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. amplus* 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. lepturn* (Kunth) C.DC. var. * lepturum*; *P. lepturum* var. *angustifolium* (C.DC.) Yunck.; *P. mollicomum* Kunth and *P. vicosamum* 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*, *iso-Eudesmol*, *trans*-Nerolidol, Germacrene D, β-Elemene, 7-epi-α-Selinene, 1,8-Cineole, β-Elemene, 7-β-Eudesmol, 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. In this region there is large ranging 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 present 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. 5-12

*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. (Capea) 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
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. 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. They were collected in different areas of Marambaia Island, Mangaratiba, 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. 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-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 (IR) 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.

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.

Retention indices (RI) were calculated using data obtained from the GC-FID of a homologous series of saturated aliphatic hydrocarbons (C₆-C₃₆, Sigma-Aldrich, Brazil) carried out in the same column and conditions used in GC-FID analysis for EOs. 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. 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 lepeterum var. angustifolium (C.DC.) Yunck. | 23°03'36.4"S 43°58'40.8"W | RB01319712 |
| PLL  | Piper lepeterum (Kunth) C.DC. var. lepeterum | 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. The results were processed using STATISTICA software version 10 (StatSoft 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* | R1calc | R1lit | 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|     |     |    |    |
| Camphene   | 956    | 954   | 0.14|     |     |     |     |     |     |    |     |     |    |    |
| β-Pinene   | 982    | 979   | 0.38|     | 0.17| 0.29| 0.34|     |     |    |     |     |    |    |
| Myrcene    | 990    | 990   | 0.15|     | 0.53|     | 0.31| 3.04| 3.27| 0.94|     |     |    |    |
| α-Phellandrene | 1006 | 1002 |     |     |     |     |     |     |     |    |     |     |    |    |
| 3-Carene   | 1013   | 1011  | 4.57| 0.19|     | 0.52|     | 0.53|     |    |     |     |    |    |
| α-Terpinene| 1013   | 1017  |     |     |     |     |     |     |     |    |     |     |    |    |
| α-Cymene   | 1028   | 1026  | 0.16|     | 0.35|     | 0.64|     |     |    |     |     |    |    |
| Limonene   | 1033   | 1029  | 0.30| 0.18| 2.62| 1.47| 1.50|     |     |    |     |     |    |    |
| β-cis-Ocimene| 1035 | 1037  | 5.54| 6.85| 2.80| 1.07| 0.46| 1.12| 0.82|     |     |     |    |    |
| β-Phellandrene| 1036 | 1029  |     |     |     |     |     |     |     |    |     |     |    |    |
| 1,8-Cineole| 1037   | 1031  | 1.75| 1.58|     |     |     |     |     |    |     |     |    |    |
| β-trans-Ocimene| 1049 | 1050  | 2.25| 2.76| 0.86|     | 0.68|     | 0.41|     |     |     |    |    |
| γ-Terpinene| 1062   | 1059  | 0.14|     |     |     |     |     |     |    |     |     |    |    |
| 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-1,2,4-Dimethyl-1,3,7-nonatriene| 1115 | 1113  | 1.75| 1.58|     |     |     |     |     |    |     |     |    |    |
| neo-allo-Ocimene| 1131 | 1132  | 0.73| 0.96| 0.30|     |     |     |     |    |     |     |    |    |
| α-Terpinol| 1197   | 1188  | 1.78|     |     |     |     |     |     |    |     |     |    |    |
| 3,5-Dimethoxytoluen*| 1269 | 1264  | 0.32|     |     |     |     |     |     |    |     |     |    |    |
| 2-Undecanone| 1291  | 1294  |     |     |     |     |     |     |     |    |     |     |    |    |
| δ-Elemene  | 1335   | 1338  | 0.72| 1.15| 0.14| 1.32| 0.53|     |     |    |     |     |    |    |
| Bicycloelemene| 1339 | 1339  | 5.28| 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| 1371 | 1371  |     |     |     |     |     |     |     |    |     |     |    |    |
| 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|     |     |    |    |
| β-Bourbonene| 1386 | 1388  | 0.77| 0.18|     |     |     |     |     |    |     |     |    |    |
| 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| 1403 | 1403  | 0.38| 1.37| 1.40|     |     |     |     |    |     |     |    |    |
| 1-Butyl-3,4-methylenedioxybenzene| 1408 | 1408  |     |     |     |     |     |     |     |    |     |     |    |    |
| 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|
| **Continua...** | | | | | | | | | | | | | | |
Chemodiversity of Essential Oils in *Piper* L. (Piperaceae) Species from Marambaia Island, Rio de Janeiro-RJ, Brazil

| Compound                        | Retention Time (min) | Relative Retention Time | Area (μg) | Area (mg) | Area (% of Total) |
|---------------------------------|----------------------|--------------------------|-----------|-----------|------------------|
| γ-Elemene                       | 1432                 | 1.05                     | 0.68      | 1.70      | 1.83             | 6.99          | 1.42          |
| β-Gurjunene                     | 1433                 | 1.01                     | 0.37      |           |                  |               |
| α-trans-Bergamotene             | 1435                 | 0.18                     | 1.38      | 5.52      | 1.70             | 0.76         | 6.18          |
| Aromandendrene                  | 1441                 | 0.52                     | 0.18      | 0.16      | 1.00             | 0.90         | 0.95          |
| trans-Murola-3,5-diene          | 1451                 | 0.25                     | 0.18      | 0.23      | 0.98             |               |
| β-trans-Farnesene               | 1454                 | 0.77                     | 0.71      | 3.60      | 7.73             |               |
| α-Humulene                      | 1458                 | 1.86                     | 2.06      | 0.79      | 1.08             | 1.19         | 0.85          | 2.50         | 1.54         | 2.68          |
| Cabreuva oxide B                | 1462                 | 1.46                     |           | 0.82      |                  |               |
| alloAromadendrene               | 1463                 | 1.46                     |           | 0.50      | 0.66             | 0.24         | 0.70          |
| trans-Cadina-1(6),4-diene       | 1474                 | 1.476                    | 0.49      | 0.43      | 0.54             |               |
| γ-Muurolene                     | 1477                 | 0.82                     | 1.25      | 0.20      | 0.16             | 1.39         | 3.12          |
| cis-4,10-epoxy-Amorphone         | 1480                 | 1.479                    | 1.37      | 2.47      | 2.69             | 0.66         | 0.32          |
| α-Curcumene                     | 1483                 | 1.480                    |           |           |                  |               |
| Germacrene D                    | 1483                 | 1.481                    | 1.99      | 4.92      | 2.27             | 9.73         | 24.70         | 1.57         | 1.12         | 4.28          | 3.76          |
| α-Amorphene                     | 1486                 | 1.481                    |           |           |                  |               |
| β-Selinene                      | 1490                 | 0.64                     | 0.76      | 5.12      | 1.03             | 0.35         | 1.20          | 0.43         | 8.90         |
| trans-Murola-4(14),5-diene      | 1493                 | 0.98                     | 0.73      | 0.37      | 0.42             | 2.47         | 2.69         | 0.66         | 0.32          |
| β-cis-Guaiene                   | 1493                 | 1.493                    |           |           |                  |               |
| 2-Tridecanone                   | 1495                 | 1.496                    |           |           |                  |               |
| α-Zingiberene                   | 1496                 | 1.493                    |           |           |                  | 7.34         | 11.10         |
| Valencene                       | 1496                 | 1.496                    |           |           |                  |               |
| Viridiflorene                   | 1497                 | 1.496                    |           |           |                  |               |
| Bicyclogermacrene               | 1497                 | 1.500                    | 18.50     | 5.32      | 6.60             | 4.21         | 1.75         | 8.20         | 9.46         | 17.66         | 2.66          |
| α-Muurolene                     | 1499                 | 1.500                    | 1.75      | 0.47      | 1.36             | 1.81         | 0.51          |
| α-Bulnesene                     | 1503                 | 1.509                    | 0.68      | 0.88      |                  |               |
| cis-cis-α-Farnesene             | 1504                 | 1.505                    |           |           | 0.32             | 0.65         | 0.41          | 0.36          |
| β-trans-Guaiene                 | 1505                 | 1.502                    |           |           | 1.78             | 3.10         | 1.67         | 4.69         | 6.81          |
| β-Bisabolene                    | 1508                 | 1.505                    | 1.85      | 1.14      | 1.71             | 0.38         | 0.95          |
| γ-Cadinene                      | 1515                 | 1.513                    | 0.44      | 1.06      | 0.37             | 0.74         | 1.77         | 0.40          |
| Cubebol                         | 1517                 | 1.515                    | 1.85      | 1.14      | 1.71             | 0.38         | 0.95          |
| δ-Cadinene                      | 1520                 | 1.523                    | 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                 | 1.522                    |           |           |                  |               |
| β-Sesquiphellandrene            | 1526                 | 1.522                    |           |           |                  |               |
| trans-γ-Bisabolene              | 1527                 | 1.531                    | 1.29      | 0.45      |                  |               |
| trans-Cadina-1,4-diene          | 1535                 | 1.534                    | 0.39      | 0.20      | 0.51             | 0.88         |
| α-Cadinene                      | 1539                 | 1.538                    |           |           |                  |               |
| cis-α-Bisabolene                | 1543                 | 1.536                    |           |           |                  |               |
| α-Calacorene                    | 1544                 | 1.545                    |           |           |                  |               |
| Elemol                          | 1552                 | 1.549                    | 0.17      | 8.41      |                  |               |
| Germacrene B                    | 1561                 | 1.561                    | 0.37      |           |                  |               |
| trans-Nerolidol                 | 1562                 | 1.563                    | 5.78      | 6.83      | 1.41             | 10.00        | 3.09         | 20.54        |
| Palustrol                       | 1573                 | 1.568                    |           |           |                  |               |
| Caryolan-8-ol                   | 1578                 | 1.572                    |           |           | 0.39             |               |
| Spathulenol                     | 1578                 | 1.579                    | 1.31      | 1.55      | 3.15             | 0.24         | 0.57         | 1.64         | 1.14         | 0.42          | 2.41          |
| Caryophyllene oxide             | 1583                 | 1.583                    | 0.24      | 0.90      | 2.37             | 1.34         | 0.29         | 0.51         | 1.37         | 0.72         | 1.65          |
| Thujopsan-2-α-ol                | 1588                 | 1.587                    | 0.79      | 0.49      | 0.29             | 0.99         | 1.99         | 0.36         | 0.53          |
| Globulol                        | 1595                 | 1.590                    | 0.17      |           | 0.62             | 1.90         | 1.20         |
| Viridifilorol                    | 1596                 | 1.592                    |           |           | 0.10             |               |
| Guaiol                          | 1600                 | 1.600                    | 13.22     | 0.46      |                  |               |

Continuation...
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. arboresum* 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.

|   |   |   |   |   |
|---|---|---|---|---|
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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 isoleptospermine (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%).

*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-Butyl-3,4-methylenedioxybenzene, α-Terpineol 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*
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*-Leptospermine (+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*-Leptospermine (+3.66), α-Eudesmol (+3.78), *trans*-γ-Bisabolene (-1.61), and 1-Butyl-3,4-methylenedioxybenzene (-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-Metilertitol 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 comparisions 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-Leptospernone 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.) Rauschert, Asteraceae). However, compared to the yields found for species in the literature it was lower. 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. 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 edaphic factors, such as seasonal and circadian rhythms modulations due to water availability, nutrition, and air pollution.

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.

The Marambaia Island, mainly in the restinga area, has a sunny tropical climate, with air temperatures in the region typical of tropical coastal areas. 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. *trans*-Caryophyllene is an important antioxidant component of many EOs used as seasoning. 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. The arylobutanoid derivative, 1-Butyl-3,4-methylenedioxybenzene, the main compound of *P. anisum* EO, has larvicidal activity and Linalool which is widely used in the food and cosmetics industry, known for its antimicrobial and parasitic properties.

For the first time it was identified as the majority 7-epi-α-Selinene in PG, Linalool and Guaiol in PAL, Germacrene D in PDV, and α-Eudesmol in PV. However, the findings for PAN, PDP, PLA, PLL and PM were similar to that described in the literature. We emphasize that PM has not presented until this report any content of arylobutanoids in the leaves, only in inflorescences and infructescences such as described before by our group. We highlight that, for the first time, the majority presence of iso-Leptospernone in the EOs of the genus *Piper* (PAL and PV). This compound is naturally occurring in EOs in the species of basal angiosperms. 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. 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. 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 *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., 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.

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 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. 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 monoterpenic compounds and 89 sesquiterpenic 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. arboresum varieties. Also, this study registered unprecedented detection of the major compound in P. gaudeichaudium, P. amalago, P. divaricatum and P. viscosum, 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.

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