Yield and Chemical Composition of Essential oil of Piperaceae in one Segment of the Semi deciduous Forest of Paraná State, Brazil, in Seasonal Samplings

Erci Marcos Del Quiqui¹, Cícero Deschamps², Wanderlei do Amaral², Roger Raupp Sipriano² and Marilia Pereira Machado²

¹Department of Science Agronomy, State University of Maringá, Umuarama, Brazil.
²Department of Plant Science, Federal University of Paraná, Curitiba, Brazil.

Abstract—Essential oils are composed of a complex mixture of various classes of substances; among them are phenylpropanoid, monoterpenes, and sesquiterpenes, belonging to the secondary metabolism of plants. However, these compounds can be influenced by seasonal factors, among others. The objective of this study is to realize the prospect of obtaining Piperaceae with aromatic potential from a segment of the semideciduous forest of the Atlantic Forest Biome in the northwestern region of Paraná State. The aim is to assess the qualitative and quantitative characteristics of its essential oil in the collection during the winter and summer seasons of year 2016. The statistical design was internally randomized in a factorial of 4 (species) x 3 (replicates). The species studied were Piper mosenii C. DC., Piper xylopioides Kunth, Piper diospyrifolium (Kunth) Kunth ex C. DC., and Piper gaudichaudianum Kunth. A total of 78 compounds were identified, 68 in winter and 71 in summer. The species presented a variation in the yield and composition of essential oils, both in winter and summer. The predominant chemical composition was sesquiterpenes followed by monoterpenes, with prominence of (E)-caryophyllene, germacrene D, bicyclogermacrene, α-pinene and β-pinene.

Keywords—Bioprospecting, genus Piper, protected areas, secondary metabolism, species aromatics.

I. INTRODUCTION

Brazil has a wide territorial area with a rich biodiversity, which provides a valuable source of plant species. Many of these are little studied and constitute a large biological collection for scientific research. The prospect of finding aromatic species in the forests of the Atlantic Forest biome, with its significant biodiversity, may represent the discovery of new essential oils with a potential for beneficial use, increasing the viability of sustainable management of this ecosystem, which is highly damaged, with a great need for conservation.

The plants are inexhaustible sources of natural products, many of them essential oils and secondary metabolites, mainly used in perfumery and cleaning products. They are also a source of active ingredients for the pharmaceutical industry (5; 33; 11). They can also be used in crop protection against pests and diseases, with the advantage of not accumulating in the environment and have a wide spectrum of action, which reduces the risk of developing resistant strains pathogenically (10).

More than 2000 plant species produce essential oils, among them are several representatives of the Piperaceae family, which possess high agronomic and commercial significance, as they are used as condiments, adornments, food, and in popular medicine. Some species of the genus Piper are also used in folk medicines, especially in Brazil, and many have proven to be of great significance due to the pharmacological activity and/or production of essential oils in their structure (13).

Piperaceae encompasses approximately 2,000 species allocated in approximately five genera. Just over 500 species are recorded in Brazil, distributed in four genera (30). The Piper genus is represented by 290 species and the Atlantic Forest is one of the centers of diversification and endemism of the genus, with about 150 species (14).

The production of essential oils in Brazil is still incipient to meet the demand. However, the national and international market has demonstrated a great interest in new essences; something that this biodiversity has a great potential to meet. In this context, the conservation units are an excellent laboratory for research and prospecting of essential oils. Various floristic studies were carried out at
the Caiuá Ecological Station, North Diamond, Paraná State, however, phytochemical and biological studies with the aromatic native species in this forest formation are scarce, which makes this research highly relevant.

The objective of this study is to evaluate, quantitatively and qualitatively, the essential oil in the seasonal samplings of Piperaceae, which is commonly found in a segment of the semideciduous forest of the Atlantic Forest Biome located in the Northwest of Paraná State.

II. MATERIAL AND METHODS

The study was conducted in the Caiuá Ecological Station, a Conservation Unit in the state, with an area of 1,427.30 ha, located in the northwestern region of Paraná State, Municipality of North Diamond, with approximate coordinates between 52º49' to 52º 53' W and 22° 34' to 22° 37' S and an altitude that varies from 240 m to 380 m. It belongs to the hydrographic basin of the Lower Paranapanema River, with part of the area occupying the banks of the reservoir of the Rosana Hydroelectric Power Station (UHE Rosana), remnant of the Paranapanema River.

According to the Koeppen climate classification, the northwestern region of Paraná State presents the Cfa type climate - mesothermal, humid, without a dry season, and with hot summers. The average temperature of the coldest month is below 18°C and the average temperature of the hottest month is above 22°C. The average annual rainfall is 1,200 to 1,400 mm, being the hottest month (February) 24 to 25°C and the coldest month (July) 17°C to 18°C. The relative humidity of the air average is 75%.

The formation of the majority of the soil in the Caiuá Ecological Station, is represented by soils derived from river sediments in portions adjacent to the Paranapanema River, with a predominance of Red Latosols, Red Argisols, Red-Yellow Argisols, and Quartzarenic Neosols, respectively. Its vegetation cover is inserted in the Atlantic Forest biome in the region of the Semideciduous Forest vegetation type, whose ecological concept is conditioned to the dual climate seasonality.

The studied species were Piper mosenii C. DC., Piper xylopioides Kunth, Piper diospyrifolium (Kunth) Kunth ex C. DC., and Piper gaudichaudianum Kunth species. These occurred commonly in the conservation unit.

The field work consisted of the collection of approximately 1 kg of plant material of each species (leaves and branch terminals) for extraction and quantification, and for determining the moisture content of the samples. The collection and transport of the plant material was prompted by the Environmental Institute of Paraná, under proper environmental authorization, with number 03/2016. The herbarium specimens were transported to the Botanical Museum Hall of Curitiba city where they were herborized and classified (Table 1).

### Table 1 - Description of species collected during the winter and summer of 2016 in the Caiuá Ecological Station, North Diamond /PR.

| Species                      | Herbarium number | Latitude     | Longitude    | Altitude |
|------------------------------|------------------|--------------|--------------|----------|
| *Piper mosenii* C. DC        | MBM 396409       | 22°36'8.5"S  | 52°53'4.7W   | 288m     |
| *Piper gaudichaudianum* Kunth | MBM 396403       | 22°36'29.3"S| 52°51'58.4"W| 277m     |
| *Piper xylopioides* Kunth    | MBM 396405       | 22°36'34.4"S| 52°51'48.7"W| 273m     |
| *Piper diospyrifolium* (Kunth) | MBM 396413      | 22°36'21.7S  | 52°52'4.8W   | 268m     |

The identification of species was performed with the aid of specialized bibliographies, comparison of herbarium specimens declared at the herbarium, and consultation with experts on the respective groups of plants of these species.

The oil extraction was done by means of hydrodistillation during four-and-a-half hours in a graduated Clevenger apparatus using 100 g of fresh leaves and one liter of distilled water, with three repetitions.

The essential oils were diluted in hexane at a ratio of 1% and 1.0 μL of the solution where it was injected, with a split flow of 1:20, in an Agilent 6890 gas chromatograph coupled with a mass selective detector Agilent 5973N. The injector was maintained at 250°C. The separation of the constituents was obtained in a capillary column HP-5MS (5%-phenyl-95%-dimethyl polysiloxane, 30 m x 0.25 mm x 0.25 μm), using helium as a carrier gas (1.0 ml min⁻¹). The temperature of the oven was scheduled to be 60°C to 240°C at a rate of 3°C
The detector of mass was operated on electronic ionization mode (70 eV), at a rate of 3.15 s⁻¹ sweeps and mass range of 40 µ to 450 µ. The transfer line was maintained at 260°C, the source of ions at 230°C, and the analyser at 150°C.

For the quantification, the diluted samples were injected into the chromatograph Agilent 7890A equipped with flame ionization detector (FID), operated at 280°C. The same column and analytical conditions described above were employed, except for the carrier gas used, which was hydrogen, at a flow rate of 1.5 mL min⁻¹. The percentage composition was obtained by electronic integration of the signal of the FID by dividing the area of each component by the total area (area %).

The identification of the constituents was obtained by comparison of their mass spectra with those of (32) and (18) and also by their linear retention indices calculated from the injection of a homologous series of hydrocarbons (C7-C26) and compared with data from the literature (1).

The results were submitted to analysis of variance and the means of treatments were compared by the Tukey test at 5% probability, using the software SISVAR (9) and the principal component analysis (PCA) using the program BioEstat v.5.

### III. RESULTS AND DISCUSSION

There was a significant difference in the essential oil content among species and at different seasons of collection (Table 2). The species *P. xylopioides* presented an average yield of oil statistically superior to others, and in the summer this content was higher. *P. mosenii*, *P. gaudichaudianum*, and *P. diospyrifolium* showed similar levels of oil in winter; however, in the summer these species differed in the levels among them, whereas, *P. xylopioides* presented the highest content followed by *P. diospyrifolium* and *P. gaudichaudianum*. *P. mosenii* did not produce oil in the summer contrary to the study of (24) conducted on the coast of Paraná State, where the species showed a low variation in the essential oil content in the winter, spring, and summer seasons.

#### Table 2 – Averages of the content of essential oils of fresh samples of species collected during the winter and summer of 2016, in the Caiuá Ecological Station, North Diamond, Paraná State, Brazil

| Species                                      | Oil content (%)* |  |  |
|----------------------------------------------|------------------|--|--|
| *Piper mosenii* C. DC                        | 0.28 b A         | --|--|
| *Piper gaudichaudianum* Kunth.               | 0.13 b A         | 0.05 c B | |
| *Piper xylopioides* Kunth                    | 0.94 a B         | 1.37 a A | |
| *Piper diospyrifolium* (Kunth) Kunth ex C. DC.| 0.13 b B        | 0.38 b A | |
| CV (%)                                       | 23.54            |   | |

* Medium followed by the same letter in column and capitalized on the line did not differ statistically among themselves by Tukey test at 5% probability.

The species *P. mosenii* and *P. gaudichaudianum* presented an essential oil yield that was higher in winter. As stated, *P. xylopioides* and *P. diospyrifolium* presented higher yield in summer.

The study on *P. gaudichaudianum* performed by (23) in a population in Santa Maria, Rio Grande do Sul State, found an average content of oil from fresh leaves of 0.38%, superior to that found in the present study, but without the effect of seasonality.

The chemical composition of essential oils is generally a characteristic of a given species and from the point of view of the chemical composition it is genetically and epigenetically controlled. The quantity, quality, and concentration of these species are influenced by the environmental components. Among the environmental factors that can be highlighted are, light intensity and photoperiod, the latitude, temperature (minimum and maximum average), soil (chemical and physical properties), wind, and the availability of water, or even a combination of some of these subfactors and seasonality (26).

Studies conducted by (24) on the *Piper* genus in the Atlantic Forest, on the coast of Paraná, showed the influence of seasonality on the yield and the constituents of essential oils. (16), (27), (29), (17), (21), (20), and (19), also identified the influence of seasonality on the chemical profile of the oils analyzed.

The chemical composition of the essential oil of the studied species identified 78 constituents, corresponding to an average of 90% of chemical compounds of the essential oil, in the identified samples (Table 3). The species *P. diospyrifolium* was the one that presented the highest number of compounds identified, with 55, followed by *P. gaudichaudianum* with 50, *P. xylopioides* with 35, and *P. mosenii* with 33 compounds.
Table 3 - Phytochemical analysis of essential oils of fresh samples of species collected during the winter and summer of 2016 in the Caiuá Ecological Station, North Diamond, Paraná State, Brazil.

| Compounds                      | IR² | IR¹ | P. mos | P. gau | P. xyl | P. dio |
|--------------------------------|-----|-----|--------|--------|--------|--------|
| 3Z-hexenol                     | 849 | 850 | ---    | 0.31   | ---    | ---    |
| α-pinene                       | 931 | 932 | ---    | 5.09   | 4.14   | 1.01   |
| β-pinene                       | 974 | 974 | ---    | 6.62   | 0.25   | 2.14   |
| 6-methyl-5-hepten-2-one        | 984 | 981 | ---    | 0.77   | ---    | ---    |
| myrcene                        | 989 | 988 | ---    | 0.26   | 1.22   | 0.23   |
| α-phellandrene                 | 1004| 1002| ---    | ---    | 1.56   | (1.16) |
| o-cymene                       | 1022| 1022| ---    | 0.13   | ---    | ---    |
| limonene                       | 1026| 1024| 0.10   | 0.72   | ---    | 7.77   |
| β-phellandrene                 | 1027| 1025| ---    | ---    | 12.50  | (8.97) |
| (Z)-β-ocimene                  | 1028| 1032| 0.18   | 0.89   | 0.24   | (0.18) |
| (E)-β-ocimene                  | 1045| 1044| ---    | 0.33   | 2.64   | (1.96) |
| γ-terpinene                    | 1055| 1054| ---    | ---    | 0.16   | (0.16) |
| linalool                       | 1100| 1095| ---    | ---    | (0.25) |       |
| δ-elemene                      | 1333| 1335| 1.27   | 7.52   | 0.39   | (0.28) |
| α-cubebene                     | 1345| 1345| 0.16   | 0.57   | ---    | 0.20   |
| cyclosativene                  | 1366| 1369| ---    | (0.55) | ---    | (0.40) |
| α-ylangene                     | 1366| 1373| ---    | ---    | ---    | (0.27) |
| α-copaene                      | 1370| 1374| 0.80   | 3.15   | ---    | 1.95   |
| β-elemene                      | 1387| 1389| 2.15   | (4.33) | 1.91   | (1.88) |
| Z-caryophyllene                | 1399| 1408| ---    | ---    | 0.77   | (0.57) |
| α-gurjunene                    | 1403| 1409| ---    | 0.54   | ---    | 0.11   |
| (E)-cariophyllene              | 1413| 1417| 16.39  | 7.40   | 2.59   | 20.56  |
| β-copaene                      | 1422| 1430| 0.69   | ---    | ---    | (0.41) |
| β-curjunene                    | 1422| 1431| ---    | 4.94   | ---    | 0.44   |
| γ-clemene                      | 1429| 1434| 0.78   | ---    | ---    | 0.27   |
| α-guaiene                      | 1433| 1437| 0.83   | ---    | ---    | ---    |
| aromadendrene                  | 1431| 1439| ---    | 1.44   | 0.30   | 0.70   |
| 6,9-guaiaadiene                | 1437| 1442| ---    | 0.18   | ---    | (0.14) |
| Compound                          | Formula          | Retention Time | Area (%) | Peak Area | Peak Height | Peak Width |
|----------------------------------|------------------|----------------|----------|-----------|-------------|------------|
| trans-μuurola-3.5-dieno          |                  | 1441 1451      | ---      | 0.40      | (0.35)      | ---        |
| α-humulene                       | C18H28O1         | 1446 1452      | 3.21     | 2.32      | (1.36)      | 0.50       | 1.66       |
| geranyl acetate                  | C20H30O2         | 1451 1453      | ---      | 5.33      | (3.66)      | ---        |
| allo-aromadendrene               | C19H30           | 1453 1458      | 0.13     | 5.49      | (4.52)      | 0.35       |
| cis-cadina-1(6).4-diene          | C19H30O           | 1456 1461      | 0.23     | 0.28      | (0.31)      | ---        | 0.21       |
| dauc-5,8-diene                   | C17H24O           | 1471 1471      | ---      | 5.24      | (6.26)      | 0.12       | (0.14) 0.18|
| Trans-cadina-1(6).4-diene        |                 | 1471 1475      | ---      | (1.68)    |             |            |
| γ-muurolene                      |                  | 1474 1478      | ---      | 0.61      | (0.56)      | 0.35       | 1.55       |
| germacrene D                     |                  | 1476 1484      | 30.36    | 1.41      | (1.73)      | 3.09       | 10.06      |
| aristolechene                    |                  | 1477 1487      | ---      | 0.13      | (0.17)      | ---        |
| β-selinene                       |                  | 1479 1489      | 0.69     | 0.13      | (0.36)      | ---        | 0.37       |
| δ-selinene                       |                  | 1484 1492      | 0.19     | 0.29      | (0.36)      | ---        |
| Trans-μuurola-4(14).5-dieno      |                  | 1484 1493      | ---      | ---       | ---         | 0.14       |
| Epi-cubebol                      |                  | 1484 1493      | ---      | ---       | ---         | (0.15)     |
| valencene                        |                  | 1486 1496      | ---      | 0.58      | (0.56)      |            |
| bicyclogermacrene                |                  | 1490 1500      | 13.46    | 4.15      | (4.81)      | 29.83      | 8.42       |
| α-muurolene                      |                  | 1495 1500      | ---      | 4.00      | (4.98)      | 0.77       | 0.55       |
| (EE)-α-farnesene                 |                  | 1499 1505      | 0.94     | 0.34      | (0.48)      | ---        |
| germacrene B                     |                  | 1497 1508      | ---      | (0.56)    | ---         |
| δ-amorphene                      |                  | 1501 1511      | ---      | ---       | ---         | 0.76       | (0.43)     |
| γ-cadinene                       |                  | 1508 1513      | 1.56     | 3.77      | (4.05)      | 1.27       | 1.57       |
| δ-cadinene                       |                  | 1518 1522      | 2.74     | 5.59      | (7.12)      | 3.69       | 2.72       |
| trans-cadina-1,4-diene           |                  | 1526 1533      | ---      | 0.57      | (0.83)      | ---        | 0.49       |
| α-cadinene                       |                  | 1532 1537      | ---      | 0.31      | (0.37)      | 0.20       | 0.53       |
| α-copaen-11-ol                   |                  | 1543 1539      | ---      | 0.19      | (0.14)      | ---        |
| α-calacorene                     |                  | 1535 1544      | ---      | 0.25      | (0.58)      | ---        |
| selina-3,7(11)-Diene             |                  | 1536 1545      | ---      | ---       | ---         | (0.40)     |
| germacrene B                     |                  | 1548 1559      | 4.53     | 0.43      | ---         | 1.31       |
| Compound                        | Value 1 | Value 2 | Value 3 | Value 4 | Value 5 | Value 6 | Value 7 | Value 8 | Value 9 | Value 10 | Value 11 | Value 12 | Value 13 |
|--------------------------------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| (E)-nerolidol                  | 1561    | 1561    | 1.42    | 6.13    | (7.11)  | 1.83    | (1.78)  | 1.72    |         |         |         |         |         |
| germacrene D-4-ol              | 1568    | 1574    | ---     | ---     | 4.52    | (8.57)  |         |         |         |         |         |         |         |
| espatulenol                    | 1569    | 1577    | 1.86    | 3.20    | (0.82)  |         |         | 0.77    |         |         |         |         |         |
| caryophyllate oxide            | 1574    | 1582    | 1.60    | 3.14    | (3.69)  | (0.90)  | 2.30    | (3.22)  |         |         |         |         |         |
| diethyl Phthate                | 1585    | 1590    | 0.15    |         |         |         |         | 0.13    |         |         |         |         |         |
| globulol                       | 1582    | 1590    | ---     | 0.10    |         | 0.26    | (0.22)  | (0.25)  |         |         |         |         |         |
| viridiflorol                   | 1582    | 1592    | 0.45    | 0.21    | (0.20)  |         |         |         |         |         |         |         |         |
| rosifoliol                     | 1594    | 1600    | ---     | 0.50    | (0.51)  |         |         |         |         |         |         |         |         |
| ledol                          | 1593    | 1602    | 0.45    |         |         |         |         | (0.40)  |         |         |         |         |         |
| humulene epoxy II              | 1600    | 1608    | 0.16    | 0.86    | (0.51)  |         |         | (0.14)  |         |         |         |         |         |
| 1-10-di-Epi-cubenol            | 1607    | 1618    | 0.17    | 0.67    | (0.77)  |         |         | 0.16    | (0.29)  |         |         |         |         |
| 10-Epi-γ-eudesmol              | 1612    | 1622    | ---     |         |         |         |         | (0.13)  |         |         |         |         |         |
| 1-Epi-cubenol                  | 1621    | 1627    | 0.93    | 1.22    | (1.59)  |         |         | 0.38    | (1.91)  |         |         |         |         |
| γ-eudesmol                     | 1623    | 1630    | ---     | 0.57    | (0.61)  |         |         |         |         |         |         |         |         |
| muurola-4.10(14)-dien-1-β-ol   | 1621    | 1630    | ---     |         | 1.13    | (0.92)  |         |         |         |         |         |         |         |
| Epi-α-cadinol                  | 1634    | 1638    | ---     |         |         |         |         | 1.55    | (1.91)  |         |         |         |         |
| caryophile-4(12). 8(13)-dien-5β-ol | 1630   | 1639    | 0.24    | 0.18    | (1.20)  |         |         |         |         |         |         |         |         |
| Epi-α-muurolol                 | 1634    | 1640    | 1.88    | 1.94    | (2.21)  | 2.67    | (3.40)  | 1.55    |         |         |         |         |         |
| α-muurolol                     | 1639    | 1644    | 1.03    | 1.89    | (2.17)  | 0.50    | (0.68)  | (0.34)  |         |         |         |         |         |
| β-eudesmol                     | 1644    | 1649    | ---     | 0.66    | (0.76)  |         |         | 0.39    |         |         |         |         |         |
| α-cadinol                      | 1647    | 1652    | 3.27    | 1.26    | (1.31)  | 4.51    | (4.86)  | 1.38    |         |         |         |         |         |
| Epi-α-bisabolol                | 1680    | 1683    | ---     | 1.88    | (1.38)  |         |         |         |         |         |         |         |         |
| Monoterpenes (%)               | 3.03    | (3.03)  | ---     | 12.50   | (12.50)| 21.87   | (18.75)| 17.50   |         |         |         |         |         |
| Oxygenated monoterpenes (%)    | ---     |         | ---     |         |         | ---     |         |         |         |         |         |         |         |
| Sesquiterpenes (%)             | 57.58   | (57.58) | ---     | 45.83   | (55.56)| 53.12   | (53.12)| 57.50   |         |         |         |         |         |
| Oxygenated sesquiterpenes (%)  | 36.36   | (36.36) | ---     | 35.42   | (35.56)| 21.88   | (25.00)| 22.50   |         |         |         |         |         |
The chemical composition of the species *P. mosenii* identified during the winter had an average of 57.58% of sesquiterpenes hydrocarbon, 36.36% of oxygenated sesquiterpenes, and 3.03% of monoterprenes and phenylpropanoids. A majority of the constituents of samples performed was identified as germacrene D (30.36%), (E)-caryophyllene (16.39%), and biciclogermacrene (13.46%), with the total compounds identified as 94.89%. During the summer an yield of oil was not observed for that species.

The chemical composition of essential oils is determined by genetic factors, however, according to (17), other factors may cause significant changes in the production of secondary metabolites. In fact, the secondary metabolites represent a chemical interface between plants and the environment. The stimuli arising from the environment in which the plant is located can redirect the metabolic pathway, causing a biosynthesis of different compounds. Among these factors, we can highlight the interactions between plant/microbial, plant/insect, and plant/plant; age and stage of development, abiotic factors such as brightness, temperature, rainfall, nutrition, time, and time of collection, as well as techniques during harvest and post-harvest. It is valid to note that these factors can present correlations between themselves and not act in isolation. They can exercise joint influence on secondary metabolism, which causes a variation in the income and composition of the essential oil analyzed.

The yield of essential oil of the species *P. gaudichaudianum* collected during the winter season showed a proportion of 45.83% of sesquiterpenes hydrocarbon, 35.42% of oxygenated sesquiterpenes, 12.50% of monoterprenes, 2.08% phenylpropanoids, and 4.14% of other components (chain of 8 and 12 carbons), with the total of compounds identified of 91.66%. Samples collected in the summer showed a proportion of 55.56% of sesquiterpenes hydrocarbon, 35.56% of oxygenated sesquiterpenes, 4.44% of monoterprenes, 2.22% phenylpropanoids and for other components (chain of 8 and 12 carbons). The majority of constituents were (E)-caryophyllene (7.40%), β-pinene (6.62%), (E)-nerolidol (6.13%), δ-cadinene (5.59%), geranyl acetate (5.33%), Dauca-5,8-diene (5.24%), α-pinene (5.09%), β-gurjene (4.94%), and bicyclogermacrene with 4.15%.

Yet for *P. gaudichaudianum*, it was observed that in the summer there was a reduction in the proportion of monoterprenes (12.50% to 4.44%) and increase of hydrocarbons of sesquiterpenes (45.83% to 55.56%) and the total compounds identified was 89.21%. The main constituents identified were (E)-caryophyllene (7.25%), β-pinene (6.62%), δ-cadinene (7.12%), (E)-nerolidol (7.11%), dauc-5,8-diene (6.26%), geranyl acetate (5.66%), β-gurjene (5.13%), and α-muurolene with 4.98%.

Among the principal constituents, a majoritarian checked in some municipalities of the state of Rio Grande do Sul/Brazil for essential oils from leaves and inflorescences of *P. gaudichaudianum*, stand out α-humulene (13.3–37.5%), β-caryophyllene (10.4–19.3%), β-pinene (5.6–7%), E-nerolidol (5.32–22.4%), E-caryophyllene (8.9%), bicyclogermacrene (7.4%), β-selinene (3.7–15.7%), α-selinene (8.9–16.6%), alloaromadendrene (7.7%), limolool (4.8%) (3; 25).

Already in a seasonal study in the municipality of Atalanta (Santa Catarina State) the constituents were observed to be β-caryophyllene (10.4–12.5%), α-caryophyllene (8.2–10.4%), δ-selinene (5.4–6.9%), δ-cadinene (6.0–7.3%); E-nerolidol (3.0–7.2%), Z-β-guaiene (5.5–5.6%), δ-cadinene (6.4–7.3%) and Valencene (4.0–5.6%) (22).

This difference in the chemical composition of the essential oil of the study population in comparison with the other studies carried out with the same *P. gaudichaudianum* extract could occur due to environmental, genetic, and biotic factors. Second, (31) stated, the chemical variability may be the result of the selection pressure of the environment and/or the ecology or characterizing a chemical adjustment to the environmental conditions prevalent.

The phytochemical composition of the essential oil from samples of *P. xylopioides* collected during the winter season showed a proportion of 53.12% of sesquiterpenes hydrocarbon, 21.88% of oxygenated...
sesquiterpenes, 21.87% of monoterpenes, and 3.13% of phenylpropanoids, and a total of 96.07% of identified compounds. The majority of the constituents were bicyclogermacrene 29.83%, β-phellandrene 12.50%, δ-elemene 7.52%, and alloaromadendrene 5.49%. In the summer we found a proportion of 53.12% of sesquiterpenes hydrocarbon, 25% of oxygenated sesquiterpenes, 18.75% of monoterpenes, and 3.13% of oxygenated monoterpenes, with a total of 96.73% of compounds identified. The proportion of constituents found by the majoritarian was bicyclogermacrene 33.45%, β-phellandrene 8.97%, and germacrene D-4-ol 8.57%.

The samples of P. diospyrifolium collected during the winter season showed the proportion of oxygenated monoterpenes of 57.50%, oxygenated sesquiterpenes of 22.50%, monoterpenes of 17.50%, and 2.50% of phenylpropanoids, with a total of 80.21% compounds identified. The majority of the constituents were (E)-caryophyllene of 20.56%, germacrene D of 10.06%, bicyclogermacrene of 8.42%, and limonene of 7.77%. Already, during the summer season we found a proportion of 58.70% of sesquiterpenes hydrocarbon, 26.08% of oxygenated sesquiterpenes, and 15.22% of monoterpenes hydrocarbons, with a total of 81.23% compounds identified, and the proportion of constituents the majoritarian found was (E)-caryophyllene of 20.65%, germacrene D 6 of 68%, bicyclogermacrene of 8.51%, and limonene of 5.22%.

The predominance of sesquiterpenes in the genus Piper was also observed by (8), (6), (28), and (12).

In the winter/2016 station, the dendrogram (Fig. 1) demonstrates the similarity of the chemistry among the Piper genus studied, where three distinct groups of Euclidean distances were observed. This can also be explained by the genetic variability among species and populations (2).

The first grouping is formed by two Species: P. mosenii and P. diospyrifolium. This cluster is characterized by (E)-caryophyllene (16.39–20.56%), germacrene D, (10.06–30.36%) bicyclogermacrene (8.42–13.46%), γ-cadinene (1.56–1.57%), β-elemene (0.77–2.15%), δ-cadinene (2.72–2.74%), (E)-nerolidol (1.42–1.72%), Õx. caryophyllene (1.60–2.30%), epi-α-muurolol (1.55–1.88%), and α-muurolol (0–1.03%). The cluster II includes the species P. xylopioides, which features (E)-caryophyllene (2.59%), germacrene D (3.09%) bicyclogermacrene (29.83%), γ-cadinene (1.27%), β-elemene (1.91%), δ-cadinene (1.27%), (E)-nerolidol (1.83%), epi-α-muurolol (2.67%), and α-muurolol (0.5%). The last group consists of (E)-caryophyllene (7.40%), germacrene D (1.41%) bicyclogermacrene (4.15%), γ-cadinene (3.77%), δ-cadinene (5.59%), (E)-nerolidol (6.13%), ox. caryophyllene (3.14%), epi-α-muurolol (1.94%), and α-muurolol (1.89%) in the species P. gaudichaudianum.

To determine the degree of variations in the phytochemical, a principal component analysis (PCA) was performed using a correlation matrix of all chemical compounds (Table 4 and Fig. 2).
Table 4 – Eigen values and accumulated variance for factors obtained from principal components analysis, based on the chemical composition of the species of Piper, studied on the basis of the composition of its essential oils during the winter season/2016.

| Compounds                | Factors |       |       |
|--------------------------|---------|-------|-------|
|                          | 1       | 2     | 3     |
| (E)-cariofilene          | 0.015   | -0.152| 0.338 |
| germacrene D             | 0.521   | 0.327 | -0.274|
| biciclogermacrene        | -0.019  | 0.653 | -0.098|
| γ-cadinene               | 0.459   | -0.135| 0.433 |
| β-elenene                | 0.414   | -0.137| -0.162|
| δ-cadinene               | -0.152  | 0.067 | -0.584|
| (E)-nerolidol            | -0.348  | 0.247 | 0.117 |
| Óx. cariofileno          | -0.124  | -0.502| -0.317|
| Epi-α-muurolol           | -0.223  | 0.105 | 0.325 |
| α-muurolol               | 0.130   | -0.238| 0.150 |
| α-cadinol                | -0.347  | -0.149| 0.058 |
| Eigenvalues              | 5.83    | 3.35  | 1.82  |
| % of variance            | 56.64   | 33.44 | 16.55 |
| Cumulative %             | 56.64   | 90.08 | 100.00|

* significance ≥ 60%

Fig. 2 - Principal Component Analysis (PCAs) for the species of Piper based on chemical composition of essential oils of fresh samples collected in the winter season/2016. New Var: (E)-caryophyllene; Var1: germacrene D; Var2: bicyclogermacrene; Var3: γ-cadinene; Var4: β-eleneno; Var5: δ-cadinene; Var6: (E)-nerolidol; Var7: Óx. caryophyllene; Var8: epi-α-muurolol; Var9: α-muurolol; Var10: α-cadinol.

Results obtained by PCA, based on 11 chemical compounds, are shown in Figure 2 and Table 4. The three factors explain 100% of the accumulated variation in the data; the first two factors being considered the most important, as they described 90.08% of the accumulated variance (Table 4). The compounds germacrene D, γ-cadinene, and β-elenene, demonstrate the relevant contributions, with 56.64% of the variation in the principal components (PC 1). Bicyclogermacrene, germacrene D, and (E)-nerolidol are compounds that contributed, by explaining 33.44% of the variance of principal components (PC 2).

For summer 2016, the dendrogram (Fig. 3) is submitted to chemical similarity of the Piper genus where three distinct groups of Euclidean distances were observed. The first grouping is formed only by the species P. mosenii, which showed an absence of the production of oil from this station. The cluster II includes the species P. gaudichaudianum and P. diospyrifolium, which present as constituents (E)-caryophyllene (7.25–20.65%), germacrene D (1.73–6.84%) Bicyclogermacrene (4.81–8.51%), γ-cadinene (7.12–3.22%), β-elenene (0.10–0.57%), δ-cadinene (0.37–0.34%), (E)-nerolidol (7.11–0.23%), caryophyllene oxide (3.39–3.22%), Epi-α-muurolol (2.21%) and α-muurolol (2.17–0.34%) and α-cadinol (1.31–1.46%).
Fig. 3 - Dendrogram for the Piper species and function of the chemical compounds of the essential oil of fresh samples collected in the summer/2016, using the Euclidean distance.

The last group consists of (E)-caryophyllene (2.34%), germacrene D (1.75%) bicyclogermacrene (33.45%), γ-cadinene (1.93%), β-elemene (1.91%), δ-cadinene (3.87%), (E)-nerolidol (1.78%), caryophyllene oxide (0.90%), epi-α-muurolol (3.40%), α-muurolol (0.68%), and α-cadinol (4.84%) in the species P. xylopioides.

Fig. 4 - Principal Component Analysis (PCAs) for the species of Piper based on chemical composition of essential oils of fresh samples collected in the summer season/2006. NewVar: (E)-caryophyllene; Var1: germacrene D; Var2: bicyclogermacrene; Var3: γ-cadinene; Var4: β-elemene; Var5: δ-cadinene; Var6: (E)-nerolidol; Var7: Ox. cCaryophyllene; Var8: Epi-α-muurolol; Var9: α-muurolol; Var10: α-cadinol.

Results obtained by PCA based on the analyzed species and 11 chemical compounds are presented in Fig. 4 and Table 5. The three factors explain 100% of the accumulated variation in the data; the first two factors being considered most important, because they described 79.15% of accumulated variance. The α-muurolol compounds, (E)-nerolidol, and germacrene D, demonstrate the relevant contributions, with 45.03% of the variation for the principal components (PC 1). Epi-α-muurolol and δ-cadinene are compounds that contributed by explaining 34.12% of the variance in the principal components (PC 2).
The different stations influenced both the quantity and the chemical compounds of the evaluated essential oils of the species of genus Piper. With the exception of P. Mosenii, compounds α-pinene, (E)-caryophyllene, aromadendrene, α-humulene, germacrene D, bicyclogermacrene, α-muurolene, γ-cadinene, δ-cadinene, α-cadinene, and (E)-nerolidol were found in other species in both stations of collection. The compounds of α-phellandrene, β-phellandrene, trans-muurola-3,5-diene, valencene, germacrene D-4-ol, and muurola-4,10(14)-dien-1-β-ol were found only in the species P. xylopioides in the collection of two seasons.

Differences in chemical constituents can be justified by the regulation of gene expression of the enzymes involved in the biosynthetic route of terpenes. In addition, climatic conditions (collections at different seasons) contributed to the chemical characterization of the essential oil of the species analyzed.

IV. CONCLUSION

The results presented here demonstrate that the environmental factor of seasonality interfered with the levels and the average percentage of the chemical constituents of essential oils.

The studied species of genus Piper can be distinguished into three groups per workstation, in accordance with the composition of the essential oil of fresh leaves.

The chemical composition of the predominant species evaluated consisted of sesquiterpenes followed by monoterpenes, with emphasis on (E)-caryophyllene, germacrene D, bicyclogermacrene, α-pinene and β-pinene, α-phellandrene, β-phellandrene, trans-muurola-3,5-diene, and Valencene; germacrene D-4-ol and muurola-4,10(14)-dien-1-β-ol were found only in the species P. xylopioides in the two seasons of collection.

With the exception of P. mosenii compounds, α-pinene, (E)-caryophyllene, aromadendrene, α-humulene, germacrene D, bicyclogermacrene, α-muurolene, γ-cadinene, δ-cadinene, α-cadinene, and (E)-nerolidol were found in the other species and in both workstation collections.

REFERENCES

[1] Adams, R. P. (2007). Identification of essential oil components by gas chromatography/mass spectrometry. Carol Stream: Allured Publishing Corporation.

[2] Amaral, W. do; Deschamps, C.; Bizzo, H. R.; Pinto, M. A. S.; Biasi, L. A.; Da Silva, L. E. (2017) Essential Oil Yield and Composition of Native Tree Species from Atlantic Forest, South of Brazil. Journal of Essential Oil Bearing Plants, v. 20, p. 1525-1535.

[3] Andrade, E. H. A. et al. (1998). Essential oils of Piper gaudichaudianum Kunth and P. regnellii (Miq.) CDC. Research note. Journal Essential Oil Research, v. 10, p. 465-467.

[4] Aparecido, L. E. de O., Rolim, G. de S., Richetti, J.; Souza, P. S. de, & Johann, Jerry Adriani. (2016). Köppen, Thornthwaite and Camargo climate...
classifications for climatic zoning in the State of Paraná, Brazil. Ciênc. agrotec., Lavras, v. 40, n. 4, p. 405-417.

[5] Básia, L. A.; Deschamps, C. (2009). Plantas aromáticas: do cultivo à produção de óleo essencial. Layer Studio Gráfico e Editora Ltda, 1ª edição, Curitiba, 2009. 106p.

[6] Costa, M. C. (2013). Investigação fitoquímica e avaliação do potencial antimicrobiano por bioautografia da Piper sp (Piperaceae). Monografia, 82f. (Graduação em Farmácia). Universidade Vale do Itajaí.

[7] EMBRAPA. Centro Nacional de Pesquisa de Solos. (2013). Sistema brasileiro de classificação de solos. 3 ed. rev. ampl. - Brasília, DF : Embrapa, 353p.

[8] Fazolín, M ; Estrela, J. L. V. (2011). Piperaceas da Amazônia com potencial de uso inseticida. In: I Seminário De Entomologia E Acarologia Agrícola Da Amazonia. Manaus (AM).

[9] Ferreira, D. F. (2011). Sisvar: a computer statistical analysis system. Ciência e Agrotecnologia (UFLA), v. 35, n.6, p. 1039-1042.

[10] Figueiredo, A. C., Barroso, J. G. & Pedro, L. G. (2007). Plantas Aromáticas e Medicainais. Fatores que afectam a produção. pp. 1-18. In: FIGUEIREDO, A.C., BARROSO, J. G, PEDRO, L. G (Eds), Potencialidades e Aplicações das Plantas Aromáticas e Medicainais. Curso Teórico-Prático, 3.a Ed., Edição da Faculdade de Ciências da Universidade de Lisboa - Centro de Biotecnologia Vegetal, Lisboa, Portugal.

[11] Figueiredo, A. C.; Pedro, L. G; Barroso, J. G. (2014). Plantas Aromáticas e Medicainais - óleos essenciais e voláteis. Revista da APH. Lisboa, n. 114, p. 29-33.

[12] Gasparetto, A.; Bella Cruz, A.; Wagner, T. M.; Bonomini, T. J.; Correa, R.; Malheiro, A. (2017) Seasonal variation in the chemical composition, antimicrobial and mutagenic potential of essential oils from Piper cernuum. Industrial Crops and Products. v.95, p. 256-263.

[13] Gogosz, A. M.; Boeger M. R. T.; Negrelle, R. R. B., Bergo, C. (2012). Anatomia foliar comparativa de nove espécies do gênero Piper (Piperaceae). Rodriguésia, v. 63, n. 2, p. 405-417.

[14] Guimarães, E.F., Carvalho-Silva, M., Monteiro, D., Medeiros, E.S., Queiroz, G.A. (2015). Piperaceae. Jardim Botânico do Rio de Janeiro: Rio de Janeiro. Disponível em: http://floradobrasil.jbrj.gov.br/jabot/floradobrasil/FB190. Acesso em 20 nov 2015.

[15] IBGE - Instituto Brasileiro De Geografia E Estatística. (1992). Manual técnico da vegetação brasileira: Série Manuais Técnicos em Geociências. Rio de Janeiro, IBGE.

[16] Ming, L. C.; Chaves, F. C. M.; Marques, M. O. M.; Meireles, M. A. A. (2002) Produção sazonal de óleo essencial em uma população natural de Piper aduncum L em Adrianópolis - PR. Horticulatura Brasileira, v. 20, n. 2, Suplemento 2.

[17] Moraes, L. A. S. (2009). Influência dos fatores abióticos na composição química dos óleos essenciais. Horticulatura brasileira. v.27, n. 2. p. 4050-4063.

[18] NIST Chemistry Webbook, edited by P. J. Linstrom and W. G. Mallard, http://webbook.nist.gov (acessado em dezembro de 2018).

[19] Oliveira, L. G. S.; Ribeiro, D. A.; Saraiva, M. E.;Macedo, D. G.; Macedo, J. G. F.; Pinheiro, P. G; Costa, J. G. M.; Souza, M. M. A.; Menezes, I. R. A. (2017) Chemical variability of essential oils of Copaifera langsdorffii Desf. in different phenological phases on a savannah in the Northeast, Ceará, Brazil. Industrial Crops and Products. n.97, p. 455-464.

[20] Ribeiro, M. S. S.; Costa, R. S.; Amorim, A. V.; Lacerda, C. F.; Dias N. S. (2016). Biométria e óleo essencial de alicrim pimenta cultivado em diferentes épocas e condições de luminosidade. Rev. Bras. Agríc. Irr. v. 10, no 6, Fortaleza, p. 1086 - 1095.

[21] Santos, K. A. S.; Silva, E. S.; Oliveira, M. R.; Borsato, A. V. (2016). Rendimento do óleo essencial de Vitex agnus castus L. em diferentes períodos de coleta. Cadernos de Agroecologia. v. 11, n. 2, p. 1-5.

[22] Santos, T. G. (2009). Composição química e atividade antimicrobiana dos óleos essenciais de três espécies do gênero Piper e de Baccharis semiserrata DC. 117p. Dissertação de Mestrado em Química, Universidade Regional de Blumenau, Blumenau.

[23] Schindler, B. (2015). Óleo essencial de Piper gaudichaudianum Kunth: Rendimento, composição química e atividade fungitóxica in vitro. Dissertação (Mestrado) UFSM - Centro de Ciências Rurais, Programa de Pós-Graduação em Engenharia Florestal, RS.

[24] Silva, L. E.; Amaral, A.; Garcia, B.; Parabocz, L. D. (2016). Bioprospecção de Espécies Nativas no Litoral do Paraná: Agregando valor à Biodiversidade Local. In. XXIV Sim Pôs Do Plantas M Edicinaias Do Brasil. Belo Horizonte – MG.

[25] Silva, P. P.; Pêres, V. F.; Saffi, J. (2006). Extração e caracterização do óleo essencial das inflorescências de Piper gaudichaudianum Kunth. Revista de Iniciação Científica da Ulbra, v. 1, n. 1, p. 25-30.

[26] Silva, P. S. S. (2013). Caracterização da composição química dos óleos essenciais de Lychnophora
pinaster Mart. em função da sazonalidade. 167 f.

Dissertação (mestrado) - Universidade Estadual Paulista, Faculdade de Ciências Agrônomicas de Botucatu.

[27] Silva, R. (2005). Crescimento e teor do óleo essencial de Aloysia triphylla (L'Hérit) Britton (Verbenaceae), em função da adubação orgânica, sazonalidade, horário de colheita, processamento pós-colheita. Dissertação, 66 f. (Mestrado em Agronomia). Universidade Federal de Lavras.

[28] Soletti, A. G. (2015). Efeitos da sazonalidade sobre a composição química, potencial antimicrobiano, citotóxico e mutagênico dos óleos essenciais e frações diclorometano e acetato de etila de *Piper amplum* e *Piper cernuum*. Tese (Doutorado) Universidade do Vale do Itajaí, Programa de Doutorado em Ciências Farmacêuticas.

[29] Souza, J. P. B. (2007). Influência da sazonalidade no perfil químico dos óleos essenciais e das substâncias fixas de *Baccharis dracunculifolia* cultivada utilizando-se cromatografia em fase gasosa e líquida. Dissertação (Mestrado) Faculdade de ciências farmacêuticas de Ribeirão Preto. Ribeirão Preto.

[30] Souza, V. C.; Lorenzi, H. (2012) Botânica sistemática: guia ilustrado para identificação das famílias de angiospermas da flora brasileira, baseado em APG II. 3. ed. Nova Odessa: Instituto Plantarum. 768p.

[31] Telascrea, M., Araújo, C. C.; Marques, M.; Facanali, R.; Moraes P. L. R., Cavalheiro, A. J. (2007) Essential oil from leaves of Cryptocarya mandioccana Meisner (Lauraceae): Composition and intraspecific chemical variability. Biochem Syst Ecol. v. 35, p. 222-232.

[32] WILEY Registry of Mass Spectral Data, 6th edn. Wiley Interscience,: New York. (1994).

[33] Yunes, R. A. (2012). Em Química de produtos naturais: novos fármacos e a moderna farmacognosia; Yunes, R. A.; Cechinel Filho V., Org.; 3a Ed. Univali, Itajaí, 384p.