Full Length Research Paper

**Piper multinodum** C.DC. (Piperaceae) essential oils chemical variation and biological activity against *Mycobacterium tuberculosis*

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Received 5 August, 2021; Accepted 14 September, 2021

**Piper multinodum** C.DC. belongs to the Piperaceae family, and is known as “Jaborandi-manso”. This species has no reported botanical, chemical, pharmacological and/or toxicological scientific studies, and has restricted occurrence in degraded Brazilian biomes. The *Piper* genus is known to be aromatic, the essential oils (EO) obtained from different organs have demonstrated significant biological activities, and can be an important tool for chemophenetic and ecological definitions. The present study aimed to characterize the chemical profile of the EO from different parts of *P. multinodum* and their activity against *Mycobacterium tuberculosis* H₃⁷Rv (ATCC, 25618). The EO were obtained by hydrodistillation and characterized by GC-MS and GC-FID. Chemical composition of the volatile mixture showed a great diversity of compounds that diverged between the vegetative and reproductive parts. The α-pinene compound was identified as being the most predominant in the leaves, infructescences and inflorescences (32.49, 67.23 and 40.23%, respectively). The branches (secondary stem) showed to be rich in sesquiterpenes and monoterpenes: α-copaene (13.24%), *E*-caryophyllene (12.32%), α-pinene (20.34%), and myrcene (11.23%). The chemical profile of stems and roots showed a low percentage of monoterpenes and sesquiterpenes, but a high percentage of arylpropanoids, with *E*-methyl-isoeugenol (77.58%) registered in the stems and euasarone (81.34%) in the root. The antmycobacterial activity showed the highest activities recorded for the EO from roots (78.51 µg/mL) and infructescences (85.91 µg/mL). In addition to the findings related to biological activity, the determination of chemical diversification between the different parts of *P. multinodum* may help to understand the ecological issues of the Piperaceae family as well as to improve the chemotaxonomic knowledge of the genus *Piper*.

**Key words:** *Piper multinodum*; essential oils; antmycobacterial activity; chemical composition.
INTRODUCTION

Piperaceae is a Pantropical family with approximately 3,700 plant species distributed worldwide. *Piper* L. is the largest genus of Piperaceae with around 2,000 species (Wanke et al., 2006; Jaramillo et al., 2008). Ethnobotanical surveys of medicinal plants report the importance of the genus *Piper* for medicinal purposes. There are secular plants cited worldwide, such as Black Pepper (*Piper nigrum* L.), Long Pepper (*Piper longum* L.) and Kava-Kava (*Piper methysticum* G. Forst) for many therapeutic purposes (Yadav et al., 2019; Takoori et al., 2019).

In Brazil, the diversity of popular use is also associated with the numbers of existing *Piper* species. There are extensive applications for this genus, such as being used as insecticides, food and condiments, in addition to medicinal, ornamental and ritualistic applications (Pereira et al., 2007; Salehi et al., 2019). Since they present similar morphological patterns, the species of this genus are easily found within several ethnospecies such as “Jaborandi” (*Piper amalago* L., *Piper crassivernium* HB & K., *Piper malicicum* Kunth and *Piper aduncum* L.), “Pariparoba” (*Piper cernuum* Vell., *Piper mikaniamum* (Kunth) Steudel and *Piper umbellatum* L.), “Betis-branco” (*Piper rivotoides* Kunth., *Piper arboreum* Aubl. and *Piper tuberculatum* Jacq.), among other species (Gogosz et al., 2012; Silva et al., 2016).

The *Piper* genus is known to be aromatic and the essential oils (EO) obtained from different organs have demonstrated significant biological activities, such as antioxidant, antibacterial, antifungal, antiprotozoal, antiproliferative in tumor cells and anti-inflammatory (Moreira et al., 2001; Scott et al., 2008; Oliveira et al., 2013; Salehi et al., 2019; Ramos and Moreira, 2019). This richness of activity is related to the chemical diversity of *Piper*’s EO, which can be an important tool for chemophenetic and ecological definitions, as well as for new and never studied species, as they help to describe the matrix of secondary metabolites specialized in each taxon (Zidorn, 2019).

Tuberculosis (TB) is characterized as a global health problem. It is an infectious disease caused by the *Mycobacterium tuberculosis* complex. It is the ninth leading cause of death, and its morbidity and mortality remain a cause for concern, especially in developing countries (Nayyar and Jain, 2005). From an epidemiological point of view, it is estimated that about 23% of the world’s population has tuberculosis in its latent form. There were approximately 10 million new cases of TB recorded in the year 2017 alone, with approximately 1.3 million deaths recorded. Among these deaths, approximately 300,000 were related to people positive for human immunodeficiency virus (HIV) (WHO, 2010; Boren et al., 2020).

The increase in cases of development of drug resistance in the treatment of TB has contributed to the worsening of this scenario worldwide, with the occurrence of multidrug-resistant, extensively resistant, and currently fully resistant *M. tuberculosis*, being a threat to the control of this disease, which requires the urgent development of new and more effective drugs (WHO, 2010; Boren et al., 2020).

In 2017, 480,000 new patients with multidrug-resistant were reported, almost half of them occurring in India, China and the Russian Federation (Nair et al., 2017; WHO, 2010; Boren et al., 2020). EO and extracts are known for their antimicrobial action against a wide range of bacterial strains (Fernandez et al., 2019; Boren et al., 2020). Both natural products and their derivatives show inhibitory activity against *M. tuberculosis* growth, and some have even been selected as prototype molecules for the development of new anti-TB agents. Some species in the genus *Piper* show promise against *M. tuberculosis* (Fernandez et al., 2019; Zodape et al., 2021).

The species *P. multinodum* C.DC., popularly known as “Jaborandi-manso”, is a plant found in Southeastern of Brazil, specifically in Brazilian biomes such as the Cerrado and the Atlantic Forest. This plant has restricted occurrence (Guimarães et al., 1994; Flora of Brazil, 2020), and, to the best of our knowledge, there are no botanical, chemical, pharmacological and/or toxicological scientific studies reported about it. This species show morphological similarities to *P. amplum* Kunth and *P. ilheusense* Yunck, which are plants of popular use and that coexist in the same region (Guimarães and Monteiro, 2006; Oliveira et al., 2016; Silva et al., 2017).

This work aims to characterize for the first time the composition of the EO from different organs of *P. multinodum* C. DC., and to report their antimycobacterial activity.

MATERIALS AND METHODS

Plant material

Different organs of *P. multinodum* C. DC., without signs of disease or herbivory, were collected in the Atlantic Forest at the Serra dos Órgãos National Park region, in the city of Teresópolis, Rio de Janeiro state, Brazil (22°27'00" S, 42°59'20" W, Elevation: 1267 m). Authorization for plant collection was given by the Chico Mendes Institute for Biodiversity Conservation (ICMBio, number 57296–1).

Samples of the fertile specimens were collected, identified and deposited with the voucher number RB01426180 at the Herbarium of the Rio de Janeiro Botanical Garden (JBRJ), Rio de Janeiro, Brazil. This study was registered at the Genetic Heritage

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Management Council under A001E32. For essential oil (EO) extraction, fresh leaves (200 g), branches (200 g), main stems (200 g), roots (200 g), infructescences (200 g) and inflorescences (200 g) were collected at 9 a.m. in December 15th, 2018.

Essential oil obtaining

The different fresh organs, including leaves for circadian rhythm study, were placed in a volumetric flask with 700 mL of distilled water, attached to a modified Cleverenger-type apparatus and heated for two hours. The yielding (%) was calculated in relation to the obtained EO (g) and the amount of used fresh plant parts (g). The experiment was carried out in triplicate. After the extraction process was completed, the EO were recovered and dehydrated with sodium sulfate (Na₂SO₄, Sigma-Aldrich, St. Louis, MO, USA). The EOs were stored in sealed amber flasks under refrigeration at -20°C until analysis (Oliveira et al., 2013; Ramos and Moreira, 2019).

Essential oil analysis

The chemical characterization and quantification of *P. multinodum* EO was performed by gas chromatography coupled to mass spectrometry (GC-MS) and a gas chromatography equipped with a flame ionization detector (GC-FID), respectively. The sample was diluted in dichloromethane (HPLC grade, Tedia, Brazil) before analysis (1 mg/mL; 1,000 ppm) (Ramos and Moreira, 2019).

A 1 μL of the solution was injected into an HP Agilent GC 6890 coupled to Agilent MS 5973 series mass selective detectors, in splitless mode, with the injector temperature set to 270°C, operating at 70 eV in positive mode. A HP-5MS capillary column (Agilent J&W; GC Columns (USA)] was used with 30 m x 0.25 mm i.d. x 0.25 μm particle size. Chromatography temperature conditions were 60 to 240°C at 3°C/min, totaling 60 min. For the separation of the constituents, helium was used as a carrier gas at 1.0 mL/min, at a rate of 1.0 s sweeps and mass range of m/z 40 – 600 atomic mass unit (µ) (Oliveira et al., 2013; Ramos and Moreira, 2019).

The GC-FID was achieved in a chromatograph equipped with a flame ionization detector [HP- Agilent 6890 GC-FID]. A 1 μL of the solution was injected under the same analytical conditions described above, except for the carrier gas used, which was hydrogen at flow rate of 1.0 mL/min (Oliveira et al., 2013; Ramos and Moreira, 2019).

The retention times (Rt) of the compounds were measured in min and were used to calculate their linear retention index, obtained from the injection of a homologous series of hydrocarbons (C₅-C₃₅, n-alkane, Sigma-Aldrich, Brazil) under the same analytical condition of the sample (Doel and Kratz, 1963; Oliveira et al., 2013; Ramos and Moreira, 2019).

The mass spectra of the constituents were compared with those from libraries (NIST, 98 and WILEY 7n) and with those published in the literature (Adams, 2017).

Antibacterial activity

The standard virulent strain of *M. tuberculosis* H₃₇Rv (ATCC, 25618) was grown in 7H9 (BACTO) culture medium, supplemented with 10% ADC, approximately 3 x 10⁷ Colony Forming Units – CFU/mL and kept in an incubator at 37°C and 5% CO₂ until the beginning of the log phase (exponential growth phase). The CFU dosage for turbidity was standardized and monitored in a spectrophotometer (Hitachi – Model U-1100) at an optical density (O.D.) of 600 nm. Subsequently, in the logarithmic growth phase, 50 μL of this suspension were plated in a 96-well microplate (1x10⁶ CFU/well). The EO samples (50 μL/well) from the different organs were previously diluted in 7H9 supplemented with ADC in a concentration two times higher than the desired final concentration and added to the microplate where the mycobacteria had already been contained. The sealed plate was incubated at 37°C and 5% CO₂ for 5 days. After this time, 10 μL per well of a 5 mg/mL solution of tetrazole 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazole (MTT) in saline phosphate buffer (PBS) was added sterilly. After three hours, 100 μL of lysis buffer was added (20% w/v sodium dodecyl sulfate (SDS)/50% dimethylformamide (DMF) in distilled water – pH 4.7) (Gomez-Flores et al., 1995).

The microplate reading was performed on a spectrophotometer at 570 nm (Hitachi – Model U-1100) (Gomes-Flores et al., 1995). Treatment with rifampicin (0.032; 0.08; 0.2 and 1 μg/mL) was used as a positive control of antimycobacterial activity in the wells containing only the bacilli. Negative control was set in wells containing bacilli and without treatment. To calculate the percentage of inhibition of mycobacterial growth, Equation (1) was used.

\[
100 - \left( \frac{O_{D_{\text{Sample}}}}{O_{D_{C^+}}} \times O_{D_{C^-}} \right)
\]

Statistical analysis

All data on the percentage of compounds in EO were described as mean ± standard deviation for three independent experiments (extraction). The data were expressed by means of variance. A statistical analysis to show differences in the antimicrobial activity of the EO was performed using the ANOVA test. A p value <0.05 was considered statistically significant (Ramos et al., 2020).

For analysis of Pearson, correlations were calculated between the main constituents and the antimicrobial activity. Principal component analyzes (PCA) and hierarchical analysis (HCA) were performed to assess a variance between different organs and respective chemical compositions of the EO (Sadgrove and Jones, 2014; Ramos et al., 2020). Results were processed using Statistica software version 10 (StartSoft Inc., Tulsa, USA).

RESULTS

The chemical composition and yield of the EO from *P. multinodum* for roots, stems, branches, leaves, infructescences, and inflorescences is presented in Table 1. Hydrodistillation of the different vegetative parts of *P. multinodum* afforded an EO with yields ranging from 0.19 to 1.32%. The reproductive organs inflorescences and infructescences showed the highest yields 1.32 and 0.93%, respectively. 33 different compounds were identified in the EO, comprising about 81 to 99%, as well as a great chemical distinction between the vegetative and reproductive parts.

Infructescences and inflorescences showed a rich composition of non-oxygenated monoterpenes α-pinene (67.23 and 40.23%, respectively), β-pinene (8.67 and 6.57%, respectively) and myrcene (9.92 and 13.23%).
Among the non-oxygenated sesquiterpenes, *E-caryophyllene* (7.67%) stood out. Arylpropanoids, such as in the original material, presented relevant percentage content in the infructescences, with a percentage of 27.25% (asaricin - 5.23%; myristicin - 6.23%; dillapiole - 5.78%; apiole - 7.67%). The EO chemical composition from leaves showed to be rich in the non-oxygenated monoterpenes α-pinene (32.49%) and myrcene (14.50%),

### Table 1. Chemical constitution and yield of essential oils from different organs of *Piper multinodium* C.D.C.

| Compounds           | Rlcalc | Rllit | Relative peak area (%)± SD |          |          |          |          |          |
|---------------------|--------|-------|-----------------------------|----------|----------|----------|----------|----------|
|                     |        |       | Roots                       | Main Stems| Branches | Leaves   | Inflorescences| Infructescences|
| α-pinene            | 929    | 932   | 0.16±0.02                   | 20.34±1.05| 32.49±2.32| 67.23±3.87| 40.23±1.33|
| camphene            | 944    | 946   |                             | 0.51±0.03 | 0.20±0.03 |          |          |
| sabinene            | 967    | 969   | 1.31±0.12                   | 2.31±0.06 | 2.4±0.06  | 1.23±0.08| 0.35±0.00 |
| β-pinene            | 975    | 974   | 3.45±0.09                   | 3.49±0.03 | 8.67±0.29 | 6.57±0.72|
| myrcene             | 987    | 988   | 11.23±0.45                  | 14.5±0.02 | 9.92±0.56 | 13.23±1.32|
| α-phelandrene       | 1000   | 1002  | 1.73±0.02                   |          |          |          |          |
| δ-3-carene          | 1007   | 1008  | 4.78±0.05                   |          |          |          |          |
| Zβ-oicimene         | 1030   | 1032  | 7.67±0.08                   | 2.40±0.23 |          |          |          |
| unidentified (MW=204)| 1036  |       | 1.15±0.06                   |          |          |          |          |
| camphene hydrate    | 1143   | 1145  | 0.70±0.02                   |          |          |          |          |
| terpiten-4-ol       | 1176   | 1174  | 1.34±0.07                   | **11.70±0.23**| 1.34±0.02|          |          |
| α-terpineol         | 1188   | 1186  | 2.57±0.12                   |          |          |          |          |
| isobornyl acetate   | 1283   | 1283  | 0.60±0.03                   |          |          |          |          |
| cyclosative         | 1367   | 1369  | 0.43±0.01                   |          |          |          |          |
| α-copaene           | 1372   | 1374  | 1.98±0.24                   |          |          |          |          |
| β-bourbonene        | 1382   | 1384  | 6.72±0.03                   | 0.53±0.02 | 0.56±0.07|
| β-copaene           | 1388   | 1389  | 0.61±0.02                   |          |          |          |          |
| *E*-caryophyllene   | 1416   | 1417  | 5.42±0.02                   | 0.60±0.03| 12.32±0.21| 4.11±0.04| 1.30±0.03| **7.67±0.05**|
| β-copaene           | 1430   | 1430  | 0.23±0.32                   |          |          |          |          |
| aromadendrene       | 1440   | 1439  | 4.32±0.23                   |          |          |          |          |
| α-humulene          | 1449   | 1452  | 1.23±0.00                   | 4.60±0.05|          |          |          |
| *allo*-aromadendrene| 1455   | 1458  | 0.32±0.00                   | 0.35±0.09|          |          |          |
| Z-murola-4,14,5-diene| 1470  | 1465  | 1.52±0.15                   |          |          |          |          |
| germacrene D        | 1480   | 1480  | 2.43±0.03                   |          |          |          |          |
| *E*-methyl-isoeugenol| 1492  | 1491  | 1.25±0.00                   | **77.58±2.21**| 5.23±0.43|          |          |
| asaricin             | 1496   | 1495  | 1.21±0.02                   |          |          |          |          |
| bicyclergmacrene    | 1498   | 1500  | 2.41±0.03                   |          |          |          |          |
| α-murolene          | 1501   | 1500  | 1.03±0.09                   |          |          |          |          |
| Zα-bisabolene       | 1505   | 1506  | 0.17±0.00                   |          |          |          |          |
| δ- amorphenone       | 1509   | 1511  | 1.69±0.04                   |          |          |          |          |
| myristicin           | 1516   | 1518  | 5.48±0.00                   |          |          |          |          |
| eusarone            | 1572   | 1572  | **81.32±1.43**              | 1.15±0.03|          |          |          |
| dillapiole          | 1622   | 1620  | 0.32±0.03                   |          |          |          |          |
| apiole              | 1675   | 1678  | 0.23±0.04                   |          |          |          |          |
| Non-Oxygenated Monoterpenes | 0.16  | 1.31  | 45.00                       | 62.36    | 87.25    | 60.38    |
| Oxygenated Monoterpenes| 1.94  | 14.97 | 1.34                       | 0.00     | 0.00     | 0.00     |
| Non-Oxygenated Sesquiterpenes | 9.29 | 1.63  | 47.36                       | 17.19    | 3.09     | 10.86    |
| Arylpropanoids       | 88.05  | 78.73 | 5.23                       | 0.00     | 2.54     | 27.25    |
| Identified Compounds in Numbers | 12  | 8    | 14                         | 17       | 12       | 12       |
| Total compounds quantified (%) | 99.44 | 96.64 | 98.93                      | 80.70    | 92.88    | 98.49    |
| Oil yielding (%)     | 0.21±0.04 | 0.11±0.03 | 0.19±0.03 | 0.63±0.04 | 1.32±0.04 | 0.93±0.09 |

Rlcalc = Calculated Retention Index (HP-5MS column); Rllit = Literature Retention Index (Adams, 2017); Main constituents in bold. SD = Standard Deviation. *All compounds were identified by MS and RI in accordance with experimental.
with a higher number of identified compounds compared to the other plant parts. Branches showed a richer composition in non-oxygenated sesquiterpenes (47.36%), with α-copaene (13.24%) and E-caryophyllene (12.32%) being predominant in this fraction. However, the main compound was identified as non-oxygenated monoterpene α-pinene (20.34%). Differently, the stems and roots showed a relatively low percentage of monoterpenes and sesquiterpenes, with an EO composition rich in arylpropanoids (E-methyl-isoeugenol -77.58% in the stems; euasarone - 81.34% in the roots). The compound E-methyl-isoeugenol was recorded in a relatively low percentage (< 2%) in the roots.

Principal component analysis (PCA) and hierarchical cluster analysis (HCA) for the different organs of P. multinodum are shown in Figures 1 and 2, respectively. The PCA showed a total variance of 76.82% (Figure 1). The first component (58.46%) was responsible for separating the chemical composition of stems and roots from the other organs with positive charges. The second component (18.36%) was responsible for separating the branches and stems from the other organs that also had positive charges. The non-oxygenated monoterpene α-pinene (-6.26) was responsible for the separation in PC1 by the high percentage content found in the inflorescences, infructescences, leaves and branches. In PC2, the positive charge of E-methyl-isoeugenol (+2.69) led to the separation of branches and stems. Despite the variations in E-methyl-isoeugenol contents, HCA analysis (Figure 2) clearly demonstrated the separation of three groups, from top to bottom: I - Rich in euasarone; II - Rich in α-pinene; III - Rich in E-methyl-isoeugenol. The separation in higher Euclidean distance of the stems is mainly due to the high percentage contents of the oxygenated monoterpene terpinen-4-ol.

Evaluation of the anti-M. tuberculosis activity of the EO from the different organs (inflorescences, infructescences, branches, stems, roots, and leaves) recorded minimum inhibitory concentration (MIC) values that are presented in Table 2 and Figure 3. The highest activities were registered for the EO obtained from roots (78.51 µg/mL) and infructescences (85.91 µg/mL).
Figure 2. Dendrogram representing the similarity relationship of the composition of essential oils from different organs of *Piper multinodum*.

| Essential oil   | MIC<sub>50</sub> (µg/mL) |
|----------------|--------------------------|
| Roots          | 78.51 ± 1.15             |
| Main Steams    | 115.00 ± 1.07            |
| Branches       | >128                     |
| Leaves         | >128                     |
| Inflorescences | >128                     |
| Infructescences| 85.91 ± 1.01             |
| Rifampicin     | 0.06 ± 1.01              |

The relative percentage of the major compounds and chemical classes were correlated with the MICs. The results of Pearson's correlations are presented in Table 3. It was possible to ascertain that euasarone (-0.713) and arylpropanoids (-0.667) showed inversely proportional and significant correlations with antibacterial activity (p < 0.05).

**DISCUSSION**

The Piperaceae family is recognized for having species with high yields of EO (Santos et al., 2001; Oliveira et al., 2013; Filly et al., 2014; Ramos et al., 2020; Kemprai et al., 2020). The contents found in the different organs of *P. multinodum* is quite adequate when compared to the...
Figure 3. Growth inhibition of *Mycobacterium tuberculosis* H₃₇Rv after treatment with *Piper multinodum* essential oils. Legend. Results of MTT assay after 5 days of incubation in the presence of samples at concentrations of 16, 32, 64, and 128 µg/mL. Positive control = *M. tuberculosis* H₃₇Rv treated with rifampicin (reference drug); negative control = *M. tuberculosis* H₃₇Rv without treatment. Statistical analysis: One-way ANOVA followed by the Tukey test *** \( p < 0.001 \), ** \( p < 0.01 \) and * \( p < 0.05 \) compared to the negative control (Mtb H₃₇Rv 1x10⁶ CFU / mL). Triplicate results represented as mean ± standard error.

Table 3. Pearson’s correlation analysis between the major compounds and chemical classes of the essential oils of *P. multinodum*.

| Compound                | Analyzed variable               | MIC₅₀ (µg/mL) | Pearson’s correlation (r) |
|-------------------------|---------------------------------|---------------|--------------------------|
|                         |                                 |               |                          |
|                         | euasarone                       | -0.713*       |                          |
|                         | E-methyl-isoeugenol             | 0.112         |                          |
| Majority compounds      |                                 |               |                          |
|                         | α-pinene                        | 0.375         |                          |
|                         | mircene                         | 0.400         |                          |
|                         | terpinen-4-ol                   | 0.062         |                          |
|                         | Non-Oxygenated Monoterpenes     | 0.477         |                          |
|                         | Oxygenated Monoterpenes         | 0.041         |                          |
|                         | Non-Oxygenated Sesquiterpenes   | 0.325         |                          |
|                         | Arylpropanoids                  | -0.667*       |                          |

* \( p < 0.05 \).

data in the Brazilian Pharmacopoeia, with commercial EO-producing species, such as, for example, inflorescences of *Matricaria chamomilla* L. (chamomile, minimum 0.4%), fruits of *Coriandrum sativum* L (coriander, minimum 0.3%), leaves of *Cymbopogon citratus* (DC.) Stapf (lemon grass, minimum 0.5%) and parts of *Mentha arvensis* L. (peppermint, minimum of 0.8%) (National Health Surveillance Agency, 2019). The species *P. multinodum* showed higher yields in the inflorescences. As previously mentioned, the organ development stages and the different vegetative parts may be a determinant for alteration of the EO yields (Figueiredo et al., 2008). The higher EO contents in reproductive organs may be linked to their ecological functions, mainly in attracting pollinators by increasing osmophores, which provide fragrance to flowers; otherwise in repelling insects by insecticidal and deterrent action, reducing herbivory (Basilio et al., 2015). A study published by the group found that *P. mollicomum* recorded higher EO yields in the inflorescences (1.80%).
than in the different vegetative parts (Ramos et al., 2020). However, *P. betleoides* C. DC. showed higher leaf EO yields (0.35%) than in the male (0.26%) and female (0.31%) inflorescences (Kemprai et al., 2020).

EO from different organs of *P. multinodum* showed different qualitative and quantitative chemical compositions. Different gene expression profiles in different organs are expected, thus, enzyme production may be privileged in a more expressive way in some tissues compared to others (Hajdari et al., 2016). Furthermore, each plant structure has different lignification characteristics (morphological) and ecological functions (attraction of pollinators, defense against pathogens and herbivory) (Abbas et al., 2017). Profiles leading to the distinction of biosynthetic pathways in different organs have been observed for *P. micromum* (Ramos et al., 2020), however, we have described this same pattern for *P. multinodum* for the first time. These findings are important for understanding ecological and chemotaxonomy approaches in the genus *Piper*.

In the aerial organs (branches, leaves, inflorescences and infructescences) of *P. multinodum*, the predominant identified compound was α-pinene. In the *Piper* genus, leaf EO have been obtained with high percentage contents of this compound, for example, *P. aequale* Vahl (39.3%) (Setzer et al., 2008); *P. amalago* L. (30.5%) (Potzernheim et al., 2006); *P. anonifolium* Kunth (53.1%) (Andrade et al., 2005); *P. lucaeanum* var. *grandifolium* Yunck (30.0%) (Marques et al., 2015); and *P. rivinoides* Kunth (73.2%) (Perigo et al., 2016).

The arylpropanoid E-methyl isoeugenol was identified in the main stems and in lower relative percentage in the infructescences of *P. multinodum*. This compound was also found in the EO from leaves of *P. marginatum* Jacq. (27.1%) (Santana et al., 2015). Still, the configurational isomer of E-methyl isoeugenol was identified in high levels in the EO of *P. divaricatum* G. Mey. (63.8 - 93.3%) (Silva et al., 2017). Ecological studies have systematically shown the role of E-methyl isoeugenol in attracting fruit flies, mainly *Bactrocera* (Macquart, 1835) and in defending against herbivores (Tan and Nishida, 2012; Royer et al., 2018). We emphasize that the main pollinators in Piperaceae are species of the order Diptera (Figueiredo and Sazima, 2000). Another arylpropanoid identified mainly in the EO from roots was euasarone (γ-asarone/isoasarone). Low contents of this compound are described for the *Piper*, for example, in the *P. sarmentosum* Roxb. species, widely used in Asia for the treatment of cough, cold, fever, rheumatism, arthralgia, diarrhea, dysentery, postpartum swelling in the feet, stomach pain, toothache, diabetes, and traumatic injuries (Qin et al., 2010; Sun et al., 2020). It is worth noting that this compound is widely found in high contents in the roots of medicinal species of basal angiosperms, such as *Asian Acorus calamus* L. (25.4%) (Vetelainen et al., 2008) and in the stems of *Aniba hostmanniana* (Nees) Mez (98.6%) (Lauraceae) from tropical America (Gottlieb and Rocha, 1972). In addition, α- and β-asarone isomers are widely studied due to their sedative, hypolipidemic, immuno-suppressive, anti-inflammatory, antioxidant, diuretic, insecticidal, antifungal, anticancer, antispasmodic, and anticonvulsant effects (Chellian et al., 2017; Bai et al., 2020). Comparative studies among the isomers by Berg et al. (2016) demonstrated for the first time that γ-asarone is not mutagenic, in contrast to a wide variety of structurally similar analogues that also occur in foods and herbal medicines. However, studies demonstrate a lack of knowledge about its efficacy and safety in humans. In contrast, the propenyl α- and β-asarone isomers were mutagenic in the presence of a metabolically active liver homogenate (Berg et al., 2020).

The use of drugs for the treatment of tuberculosis has been the same for decades. The number of bacteria resistances tends to increase significantly and the search for new and interesting prototypes from natural products has been in the spotlight. The results found for *P. multinodum* are extremely relevant, with MIC values below 100 µg/mL. It is reported that MIC < 100 µg/mL are extremely desired for extract, fraction or isolated compound as possible candidates against *M. tuberculosis*, while values between 100 and 200 µg/mL are considered moderate candidates (Bernuci et al., 2016). Our results for *P. multinodum* showed that the presence of the arylpropanoid eusarone was significant in increasing the antimycobacterial activity as demonstrated by Pearson’s correlation. In a study about the *M. tuberculosis* strain H37Rv, a mixture of Taiwanese arylpropanoids A and B isolated from *P. tainwense* Lin & Lu showed MIC of 30.0 µg/mL (Chen et al., 2014). Besides, samples rich in non-oxygenated sesquiterpenes from *P. cernuum* Vell., *P. diospyrifolium* Kunth, *P. mosenii* C. DC. and *P. rivinoides* Kunth with *Ecaryophyline* contents ranging from 9 - 10% showed MIC values between 125 and 250 µg/mL (Bernuci et al., 2016). This study is the first time this biological activity has described for eusarone (> 80%) at high contents. Looking at the data sets, it is possible to formulate some hypotheses to explain the results:

a) Methoxylation on aromatic rings is very important for antimycobacterial activity. This hypothesis is sustained from the result obtained from the mixture with majority E-methyl-isoeugenol that was reduced when compared to the EO samples obtained from the roots and inflorescences.
b) The presence of methylenedioxy groups on aromatic rings may be important for enhancing the antimycobacterial activity. The contents (~24%) of arylpropanoids with methylenedioxy group in the EO demonstrated activity at 85.9 µg/mL. This indicates that higher contents of compounds with a substitution pattern with methylenedioxy groups could significantly improve the MIC.

Andrade-Ochoa et al. (2015) studied the quantitative structure-activity relationship (QSAR) with twenty-five...
pure commercial EO. This study concluded that octanol-water partition ratio (LogP) and phenolic group (nArOH) as the main properties and structural elements contributing to antimycobacterial activity. The most active arylpropanes with MIC values of 3.12 and 8.16 μg/mL in this study were cinnamaldehyde and cinnamic acid.

**Conclusion**

Investigation of the chemical variability of EO from *P. multinodum* performed for the first time in this study revealed a diverse chemical composition among the different organs of this plant. An important antimycobacterial activity recorded for EO from roots and infructescences may be a starting point for future studies. The distinction of biosynthetic pathways observed for the first time for *P. multinodum* is also an important finding for the understanding of chemotaxonomic knowledge in the genus *Piper*, which can also help in the understanding of ecological and chemophenetics issues related to the Piperaceae species.

**CONFLICT OF INTERESTS**

The authors have not declared any conflict of interests.

**ACKNOWLEDGEMENTS**

The authors thank CAPES, FAPERJ, PROEPCNPq (407845/2017-8) for their financial support.

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