane	1480	1479			0.37									
α-Curcumene	1483	1480										3.36		
Germacrene D	1483	1481	**9.63**	**8.32**	1.99	4.92	2.27	**9.73**	**24.70**	1.57	1.12		4.28	3.76
α-Amorphene	1486	1481								7.50				
β-Selinene	1490	1490	0.64	0.76			5.12	1.03	0.35	1.20			0.43	8.90
trans-Muurola-4(14),5-diene	1493	1493	0.98	0.73	0.37			0.42	2.47	2.69	0.66		0.32	
β-*cis*-Guaiene	1493	1493				0.14								
2-Tridecanone	1495	1496											0.50	
α-Zingiberene	1496	1493									7.34	11.10		
Valencene	1496	1496					5.02							
Viridiflorene	1497	1496												5.17
Bicyclogermacrene	1497	1500	**18.50**	5.32	6.60	4.21		1.75	**8.20**	**9.46**	**17.66**		2.66	
α-Muurolene	1499	1500		1.75	0.47			1.36	1.81				0.51	
α-Bulnesene	1503	1509	0.68	0.88										
cis,cis-α-Farnesene	1504	1505									0.41		0.36	
β-*trans*-Guaiene	1505	1502						0.32	0.65					
β-Bisabolene	1508	1505					1.78	3.10			1.67	4.69		6.81
γ-Cadinene	1515	1513	0.44	1.06	0.37			0.74	1.77				0.40	
Cubebol	1517	1515	1.85	1.14	1.71				0.38				0.95	
δ-Cadinene	1520	1523	3.81	6.07	2.40	0.35	2.99	2.18	6.20		0.51		2.17	4.34
7-*epi*-α-Selinene	1521	1522								**29.48**				
β-Sesquiphellandrene	1526	1522									0.99			
trans-γ-Bisabolene	1527	1531					1.29	0.45			**17.32**	**38.65**		
trans-Cadina-1,4-diene	1535	1534		0.39	0.20				0.51		0.88			
α-Cadinene	1539	1538							0.49					
cis-α-Bisabolene	1543	1536										0.28		
α-Calacorene	1544	1545						0.94						
Elemol	1552	1549		0.17	8.41									
Germacrene B	1561	1561			0.37					3.92				
trans-Nerolidol	1562	1563	5.78	6.83			1.41	10.00	3.09				**20.54**	
Palustrol	1573	1568											0.26	
Caryolan-8-ol	1578	1572						0.39						
Spathulenol	1578	1579	1.31	1.55	3.15	0.24	0.57		1.64		1.14		0.42	2.41
Caryophyllene oxide	1583	1583		0.24	0.90		2.37	1.34	0.29		0.51	1.37	0.72	1.65
Thujopsan-2-α-ol	1588	1587	0.79	0.49	0.29			0.99	1.99			0.36	0.53	
Globulol	1595	1590		0.17			0.62	1.90	1.20					
Viridiflorol	1596	1592											0.10	
Guaiol	1600	1600			**13.22**				0.46					

Continua...

Continuação...

Ledol	1607	1602											1.81	
Rosifoliol	1608	1600							0.79					
Khusimone	1609	1604						2.86	0.35					
iso-leptospermone	1612	1622					**26.44**							5.43
Humulene epoxide II	1612	1608						0.36				2.28	0.45	
α-*epi*-7-epi-5-Eudesmol	1615	1607			2.20									
Dillapiole	1621	1620											2.68	
Di-*epi*-1,10-cubenol	1623	1619	0.49						0.61					
epi-Cedrol	1624	1618						0.57						
1-*epi*-Cubenol	1630	1628	1.57	1.80					1.26				0.78	
trans-iso-Longifolanone	1631	1626			1.76			3.09						
Eremoligenol	1632	1631			0.77		1.48							
γ-Eudesmol	1634	1632					2.43							3.52
cis-Cadin-4-en-7-ol	1637	1636						0.63	0.49					
Gossonorol	1640	1637									0.23	1.53		
Caryophylla-4(12),8(13)-dien-5α-ol	1641	1640						0.84						
epi-α-Cadinol	1645	1640	1.14	3.02				0.83	1.49		0.36	0.74	1.34	
epi-α-Muurolol	1647	1642	1.13	3.28				1.10	1.68				0.32	
α-Muurolol	1647	1646	2.46	2.51	1.53		1.20	0.72	0.68				0.63	
Cubenol	1650	1646			0.44									
α-Eudesmol	1658	1653					**11.57**							**17.35**
α-Cadinol	1658	1654	2.40	**8.89**	2.78			2.09	3.39	1.32	0.21		1.50	
*neo*Intermedeol	1662	1660						1.32						
*allo*Himachalol	1669	1662			6.15									
epi-β-Bisabolol	1672	1671									2.84			
β-Bisabolol	1674	1675						2.05				**9.49**		
Apiole	1677	1678											1.14	
epi-α-Bisabolol	1687	1684						0.98				0.81		
α-Bisabolol	1688	1685					3.12	1.45						4.79
Non-oxigenated Monoterpenes	14.34	13.76	4.10	22.71	2.05	5.78	3.66	2.41	3.72	3.27	17.64	8.30		
Oxigenated Monoterpenes	0.00	0.43	17.79	0.33	5.43	0.23	0.00	7.43	0.00	0.00	16.78	1.43		
Non-oxigenated Sesquiterpenes	64.55	51.37	32.38	18.08	38.78	57.17	73.23	82.85	88.41	76.53	26.23	55.10		
Oxigenated Sesquiterpenes	18.92	30.09	43.68	0.24	51.21	34.33	19.79	1.32	5.29	16.58	30.35	35.15		
Arylpropanoids	0.00	0.38	0.00	58.36	1.37	0.00	0.00	1.40	0.00	0.00	3.82	0.00		
Other Compounds	1.75	1.58	0.32	0.13	0.00	0.00	0.00	0.00	0.00	0.00	1.37	0.00		
Total compounds in the essential oil (%)	99.56	97.61	98.27	99.85	98.84	97.51	96.68	95.41	97.42	96.38	96.19	99.98		
Oil yielding (%)	0.09	0.12	0.14	0.11	0.07	0.09	0.06	0.20	0.07	0.04	0.56	0.03		

[a]All compounds were identified by MS and RI in accordance with experimental. [a]Compound listed in order of elution. RIcalc = Calculated Retention Index (HP-5MS column); RIlit = Literature Retention index (Adams[26]); Main constituents in bold. [b]Identified by Shinoda et al.[66]; [c]Identified by Fernández-Álvarez et al.[67]; [d]Identified by Moreira et al.[68]; *Maybe a contaminant compound from solvent; PAA – *Piper arboreum* var. *arboreum*; PAH – *P. arboreum* var. *hirtellum*; PAL – *P. amalago*; PAN – *P. anisum*; PAP- *P. amplum*; PDP- *P. diospyrifolium*; PDV- *P. divaricatum*; PG – *P. gaudichaudianum*; PLA – *P. lepturum* var. *angustifolium*; PLL – *P. lepturum* var. *lepturum*; PM – *P. mollicomum*; PV - *P. vicosanum*.

The *Piper* species had an essential oil content ranging from 0.03 to 0.56% (Table 2). *P. mollicomum* (PM) *and P. gaudichaudianum* (PG) presented the highest percentages of EOs yield (0.56% and 0.20%, respectively).

The EO from *Piper* species showed in total 117 compounds (Table 2). *P. diospyrifolium* (PDP)*, P. divaricatum* (PDV)*, P. arboreum* var. *hirtellum* (PAH) and *P. mollicomum* (PM) had the highest numbers ranging from 43 to 47 compounds, while other species ranged from 17 to 40.

Piper EOs from Marambaia Island were pronounced by great relative percentage of sesquiterpenes (18.32 - 93.70%), mainly non-oxygenated ones (28.08 - 88.45%). Exception for *P. anisum* (PAN) which showed high levels of arylpropanoids (58.36%), followed by PM with 3.82%. Qualitatively, the non-oxygenated monoterpenes α-Pinene; non-oxygenated sesquiterpenes Bicycloelemene, α-Copaene, β-Elemene, α-Humulene, Germacrene D and *trans*-Caryophyllene, as well as oxygenated sesquiterpenes Spathulenol and *trans*-Caryophyllene oxide were registered in most *Piper* species.

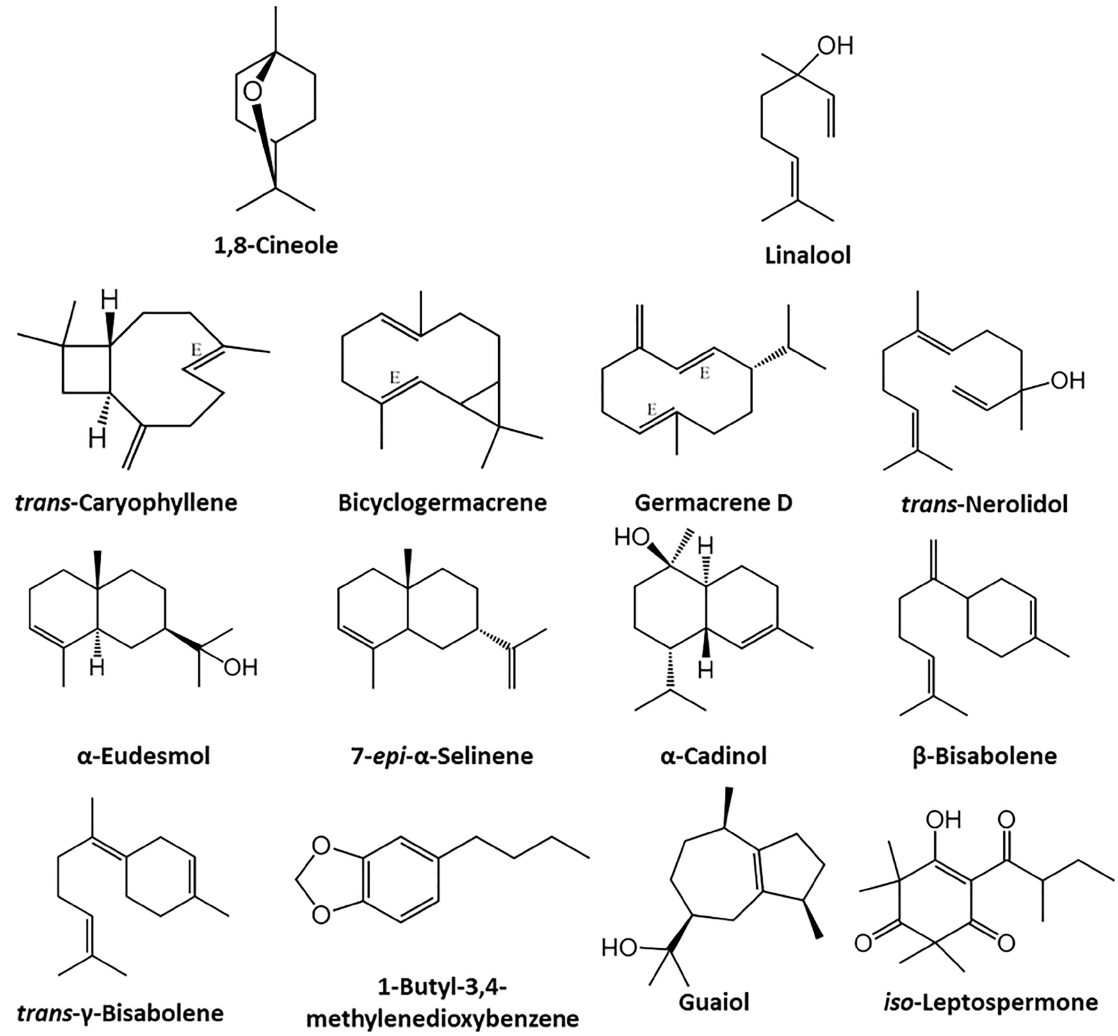

Figure 1. Major compounds identified in essential oil of 10 *Piper* sp. and 2 varieties from Marambaia Island, Mangaratiba, Rio de Janeiro, Brazil

P. amalago (PAL) EO showed as major compound the oxygenated acyclic monoterpene Linalool (17.79%) and the oxygenated bicyclic sesquiterpene Guaiol (13.22%). *P. anisum* (PAN) presented an unusual compound in the EO the arylbutanoid derivative 1-Butyl-3,4-methylenedioxybenzene (58.36%), previously described for this plant. *P. amplum* (PAP) showed cyclic triketone *iso*-leptospermone (26.44%) and α-Eudesmol (11.57%). PDP EO mostly consisted of *trans*-Nerolidol (10.00%), Germacrene D (9.73%) and β-Elemene (9.51%). PDV EO showed the monocyclic sesquiterpene Germacrene D (24.74%). PG EO is marked by the presence of 7-*epi*-α-Selinene (29.48%); PM by the oxygenated monoterpene 1,8-Cineole (15.00%) and *P. vicosanum* (PV) by the sesquiterpenes α-Eudesmol (17, 35%) and α-Copaene (12.34%).

Piper arboreum var. *arboreum* (PAA) and *Piper arboreum* var. *hirtelum* (PAH) showed agreement in the presence of the major bicyclic sesquiterpene *trans*- Caryophyllene (13.71 and 13.22%); and significantly difference (> 5%) only in the relative percentage of Bicyclogermacrene (18.50 and 5.32%) and α-Cadinol (2.40 and 8.89%), respectively.

P. lepturum var. *angustifolium* (PLA) and *P. lepturum* var. *lepturum* (PLL) showed high relative percentage of *trans*-γ-Bisabolene (17.32 and 38.65%, respectively), distinguishing their EO by the presence of Bicyclogermacrene (17.66%) for PLA and β-Bisabolol (9.49%) for PLL.

Some of the compounds were found to be exclusive of specific species, for example, *allo*-Himachalol and α-*epi*-7-*epi*-5-Eudesmol in PAL; Valencene in PAP; 1-Butil-3,4-metilenodioxibenzene, α-Terpinene and α-Phellandrene in PAN; Neointermedeol and α-calacorene in PDP; Rosifoliol and α-Cadinene in PDV; 7-*epi*-α-Selinene and α-Amorphene in PG; *epi*-β-Bisabolol and β-Sesquiphellandrene in PLA; α-Curcumene and α-*cis*-Bergamotene in PLL; Dillapiole, Ledol and α-Terpineol in PM, and Viridiflorene in PV.

Using the chemical profile of the majority composition (> 5%) of the EOs of the ten *Piper* sp. and two varieties from Marambaia Island, it was possible to apply chemometric analysis as shown in the heatmap in Figure 2, principal component analysis (PCA) in figure 3 and cluster analysis in figure 4.

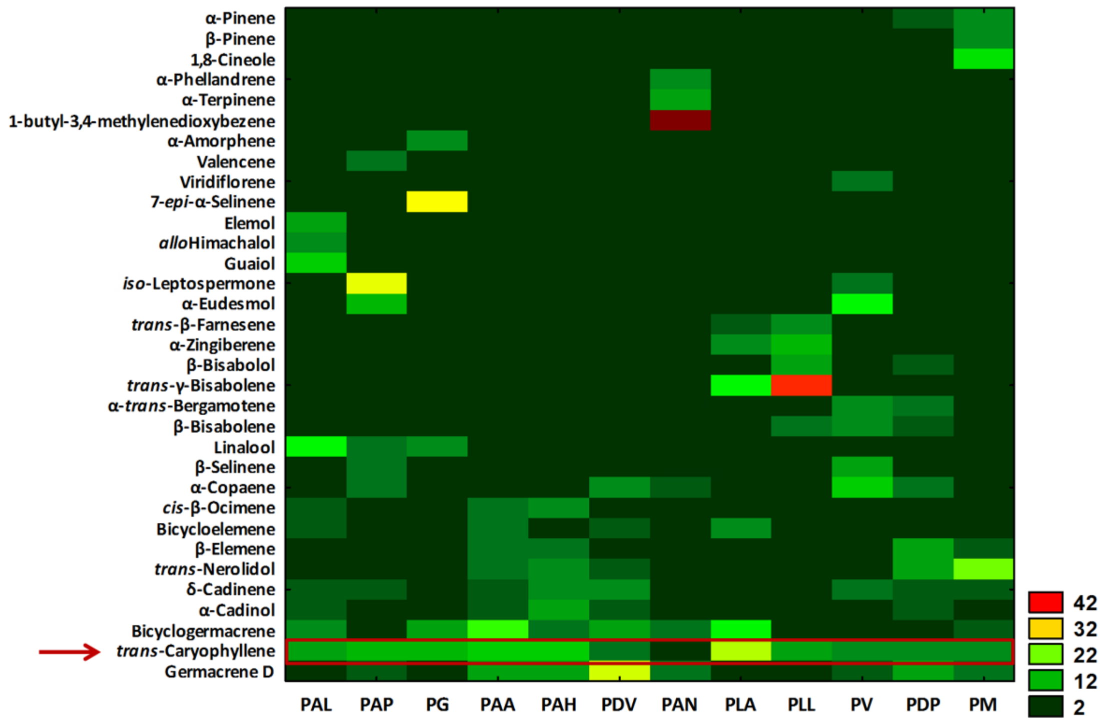

Figure 2. Heatmap (Two-Way Joining) based on the chemical profile of the samples (12 essential oils from *Piper* sp. obtained from Marambaia Island, Mangaratiba, Rio de Janeiro, Brazil). PAA – *P. arboreum* var. *arboreum*; PAH – *P. arboreum* var. *hirtellum*; PAL – *P. amalago*; PAN – *P. anisum*; PAP - *P. amplum*; PDP- *P. diospyrifolium*; PDV- *P. divaricatum*;PG – *P. gaudichaudianum*; PLA – *P. lepturum* var. *angustifolium*; PLL – *P. lepturum* var. *lepturum*; PM – *P. mollicomum*; PV - *P. vicosanum*

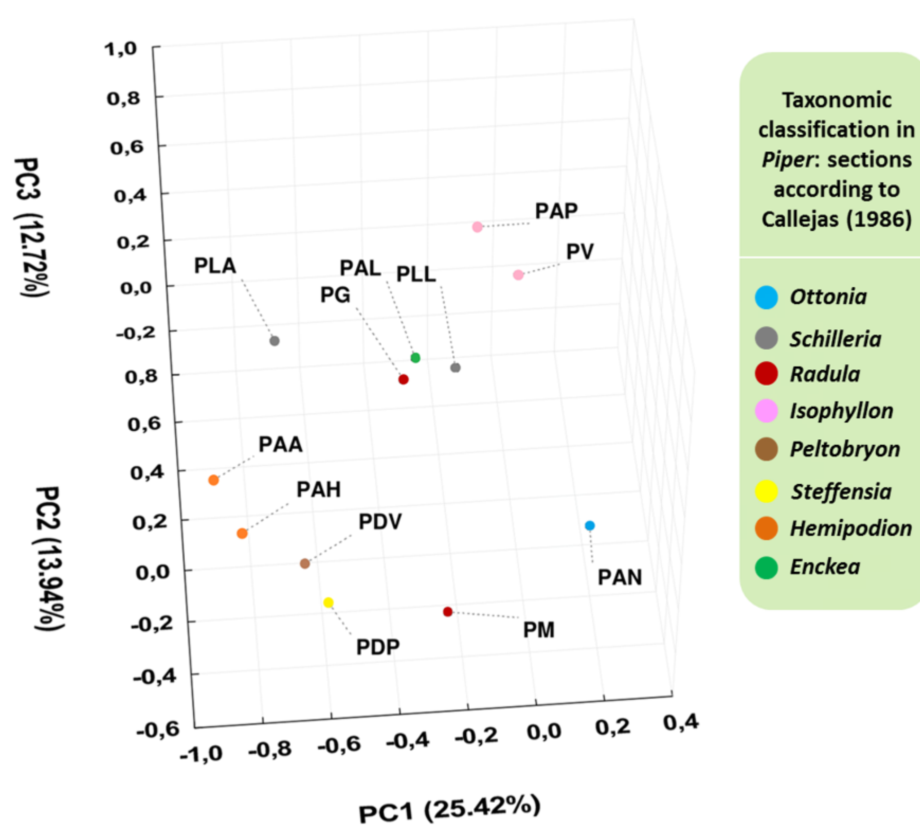

Figure 3. Triplot graph of principal component analysis based on the chemical profile of samples of 12 essential oils from *Piper* sp. obtained from Marambaia Island, Mangaratiba, Rio de Janeiro (Brazil), and correlated with taxonomic classification by Callejas (1986). PAA – *P. arboreum* var. *arboreum*; PAH – *P. arboreum* var. *hirtellum*; PAL – *P. amalago*; PAN – *P. anisum*; PAP- *P. amplum*; PDP- *P. diospyrifolium*; PDV- *P. divaricatum*;PG – *P. gaudichaudianum*; PLA – *P. lepturum* var. *angustifolium*; PLL – *P. lepturum* var. *lepturum*; PM – *P. mollicomum*; PV - *P. vicosanum*

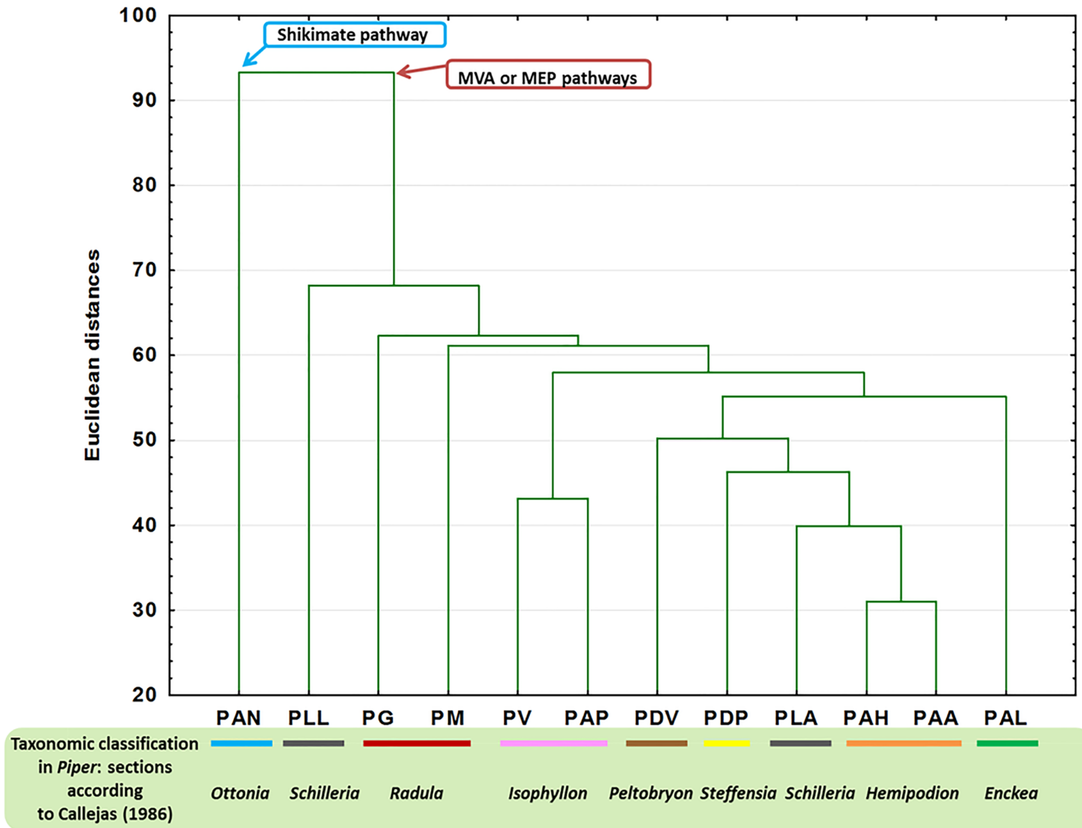

Figure 4. Cluster analysis chart based on the chemical profile of samples of 12 essential oils *Piper* sp. obtained from Marambaia Island, Mangaratiba, Rio de Janeiro (Brazil), and correlated with taxonomic classification by Calleja (1986). PAA – *P. arboreum* var. *arboreum*; PAH – *P. arboreum* var. *hirtellum*; PAL – *P. amalago*; PAN – *P. anisum*; PAP- *P. amplum*; PDP- *P. diospyrifolium*; PDV- *P. divaricatum*;PG – *P. gaudichaudianum*; PLA – *P. lepturum* var. *angustifolium*; PLL – *P. lepturum* var. *lepturum*; PM – *P. mollicomum*; PV - *P. vicosanum*

In figure 2, through color changes, a pattern of chemodiversity for the island's *Piper* genus based on the presence in all samples of the non-oxygenated sesquiterpene *trans*-Caryophyllene (1.20 - 22.45%), such as a predominance of compounds from farnesyl pyrophosphate (sesquiterpenes). The species with the lowest relative percentage (1.20%) in the EO was *P. anisum*, as its main compounds produced from the shikimate biosynthetic pathway. In proportion to the terpenic production in this EO, the compound has relevant value.

The compounds Germacrene D and Bicyclogermacrene are also produced by almost all species with lower percentages. This notation points out for evaluations at different time scales (seasonal and circadian) to understand the biosynthetic tendencies for the formation of humulil (*trans*-Caryophyllene) or germacryl (Germacrene D and Bicyclogermacrene) cations.

The results obtained in figure 3 (PCA) and 4 (Cluster) were correlated with the taxonomic classifications based on the morphology proposed by Callejas[27]. The main component analysis (Figure 3) was constructed in three-dimensional axis to better support the data set with a total variance of 52.08%. It is possible to observe from the variance the chemical diversity presented in *Piper* EOs from Marambaia Island. In PC1, the negative charges of *trans*-Caryophyllene (-6.23), Bicyclogermacrene (-4.21) and Germacrene D (-4.19) exerted greater force for species separation. However, PV and PAN registered positive charges on PC1 driven by the positive charge of *iso*-Leptospermone (+0.737) and 1-Butyl-3,4-methylenedioxybenzene (+1.75), respectively. The separations in PC2 are mainly justified by the negative charges of *trans*-Nerolidol (-3.03) and Germacrene D (-3.00) and positive charges of *trans*-γ-Bisabolene (+3.80) and Linalool (+1,59). In PC3 the positive and negative charges that justify the separations are mainly *iso*-Leptospermone (+3.66), α-Eudesmol (+3.78), *trans*-γ-Bisabolene (-1.61), and 1-Butyl-3,4-methylenedioxybezene (-1.49). Additionally, it is noted that PC1 separated the members of the ***Isophyllon* taxonomic section** (PAP and PV); PC2 separated the members (PG and PM) from the ***Radula* taxonomic section** and brought PM closer to PDP, which belongs to the ***Steffensia* taxonomic section**. However, the first three components were not sufficient to separate the varieties of *P. lepturum* (PLA and PLL).

Figure 4 (cluster) generated by the Unweighted Pair Group Methods with Arithmetic Average (UPGMA) using the Euclidean distances, it is possible to observe, from left to right, that in greater distances, there was separation of the samples that express the difference from the predominance of the different biosynthetic pathway compounds in the EOs. It was possible to register that the PAN, belonging to the *Ottonia* section, had the predominance of compound produced from shikimate pathway (1-Butyl-3,4-methylenedioxybenzene).

The other species showed the production mainly of the compounds from mevalonate (MVA) or 2-*C*-Metileritritol 4-phosphate (MEP) pathways, mainly, high percentages of *trans*-Caryophyllene. Unlike the results of the PCA, complete distancing of PLL and PLA species from the *Schilleria* taxonomic section was observed. PLL showed exclusively high levels of *trans*-β-Farnesene and β-Bisabolol in comparisons with the other samples, however, this species and its variety present a composition rich in bisabolyl cation derivatives. Further study will be needed to precisely define the chemical distinctions of these species by EO or by compounds present in crude extractions or fractions (metabolomic studies).

Likewise, the *Schilleria* section different only due to the smaller Euclidean distance, the members of the *Radula* section were separated, with PG rich in 7-*epi*-α-Selinene and PM rich in 1,8-Cineole. The high content of α-Eudesmol section *Isophyllon* may indicate the occurrence of a chemical marker, although PAP and PV species are also rich in *iso*-Leptospermone and α-Copaene, respectively. The groups PDV, PDP, PLA, PAH and PAA showed to be rich in Germacrene D that led to the separation of PAL which is rich in Linalool and Guaiol. Similar to PLA and PLL, chemical convergence in the composition of EOs made it impossible to separate the *P. arboreum* varieties.

4. Discussion

The results showed that the EO yield values are high in relation to other commercial species, such as chamomile (*Chamomilla recutita* (L.) Rauschter, Asteraceae). However, compared to the yields found for species in the literature it was lower. [20,28,29,30-32] It is known that EOs are associated with communication between plants, in attracting pollinators and seed disseminators and in defense against other plants, microorganisms and herbivory. In addition, several factors interfere in the qualitative and quantitative production of essential oils.[33,34] These changes are associated with the genotype of the species that, throughout the evolutionary process, made several plastic adaptations, enabling greater variations in EOs because of interactions with biotic and edaphoclimatic factors, such as seasonal and circadian rhythms modulations due to water availability, nutrition, and air pollution. [35,36]

The range in which temperature variations occur is one of the factors that most influence the development of the species, thus affecting the production of secondary metabolites. The formation of volatile oils, in general, seems to increase at higher temperatures, although very hot days lead to an excessive loss of these metabolites. In addition, water stress and saline soils, typical of restingas, often have significant consequences on the qualitative and quantitative yields of secondary metabolites in plants. There are several reports that these conditions generally lead to an increase in the production of various types of secondary metabolites.[34-36] The Marambaia Island, mainly in the restinga area, has a rainy tropical climate, with the air temperatures in the region typical of tropical coastal areas.[2] The monthly average temperatures are higher in relation to dense and stationary rain forests. In November, when the plants were collected, the average temperature was 24.5 °C. These factors may be related to the low yields found in the species of *Piper* in this area.

Many of the identified compounds are used in the pharmaceutical and cosmetic industry, presenting promising results and applications, for example, *trans*-γ-Bisabolene is used for the development of antiperspirants and deodorants.[37] *trans*-Caryophyllene is an important antioxidant component of many EOs used as seasoning.[37] Compound α-Pinene, a common constituent of several EOs, is used in the manufacture of insecticides and solvents; α-Terpinene shows antimicrobial and antifungal action.[38] The arylbutanoid derivative, 1-Butyl-3,4-methylenedioxybenzene, the main compound of *P. anisum* EO, has larvicidal activity[39,68], and Linalool which is widely used in the food and cosmetics industry, known for its antimicrobial and parasitic properties.[40,41]

For the first time it was identified as the majority 7-*epi*-α-Selinene in PG [42-48], Linalool and Guaiol in PAL[42,43,49-52], Germacrene D in PDV[53], and α-Eudesmol in PV[54,55]. However, the findings for PAN, PDP, PLA, PLL and PM were similar to that described in the literature.[20,31,39,42-59] We emphasize that PM has not presented until this report any content of arylpropanoids in the leaves, only in inflorescences and infructescences such as described before by our group.[20]

We highlight that, for the first time, the majority presence of i*so*-Leptospermone in the EOs of the genus *Piper* (PAL and PV). This compound is naturally occurring in EOs in the species of basal angiosperms.[60,61] The hypothesis is that chemical production may be related to the process of chemical convergence, in the evolutionary histories of the species PAL and PV, which are already very close morphologically and belong to the same taxonomic section (*Isophyllon*).

Some authors already described importance of chemical phenotypic plasticity as a tool to elucidate phylogenetic relationships, mainly from descriptions of the matrix of natural products of a given taxon, and use them for a phenetic characterization of clades, mainly in EOs.[22,23,62,63] There are great difficulties in having secondary metabolites as markers. In many studies, the mixture of compounds may show chemical agreement and divergence in relation to the taxonomic groups. It is known that these changes are due to the different pressures and adaptations to the abiotic and biotic factors acquired over the evolution of the species in their environment.[23] This could justify in general the maintenance of chemical production by some biosynthetic pathways for varieties in *P. arboreum* (PAH and PAA), as well as for *P. lepturum* (PLL and PLA). According to Machado and collaborators[64] *Piper* species are difficult to define because they are very similar in their external morphology, making their identification difficult.

The emergence of variation within taxon makes this challenge even more difficult. Pereira et al.[31] in their work tried to differentiate EOs from PLL and PLA collected in the city of Rio de Janeiro (Rio de Janeiro, Brazil). Authors observed that PLL showed higher percentage of α-Guaiene and PLA presented β-Bisabolene. This fact was not reported on Marambaia Island, in which conservation of precursors was reported. We suggest a deeper ecological chemical approach to differentiation, and we emphasize that it was possible by anatomical and histochemical analysis to differentiate these species.[64]

Light is shed for chemical plasticity presented in the *Ottonia* (PAN) section, and the shikimate derivatives in EOs may be an interesting chemical marker for the evaluation and identification of this species. We believe that more in-depth evaluations around the understanding of phenoplasticity with species in this section should be performed at different spatial and temporal scales to confirm this hypothesis.

The presence of *trans*-Caryophyllene as marked in *Piper* may be important in the process of chemical communication for the genus in Marambaia Island. However, it needs to be better evaluated. It is known that deciphering chemodiversity patterns is a current challenge in ecological chemical studies, as these exist and are important for the maintenance and survival of species in their natural environment. Kessler and Kalske[21] describe that the more common, or more apparent, a species is in its community, the less noise should impact its communication systems. In addition, the strength of natural selection in chemical communication by antagonists should increase the more dominant a species becomes. For better understanding, *trans*-Caryophyllene together with Limonene, Linalool and Arylpropanoid derivatives make up about 50-70% of the floral emissions of all studies, serving as an indicator to define this marker, highlights the importance of this study.[21,65] However, a future study of the seasonality in the area will be important to better analyze the changes in the chemical compositions of the *Piper* species from the Marambaia Island.

5. Conclusions

This is the first study on the chemical composition of the EOs of *Piper* species from Marambaia Island, Rio de Janeiro. Nineteen monoterpene compounds and 89 sesquiterpene compounds were identified, showing an important chemical diversity. It is also the first known study comparing the chemical composition of the EOs from the leaves of *P. arboreum* varieties. Also, this study registered unprecedented detection of the major compound in *P. gaudichaudianum*, *P. amalago*, *P. divaricatum* and *P. viscosanum*, in addition to reporting the existence of arylpropanoids for the first time in the EO of *P. mollicomum* from leaves. It was permissible to describe *trans*-Caryophyllene as a possible chemical marker to decode *Piper* chemodiversity on the Island. Important correlations between sections and the chemical composition of EOs were achieved for *Piper* species from Marambaia, bringing some light to chemotaxonomic features in Piperaceae.

Acknowledgements

The authors thank CAPES, FAPERJ, PROEP-CNPq for their financial support, and the Brazilian Navy for their support and accomodations.

References

1. Pereira, M. G.; Menezes, L. F. T. D.; Schultz, N.; Aporte e decomposição da serapilheira na Floresta Atlântica, Ilha da Marambaia, Mangaratiba, RJ. *Ciência Florestal* **2008**, *18*, 443. [CrossRef]
2. Goncalves, N. B.; Nettesheim, F. C.; Conde, M.; Tree community variation in a tropical continental island according to slope aspect and human interference. *Anais da Academia Brasileira de Ciências* **2018**, *90*, 17. [CrossRef] [PubMed]
3. Lourenço, E. C.; Costa, L. M.; Silva, R. M.; Esbérard, C. E. L.; Bat diversity of Ilha da Marambaia, Southern Rio de Janeiro State, Brazil (Chiroptera, Mammalia). *Brazilian Journal of Biology* **2010**, 70, 511. [CrossRef] [PubMed]
4. Yuncker, T. G.; The Piperaceae of Brazil. *Hoehnea* **1972**, *2*, 19.
5. Guimarães, E. F.; Mautone, L.; Magalhães, H. G.; Guimarães, L. A.; Estudos taxonômico e farmacoquímico e bioensaios de *Piper lhotzkyanum* Kunth (Piperaceae), uma espécie ocorrente em Minas Gerais. *Daphne* **1992**, *2*, 3. [Link]
6. Tebbs, M. C. Piperaceae.; In: *Flowering Plants· Dicotyledons*. Springer, Berlin, Heidelberg **1993**, 516. [CrossRef]
7. Jaramillo, M. A.; Manos, P. S.; Phylogeny and patterns of floral diversity in the genus *Piper* (Piperaceae). *American Journal of Botany* **2001**, *88*, 706. [CrossRef] [PubMed]
8. Chase, M. W.; Christenhusz, M. J. M.; Fay, M. F.; Byng, J. W.; Judd, W. S.; Soltis, D. E.; Stevens, P. F.; An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. *Botanical Journal of the Linnean Society* **2016**, *181*, 1. [CrossRef]
9. Queiroz, G. A.; Guimarães, E. F.; *Piper* L.(Piperaceae) do Leste Metropolitano, RJ, Brasil. *Brazilian Journal of Development* **2020**, *6*, 11. [CrossRef]
10. Flora do Brasil. *Piperaceae*. Disponível em: <http://reflora.jbrj.gov.br/reflora/floradobrasil/FB190>. Acesso em 05 fev. 2021.
11. Weng, J. K.; Philippe, R. N.; Noel, J. P.; The rise of chemodiversity in plants. *Science* **2012**, *336*, 1667. [CrossRef] [PubMed]
12. Müller, C.; Bräutigam, A.; Eilers, E.; Junker, R.; Schnitzler, J. P.; Steppuhn, A.; Wittmann, M.; Ecology and evolution of intraspecific chemodiversity of plants. *Research Ideas and Outcomes* **2020**, *6*, e49810. [CrossRef] [PubMed]
13. Fonseca-Kruel, V. S.; Peixoto, A. L.; Ethnobotany of Arraial do Cabo Marine Extractive Reserve, Rio de Janeiro, Brazil. *Acta Botanica Brasilica* **2004**, *18*, 177. [CrossRef]
14. Gilbert, B.; Favoreto, R.; *Piper umbellatum* L.= *Pothomorphe umbellata* (L.) Miq. *Revista Fitos*, **2010**, 5, 2. [Link]
15. Oliveira, G. L.; Moreira, D. D. L.; Mendes, A. D. R.; Guimarães, E. F.; Figueiredo, L. S.; Kaplan, M. A. C.; Martins, E. R.; Growth study and essential oil analysis of *Piper aduncum* from two sites of Cerrado biome of Minas Gerais State, Brazil. *Revista Brasileira de Farmacognosia* **2013**, *23*, 743. [CrossRef]

16. Silva, J. K.; Trindade, R.; Alves, N. S.; Figueiredo, P. L.; Maia, J. G. S.; Setzer, W. N.; Essential oils from neotropical *Piper* species and their biological activities. *International Journal of Molecular Sciences* **2017**, *18*, 2571. [CrossRef] [PubMed]
17. Salehi, B.; Zakaria, Z. A.; Gyawali, R.; Ibrahim, S. A.; Rajkovic, J.; Shinwari, Z. K.; Setzer, W. N.; *Piper* species: A comprehensive review on their phytochemistry, biological activities and applications. *Molecules* **2019**, *24*, 1364. [CrossRef] [PubMed]
18. Fonseca, A. C. C. D.; de Queiroz, L. N.; Sales Felisberto, J.; Ramos, Y. J.; Mesquita Marques, A.; Wermelinger, G. F.; Robbs, B. K.; Cytotoxic effect of pure compounds from *Piper rivinoides* Kunth against oral squamous cell carcinoma. *Natural Product Research* **2020**, *10*, 1. [CrossRef] [PubMed]
19. Silva Novaes, A.; Silva Mota, J.; Barison, A.; Veber, C. L.; Negrão, F. J.; Kassuya, C. A. L.; Barros, M. E.; Diuretic and antilithiasic activities of ethanolic extract from *Piper amalago* (Piperaceae). *Phytomedicine* **2014**, *21*, 523. [CrossRef] [PubMed]
20. Ramos, Y. J.; Brito Machado, D.; Queiroz, G. A.; Guimarães, E. F.; Defaveri, A. C. A.; Moreira, D. L.; Chemical composition of the essential oils of circadian rhythm and of different vegetative parts from *Piper mollicomum* Kunth-A medicinal plant from Brazil. *Biochemical Systematics and Ecology* **2020**, *92*, 104116. [CrossRef]
21. Kessler, A.; Kalske, A. Plant secondary metabolite diversity and species interactions.; *Annual Review of Ecology, Evolution, and Systematics* **2018**, *49*, 115. [CrossRef]
22. Zidorn, C.; Plant chemophenetics – A new term for plant chemosystematics/plant chemotaxonomy in the macro-molecular era. *Phytochemistry* **2019**, *163*, 147. [CrossRef]
23. Sadgrove, N. J.; Telford, I. R.; Padilla-González, G. F.; Greatrex, B. W.; Bruhl, J. J.; GC–MS 'chemophenetics' on Australian pink-flowered *Phebalium* (Rutaceae) using herbarium leaf material demonstrates phenetic agreement with putative new species. *Phytochemistry Letters* **2020**, *38*, 112. [CrossRef]
24. Wasicky, R.; Uma modificação do aparelho de Clevenger para extração de óleos essenciais. *Revista Faculdade de Farmácia e Bioquímica* **1963**, *1*, 77.
25. Dool, H. V. D.; Kratz, P. D.; A generalization of retention index system including linear temperature programmed Gas-Liquid Partition Chromatogaphy. *Journal of Chromatography* **1963**, *11*, 463. [CrossRef] [PubMed]
26. Adams, R. P.; Identification of essential oil components by gas chromatography/quadrupole mass spectroscopy. 4a. ed., *Carol Stream*: Illinois, 2007.
27. Callejas, R.; *Taxonomic revision of Piper subgenus Ottonia* (Piperaceae). Tese de Doutorado. City University of New York, 1986.
28. Navickiene, H. M. D.; Morandim, A. D. A.; Alécio, A. C.; Regasini, L. O.; Bergamo, D. C. B.; Telascrea, M.; Kato, M. J.; Composition and antifungal activity of essential oils from *Piper aduncum, Piper arboreum* and *Piper tuberculatum*. *Química Nova* **2006**, *29*, 467. [CrossRef]
29. de BF Ferraz, A.; Balbino, J. M.; Zini, C. A.; Ribeiro, V. L. S.; Bordignon, S. A.; von Poser, G.; Acaricidal activity and chemical composition of the essential oil from three *Piper* species. *Parasitology Research* **2010**, *107*, 243. [CrossRef] [PubMed]
30. Simeone, M. L. F.; Mikich, S. B.; Côcco, L. C.; Hansel, F. A.; Bianconi, G. V.; Chemical composition of essential oils from ripe and unripe fruits of *Piper amalago* L. var. medium (Jacq.) Yunck and *Piper hispidum* Sw. *Journal of Essential Oil Research* **2011**, *23*, 54. [CrossRef]
31. Pereira, F. G.; Santos, P. R. D.; Guimarães, E. F.; Kaplan, M. A. C.; Composition of the essential oils from leaves of *Piper lepturum* Kunth (C. DC.) var. *lepturum* and *Piper lepturum* var. *angustifolium* (Miq.) Yunck. from Brazil. *Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas* **2016**, *15*, 88. [Link]
32. Schindler, B.; Silva, D. T.; Heinzmann, B. M.; Efeito da sazonalidade sobre o rendimento do óleo essencial de *Piper gaudichaudianum* Kunth. *Ciência Florestal* **2018**, *28*, 263. [CrossRef]
33. Nalepa, T.; Carvalho, R. I. N.; Produção de biomassa e rendimento de óleo essencial em camomila cultivada com diferentes doses de cama-de-aviário. *Scientia Agraria* **2007**, *8*, 161. [Link]
34. Gobbo-Neto, L.; Lopes, N. P.; Plantas medicinais: fatores de influência no conteúdo de metabólitos secundários. *Química Nova* **2007**, *30*, 374. [CrossRef]
35. Schwob, I.; Bessiere, J. M. Masotti V. V.; Viano J. J.; Changes in essential oil composition in saint john, s wort (*Hypericum perforatum* L.) aerial parts during its phonological cycle. *Biochemical Systematics and Ecology* **2004**, *32*, 735. [CrossRef]
36. Pritarević, I.; Kuftinec, J.; Blažević, N.; Kuštrak, D.; Seasonal variation of essential oil yield and composition of dalmatian sage, *Salvia officinalis*. *Journal of Natural Products* **1984**, *47,* 409. [CrossRef] [PubMed]
37. Cosentino, S.; Tuberoso, C. I. G.; Pisano, B.; Satta, M.; Mascia, V.; Arzedi, E; Palmas, F.; *In-vitro* antimicrobial activity and chemical composition of Sardinian *Thymus* essential oils. *Letters in Applied Microbiology* **1999**, *29*, 130. [CrossRef] [PubMed]
38. Sibanda, S.; Chigwada, G.; Poole, M.; Gwebu, E. T.; Noletto, J. A.; Schimidt, J. M.; Rea, A. I; Satzer, W. N.; Composition and bioactivity of the leaf essential oil of *Heteropyxis dehniae* from Zimbabwe. *Journal of Ethnopharmacology* **2004**, *92*, 107. [CrossRef] [PubMed]
39. Batista, D.; Campos, P.; Silva, V. R.; Santos, L. D. S.; Bezerra, D. P.; Soares, M. B.; Ribeiro, P. R.; *Piper anisum* as a promising new source of bioactive metabolites. *Chemical Papers* **2020**, *74*, 1505. [CrossRef]
40. Letizia, C. S.; Cocchiara, J.; Lalko, J.; Api, A. M.; Fragrance material review on linalool. *Food and Chemical Toxicology* **2003**, *41*, 943. [CrossRef] [PubMed]
41. Villamizar, L. H.; Cardoso, M. D. G.; Andrade, J. D.; Teixeira, M. L.; Soares, M. J.; Linalool, a *Piper aduncum* essential oil component, has selective activity against *Trypanosoma cruzi* trypomastigote forms at 4 °C. *Memórias do Instituto Oswaldo Cruz* **2017**, *112*, 131. [CrossRef] [PubMed]
42. Perigo, C. V.; Torres, R. B.; Bernacci, L. C.; Guimarães, E. F.; Haber, L. L.; Facanali, R.; Vieira, M. A. R.; Quecini, V.; Marques, M. O. M.; The chemical composition and antibacterial activity of eleven *Piper* species from distinct rainforest areas in Southeastern Brazil. *Industrial Crops and Products* **2016**, *94*, 528. [CrossRef]

43. Morandim-Giannetti, A. A.; Pin, A. R.; Pietro, N. A.S.; Oliveira, H. C.; Mendes-Giannini, M. J. S.; Alecio, A. C.; Kato, M. J.; Oliveira, J. E.; Furlan, M.; Composition and antifungal activity against *Candida albicans*, *Candida parapsilosis*, *Candida krusei* and *Cryptococcus neoformans* of essential oils from leaves of *Piper* and *Peperomia* species. *Journal of Medicinal Plant Research* **2010**, *4*, 1810. [Link]
44. Bernuci, K. Z.; Iwanaga, C. C.; Fernadez-Andrade, C. M.; Lorenzetti, F. B.; Torres-Santos, E. C.; Faiões, V. D.; Gonçalves, J. E.; Amaral, W.; Deschamps, C.; Scodro, R. B.; Cardoso, R. F.; Evaluation of chemical composition and antileishmanial and antituberculosis activities of essential oils of *Piper* species. *Molecules* **2016**, *21*, 1698. [CrossRef] [PubMed]
45. Andrade, E. H.; Zoghbi, M. D.; Santos, A. S.; Maia, J. G.; Essential oils of *Piper gaudichaudianum* Kunth and *P. regnellii* (Miq.) C.DC. *Journal of Essential Oil Research* **1998**, *10*, 465. [CrossRef]
46. Morais, S. M.; Facundo, V. A.; Bertini, L. M.; Cavalcanti, E. S.; dos Anjos Júnior, J. F.; Ferreira, S. A.; de Brito, E. S.; de Souza Neto, M. A.; Chemical composition and larvicidal activity of essential oils from *Piper* species. *Biochemical Systematics and Ecology* **2007**, *35*, 670. [CrossRef]
47. Péres, V. F.; Moura, D. J.; Sperotto, A. R. M.; Damasceno, F. C.; Caramão, E. B.; Zini, C. A.; Saffi, J.; Chemical composition and cytotoxic, mutagenic and genotoxic activities of the essential oil from *Piper gaudichaudianum* Kunth leaves. *Food and Chemical Toxicology* **2009**, *47*, 2389. [CrossRef] [PubMed]
48. Sperotto, A. R. M.; Moura, D. J.; Péres, V. F.; Damasceno, F. C.; Caramão, E. B.; Henriques, J. A. P.; Saffi, J.; Cytotoxic mechanism of *Piper gaudichaudianum* Kunth essential oil and its major compound nerolidol. *Food and Chemical Toxicology* **2013**, *57*, 57. [CrossRef] [PubMed]
49. Potzernheim, M.; Bizzo, H. R.; Agostini-Costa, T. S.; Vieira, R. F.; Carvalho-Cilva, M.; Gracindo, L. A. M. B.; Chemical characterization of seven *Piper* species (Piperaceae) from Federal District, Brazil, based on volatile oil constituents. *Revista Brasileira de Plantas Medicinais* **2006**, *8*, 10. [Link]
50. Setzer, W. N.; Park, G.; Agius, B. R.; Stokes, S. L.; Walker, T. M.; Haber, W. A.; Chemical compositions and biological activities of leaf essential oils of twelve species of *Piper* from Monteverde, Costa Rica. *Natural Product Communications* **2008**, *3*, 1367. [CrossRef]
51. de Ferraz, A. B. F.; Balbino, J. M.; Zini, C. A.; Ribeiro, V. L.; Bordignon, S. A.; Von-Poser, G.; Acaricidal activity and chemical composition of the essential oil from three *Piper* species. *Parasitology Research* **2010**, *107*, 243. [CrossRef] [PubMed]
52. Mota, J. S.; Souza, D. S.; Boone, C. V.; Lima Cardoso, C. A.; Bastos Caramão, E.; Identification of the volatile compounds of leaf, flower, root and stem oils of *Piper amalago* (Piperaceae). *Journal of Essential Oil Bearing Plants* **2013**, *16*, 11. [CrossRef]
53. Almeida, J. G. L.; Silveira, E. R.; Pessoa, O. D. L.; Nunes, E. P.; Essential oil composition from leaves and fruits of *Piper divaricatum* G. Mey. *Journal of Essential Oil Research* **2009**, *21*, 228. [CrossRef]
54. Brait, D. R.; Vaz, M. S.; Arrigo, J. S.; Carvalho, L. N.; Araújo, F. H.; Vani, J. M.; da Mota, J. S.; Cardoso, C. A.; Oliveira, R. J.; Negrão, F. J.; Toxicological analysis and anti-inflammatory effects of essential oil from *Piper vicosanum* leaves. *Regulatory Toxicology and Pharmacology* **2015**, *73*, 699. [CrossRef]
55. Mesquita, J. M. O.; Oliveira, A. B.; Braga, F. C.; Lombardi, J. A.; da Cunha, A. P.; Salgueiro, L.; Cavaleiro, C.; Essential oil constituents of *Piper vicosanum* Yunker from the Brazilian Atlantic forest. *Journal of Essential Oil Research* **2006**, *18*, 392. [CrossRef]
56. Vieira, S. C.; Paulo, L. F.; Svidzinski, T. I.; Dias Filho, B. P.; Nakamura, C. V.; Souza, A. D.; Young, M. C.; Cortez, D. A.; Antifungal activity of *Piper diospyrifolium* Kunth (Piperaceae) essential oil. *Brazilian Journal of Microbiology* **2011**, *42*, 1001. [CrossRef] [PubMed]
57. Morandim, A. D.; Pin, A. R.; Pietro, N. A.; Alecio, A. C.; Kato, M. J.; Young, C. M.; de Oliveira, J. E.; Furlan, M.; Composition and screening of antifungal activity against *Cladosporium sphaerospermum* and *Cladosporium cladosporioides* of essential oils of leaves and fruits of *Piper* species. *African Journal of Biotechnology* **2010**, *9*, 6135. [Link]
58. Duarte, M. C. T.; Leme, E. E.; Delarmelina, C.; Soares, A. A.; Figueira, G. M.; Sartoratto, A.; Activity of essential oils from Brazilian medicinal plants on *Escherichia coli*. *Journal of Ethnopharmacology* **2007**, *111*, 197. [CrossRef] [PubMed]
59. Souza, S. P.; Valverde, S. S.; Costa, N. F.; Calheiros, A. S.; Lima, K. S. C.; Frutuoso, V. S.; Lima, A. L. S.; Chemical composition and antinociceptive activity of the essential oil of *Piper mollicomum* and *Piper rivinoides*. *Journal of Essential Oil Research* **2014**, *8*, 788. [Link]
60. Alvarez Costa, A.; Naspi, C. V.; Lucia, A.; Masuh, H. M.; Repellent and larvicidal activity of the essential oil from *Eucalyptus nitens* against *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae). *Journal of Medical Entomology* **2017**, *54*, 670. [CrossRef] [PubMed]
61. Christoph, F.; Kubeczka, K. H.; Stahl-Biskup, E.; The composition of commercial manuka oils from New Zealand. *Journal of Essential Oil Research* **1999**, *11*, 705. [CrossRef]
62. Evergetis, E.; Haroutounian, S. A.; Volatile systemtics: A novel biochemical interpretation of essential oil compounds enhances their chemophenetic significance. *Biochemical Systematics and Ecology* **2020**, *92*, 104087. [CrossRef]
63. Nikolić, J. S.; Zlatković, B. K.; Jovanović, S. Č.; Stojanović, G. S.; Marin, P. D.; Mitić, Z. S.; Needle volatiles as chemophenetic markers in differentiation of natural populations of *Abies alba*, *A. x borisii-regis* and *A. cephalonica*. *Phytochemistry* **2021**, *183*, 112612. [CrossRef] [PubMed]
64. Machado, N. S. D. O.; Pereira, F. G.; Santos, P. R. D. D.; Costa, C. G.; Guimarães, E. F.; Comparative anatomy of the leaves of *Piper lepturum* (Kunth) C. DC. var. *lepturum* and *Piper lepturum* var. *angustifolium* (C. DC.) Yunck. *Hoehnea*. **2015**, *42*, 1. [CrossRef]
65. Knudsen, J. T.; Eriksson, R.; Gershenzon, J.; Ståhl, B.; Diversity and distribution of floral scent. *The Botanical Review* **2006**, *72*, 1. [CrossRef]
66. Shinoda, N.; Shiga, M.; Nishimura, K.; Constituents of yuzu (*Citrus junos*) oil. *Agricultural and Biological Chemistry* **1970**, *34*, 2. [CrossRef]
67. Fernández-Álvarez, M.; Lores, M.; Llompart, M.; García-Jares, C.; Cela, R.; The photochemical behaviour of five household pyrethroid insecticides and a synergist as studied by photo-solid-phase microextraction. *Analytical and Bioanalytical Chemistry* **2007**, *388*, 5 [CrossRef] [PubMed]

68. Moreira, D. L.; Kaplan, M. A. C.; Guimarães, E. F.; 1-Butyl-3, 4-methylenedioxybenzene as the major constituent of essential oil from *Ottonia anisum* Sprengel (Piperaceae). *Journal of Essential Oil Research* **1997**, *9*, 5. [CrossRef]