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Piper rivinoides Kunth: A medicinal plant that preserves bioactive chemical substances in its essential oil throughout the seasons

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The chemical composition and seasonal variation of essential oils (EO) extracted from the aerial parts of the traditional medicinal plant *Piper rivinoides* were analyzed using gas chromatography (GC) coupled with mass spectrometry (MS) and GC coupled with flame ionization detector (GC-FID) technique, respectively. The wild plants were collected from two different sites in the Atlantic Forest. The analysis allowed us to identify 96.60 to 99.80% of the EO composition. The major compounds with the highest relative percentage for both specimens, regardless of the season, were the bioactive monoterpenes α-pinene and β-pinene, which ranged from 34.78 (summer) to 53.87% (winter) and from 15.24% (autumn) to 47.71% (winter), respectively. The seasonal stability of the major compounds in the two study specimens throughout the year indicates that the phenological cycle influences biosynthesis more than abiotic factors. This type of chemical phenotypic stability is rarely observed in species belonging to the Piperaceae family, which is characterized by high chemical variability. Furthermore, this stability is favorable, and *P. rivinoides* has the potential to be a source of bioactive compounds.

Key words: α-pinene, β-pinene, Atlantic Forest, aromatic plant, volatile compounds.

INTRODUCTION

Piper rivinoides Kunth is a native species of Brazil used for medicinal, ritual, and aromatic purposes. It is popularly known as "Betis-Branco", "Aperta-ruão", "Murta" or "Ruão". *P. rivinoides* is widely distributed throughout the Brazilian territory, and is described as a glabrous and ciophilous shrub that grows up to 3-6 m tall, has a well-woody trunk, shiny ovate leaves with a symmetrical base, showy and triangular inflorescences, and fruit clusters of the erect spike type(Yuncker, 1972; Flora do Brasil, 2020; Queiroz and Guimarães, 2020).

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P. rivinoides has the liturgical name *"Èwé Boyí Funfun*" according to ethnobotanical surveys in Brazil, and its use in cleansing baths in religious rituals originated in Africa. *P. rivinoides* has been used medicinally to treat wounds, ulcers, vaginal discharge, bleeding, and even oral problems. Furthermore, this plant is sold in folk markets and collected from wild specimens (Barros, 2010; Mandarino and Gomberg, 2009; Albuquerque et al., 2007).

Previous research with the extracts of this species revealed that the hexane fraction contains a high concentration of bioactive benzofuran neolignans (eupomatenoids and conocarpan) and pharmacological potential against *Candida albicans* and *Leishmania amazonensis* (Moreira et al., 2016; Marques et al., 2014).

Literature data on EO, obtained by hydrodistillation from fresh leaves of *P. rivinoides*, have shown a great chemical diversity rich in monoterpenes, sesquiterpenes, and arylpropanoids, which may vary depending on the collection site (Bernuci et al., 2016; Perigo et al., 2016; Leal et al., 2019). Aspects of the pharmacological potential of these EO indicate their antinociceptive (Souza et al., 2014), antifungal (Bernuci et al., 2016; Leal et al., 2019), antimicrobial (Leal et al., 2019), and cytotoxic (Fonseca et al., 2020; Machado et al., 2021) activity.

Recently, the apoptotic effect of β-pinene (main component) and EO of *P. rivinoides* in oral squamous cell carcinoma (Machado et al., 2021) was described. This study confirmed the plant's long history of traditional medicinal use in the treatment of oral problems (Barros, 2010). Essential oil research is of interest in the production of herbal raw materials due to their functions as medicines, cosmetics, and other products. Because of the low cost of extraction, processing is advantageous in low-income areas and allows for large-scale production with low operating costs, which would become profitable through incentive programs and public project funding (Araújo et al., 2021).

Despite the fact that *P. rivinoides* is widely used in traditional medicine, research on the seasonal variation profile of EO from this plant is limited. It is well known that biotic and abiotic factors influence the large chemical phenotypic plasticity of Piperaceae species, and that these variations in the composition of plant chemistry can lead to changes in their respective biological potential (Monzote et al., 2010; Salehi et al., 2019).

Based on the foregoing, this study aims to evaluate the effect of seasonality on the chemical profile of EO of wild specimens of the medicinal plant *P. rivinoides* collected from two different sites in fragments of the Atlantic Forest with varying altitudes and climatic patterns. Determining the chemical nature of EO of *P. rivinoides* in different months over a year and at two different collection sites can help us understand the chemotaxonomics and chemophenetics of the plant and the genus *Piper*. Furthermore, this study provides tools for determining the best time to collect plant material that can provide an EO with high-quality secondary metabolites and, consequently, biological activities.

MATERIALS AND METHODS

Plant

Samples of fresh leaves of wild *P. rivinoides* were collected from two specimens in different regions of the Atlantic Forest in Rio de Janeiro State: (a) Tijuca National Park, Rio de Janeiro/RJ (PRR) (S 22° 57' 09. 94"; E 43° 01 '17. 19", elevation 205 m (permit SISBIO n. 57296-1) and (b) Serra da Tiririca State Park, Niterói/RJ (PRN) (S 22° 97' 18.24"; E 43° 24' 23.78", elevation 28 m (INEA permit n° 07/ 0002.4729/ 2019). Dr. Elsie Franklin Guimares performed the botanical identification of the plant material at the Research Institute of the Botanical Garden of Rio de Janeiro, and the herborized specimens were deposited in the Herbarium of the College of the State of Rio de Janeiro (HRJ) under voucher numbers 13404 for PRR and 13403 for PRN. In each season of 2019, one specimen was collected twice: summer (January and February), autumn (April and May), winter (July and August), and spring (October and November), always in the morning between 9:00 and 10:00 am. Data on abiotic factors, including average temperature (°C), humidity (%), and precipitation (mm), were obtained from the Brazilian Institute of Metrology and Research (INMET) and are presented in Figure 1.

Extraction of essential oil

For 2 h, approximately 100 g of fresh leaves were hydrodistilated in a modified Clevenger apparatus (Oliveira et al., 2014). The EO was separated from the aqueous phase and dried with anhydrous sodium sulfate. The total yield was expressed as a percentage, considering the weight of EO (g) per 100 g of fresh plant material. The EO was stored in sealed amber vials under refrigeration at -20°C until analysis (Oliveira et al., 2014).

Essential oil analysis

Chemical characterization and quantification of the EO of *P. rivinoides* was performed by gas chromatography (GC) coupled with mass spectrometry (MS) and GC coupled with flame ionization detector (GC-FID). GC-FID analyses were performed in triplicate to increase accuracy. The EO was diluted in dichloromethane (1 mg/mL) [HPLC grade, Tedia, Brazil] before analysis.

GC-MS and GC-FID conditions are as follows: GC-MS analyses were performed using a gas chromatograph HP Agilent GC 6890 coupled to a mass spectrometer Agilent MS 5973N, with an ionization energy of 70 eV (positive mode). EO solution was injected at 1 µL (splitless), with an injector temperature of 270°C and equipped with HP-5MS (30 m × 0.25 mm i.d. × 0.25 µm film thickness) capillary column [Agilent J & W; GC columns (USA)], temperature programming from 60 to 240°C, with an increase of 3°C/min (60 min total run), using helium (~99.99%) as carrier gas at a constant flow rate of 1.0 mL/min and mass range m/z 40-600 atomic mass units (u). GC-FID analyses were performed using a gas chromatograph HP-Agilent 6890 GC-FID. EO solution was injected at 1 µL (splitless) with an injector temperature of 270°C, equipped with HP -5MS (30 m × 0.25 mm i.d. × 0.25 µm film thickness) capillary column [Agilent J & W; GC Columns (USA)], temperature programming from 60 to 240°C, with an increase of 3°C/min (60 min total run), using hydrogen as carrier gas at a constant flow rate of 1.0 mL/min (Oliveira et al., 2014).

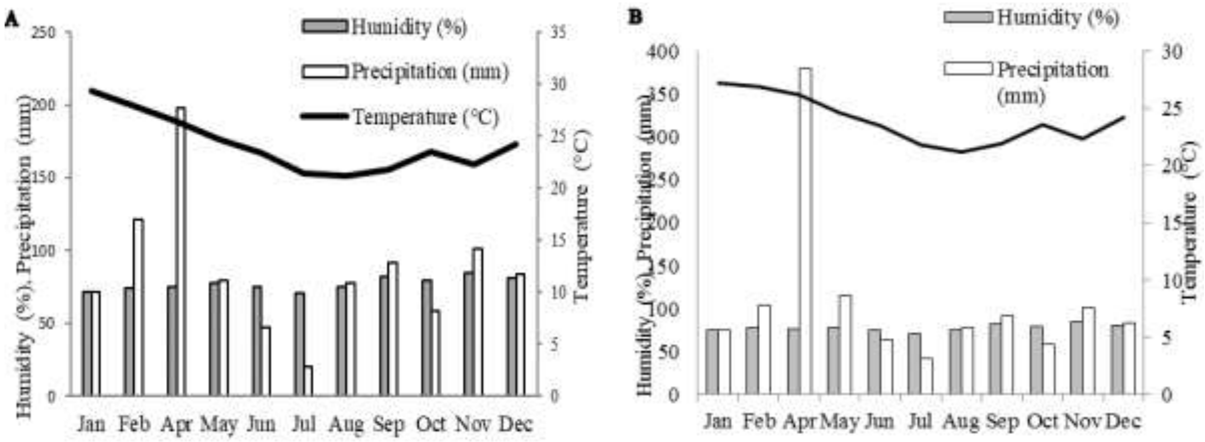

Figure 1. Climatic data of mean temperature, precipitation, and relative humidity (A) Serra da Tirirca-Niterói/RJ e (B) Rainforest of Tijuca/RJ.
Source: INMET (National Institute of Meteorology)

Compounds identification and statistical analysis

Retention indices (RI) were calculated from the retention time of a homologous series of saturated aliphatic hydrocarbons (C_8-C_{28}, Sigma-Aldrich, Brazil) obtained with CG-FID under the same analytical conditions as EO (Dool and Kratz, 1963). Compounds in the EO were identified by comparing their mass spectra with database records (WILEY 7n, NIST) and by comparing the calculated RIs with those from the literature (Adams, 2017). Furthermore, co-injection with authentic standards was performed wherever possible, as described previously. All data on the percentage of compounds in the EO were reported as mean ± standard deviation for three independent experiments (extraction).

Principal component analysis (PCA) and hierarchical analysis (HCA) graphs were constructed for chemometric analysis, in which the chemical composition of EO was treated as operational taxonomic units. For this purpose, the percentage values (% area) were extracted from the CG data, transformed into a sinusoidal arc of the root of *p*, and converted into a matrix, excluding unidentified compounds (Catinella et al., 2021). The Euclidean distance was combined with the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) method to generate the dendrogram. Multiple comparisons between the main components of EO and abiotic factors (temperature, humidity, precipitation) were performed using Pearson correlation ($p < 0.05$). All results were processed using STATISTICA version 10 software (StartSoft Inc., Tulsa, USA).

RESULTS AND DISCUSSION

Essential oil yield

A total of 16 colorless samples were obtained from EO. These results are presented in Table 1. The yields found in this study for wild *P. rivinoides* ranged from 0.16 to 1.36 (w/w) (Table 1), higher than others previously reported in the literature (0.05 (Souza et al., 2014) and 0.63 (Perigo et al., 2016)). Monthly yields varied similarly at both collection sites, with no site dominating ($p > 0.05$) (Figure 2).

There was a considerable increase in the EO yield in the spring, which coincides with the reproductive phase of the plant (flowering). A tendency to increase yield was also noticed as the temperature decreased. So, a lower quantity of EO was obtained in the hottest months of the year (summer). Similar results were found for other Piperaceae species such as *Piper mosenii* C. DC and *Piper gaudichaudianum* Kunth (Del Quiqui et al., 2019). Other reports in the literature also corroborate these findings (El Beyrouthy et al., 2015; Marques et al., 2014).

Pearson's analysis did not show correlations between EO yield and precipitation and temperature. However, there was a moderate correlation between EO yield and humidity for both locations (r = 0.5954 PRN and r = 0.6588 PRR), but significant only for the results of PRN ($p < 0.05$). According to the literature, in humid environments where water is not a limiting factor, as in rainforests, there is an increase in the rate of plant transpiration, which may explain this correlation. When the rate of metabolism increases the rate of volatilization decreases, then the EO yield is directly affected (Queiroz et al., 2017). AHC of bioclimatic data suggested that general volatile differentiation in *P. rivinoides* from Rio de Janeiro essentially does not represent an adaptive response to bioclimatic, or geological conditions. Therefore, there was no clear pattern of correlation between climate and EO yield for *P. rivinoides*, suggesting the biggest connection to the vegetative cycle than climatic variations, a fact previously described for other plants (El Beyrouthy et al., 2015; Shadi and Saharkhiz, 2016).

In the spring, which coincides with the reproductive phase of the plant (flowering), there was a significant increase in EO yield. A tendency to increase yield was also observed when temperatures decreased. Thus, a lower amount of EO was obtained during the hottest months of the year (summer). Similar results were found for other Piperaceae species such as *P. mosenii* C. DC

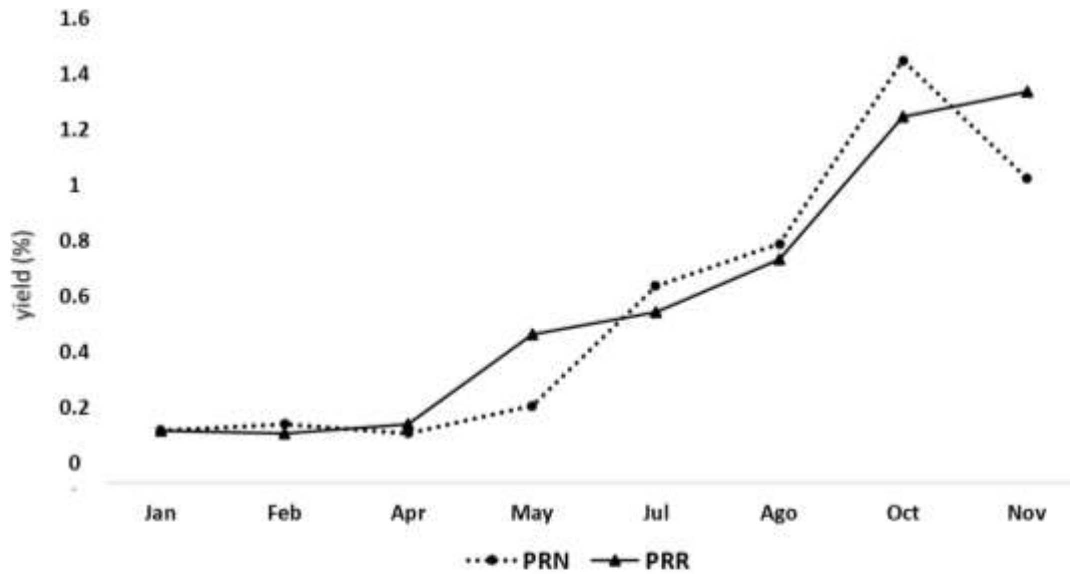

Figure 2. Variation rate of the EO yield of wild *Piper rivinoides* from the state of Rio de Janeiro.
Source: Authors 2022

and *P. gaudichaudianum* Kunth (Del Quiqui et al., 2019). Other reports in the literature also confirm these results (El Beyrouthy et al., 2015; Marques et al., 2014).

Pearson analysis showed no correlations between EO yield and rainfall and temperature. However, there was a moderate correlation between EO yield and moisture for both sites (r = 0.5954 PRN and r = 0.6588 PRR), but it was significant only for the results of PRN ($p < 0.05$). The literature states that in humid environments where water is not a limiting factor, such as rainforests, plant transpiration rate increases, which could explain this correlation. When the metabolic rate increases, the rate of volatilization decreases, affecting the EO yield directly (Queiroz et al., 2017). The AHC of bioclimatic data suggests that the overall differentiation in volatility in *P. rivinoides* from Rio de Janeiro is essentially not an adaptive response to bioclimatic or geological conditions. Therefore, there was no clear pattern of correlation between climate and EO yield in *P. rivinoides*, suggesting that the greatest link is to the vegetative and reproductive cycles than to climatic variation, a fact previously described for other plants (El Beyrouthy et al., 2015; Shadi and Saharkhiz, 2016).

Chemical profile of the essential oils

The relative percentage of compounds is shown in Table 1. Forty-one compounds were identified in summer, 37 in fall, 24 in winter, and 21 in spring, representing 98.74, 97.56, 97.58, and 98.56%, respectively, of the total compounds identified.

Both EO had a rich fraction of cyclic non-oxygenated monoterpenes and a minority fraction of sesquiterpenes. However, some compounds were not detected in all individuals, with slight qualitative (richness) and quantitative (content) differences. The compounds with the highest relative content for both specimens, regardless of season, were α-pinene and β-pinene, ranging from 34.78 to 53.87% and from 15.24 to 47.71%, respectively. These results are interesting from a pharmacological point of view, since the main difficulty in the preparation of drugs from natural products is the relative complexity of obtaining bioactive compounds in high concentrations and with chemical stability (Araújo et al., 2021; WHO, 2011).

A large relative percentage of non-oxygenated cyclic terpenes (bicyclogermacrene and limonene) was also found in all seasons. The largest relative percentage of α-pinene and β-pinene is like other results obtained in species of the genus Piper such as Piper anonifolium Kunth (45.0% α-pinene and 18.0% 32.2 β-pinene), Piper bredemeyeri Jacq. (20.3% α-pinene and β-pinene), Piper ovatum Vahl. (23.1% α-pinene and 14.2% β-pinene) (Da Silva et al., 2017), Piper solsianum C. DC (22.7% α-pinene and 3.5% β-pinene), and Piper amplum Kunth (18.1% α-pinene and 0.9% β-pinene) (Perigo et al., 2016). These monoterpenes are also described for other species of the Piperaceae family, such as Piper hainanense Hemsl. (7.14% α-pinene and 0.14% β-pinene), Piper pseudofuligineum C. DC. (6.73% α-pinene); Piper laetispicum C. DC. (6.09% α-pinene and 0.1% β-pinene) (Hao et. al., 2018); and Piper albispicum C. DC (14.6% α-pinene and 15.6% β-pinene (Nguyen et al., 2022). However, the percentages are relatively in the minority for these species.

Table 1. Chemical constitution and data on leaf essential oils from the wild *Piper rivinoides* Kunth growing in Rainforest of Tijuca (PRR) and Serra da Tiririca-Niterói (PRN).

Compound	RI Lit	RI cal	Relative Percentage (% ± SD)															
			January		February		April		May		July		August		October		November	
			PRN	PRR	PRN	PRR	PRN	PRR	PRN	PRR	PRN	PRR	PRN	PRR	PRN	PRR	PRN	PRR
No-oxygenated Hydrocarbon			***89.03***	***84.7***	***82.19***	***81.28***	***83.82***	***81.26***	***86.06***	***83.08***	***92.28***	***93.08***	***90.34***	***93.27***	***83.15***	***84.9***	***79.66***	***83.09***
□-Thujene	924	926	-	-	0.06±0.03	0.05±0.01	0.9±0.06	0.05±0.02	0.35±0.12	-	0.08±0.04	0.03±0.02	0.07±0.02	0.12±0.05	-	0.24±0.10	-	-
□-Pinene	932	933	34.78±0.34	39.74±0.16	49.53±0.26	37.35±0.13	49.37±0.38	37.33±0.22	49.52±0.12	35.57±0.02	51.88±0.19	38.70±0.22	53.87±0.11	38.06±0.01	51.64±0.21	35.86±0.21	46.32±0.21	36.93±0.35
Camphene	946	945	0.86±0.02	1.01±0.01	1.16±0.02	0.35±0.30	1.54±0.02	1.03±0.05	2.11±0.08	1.27 0.03	1.44±0.02	1.01±0.04	1.28±0.20	1.03±0.03	1.09±0.02	0.70±0.01	1.12±0.11	0.64±0.06
Sabinene	969	970	-	0.17±0.01	0.48±0.10	-	0.15±0.22	0.18±0.13	0.18±0.03	0.32 0.03	0.18±0.06	0.92±0.06		0.15±0.13	0.35±0.03	0.21±11	0.90±0.10	0.08±0.01
□-Pinene	974	975	44.30±0.22	35.61±0.32	16.2±0.04	38.23±0.03	15.26±0.05	37.34±0.22	16.30±0.33	39.69±0.24	18.13±0.14	44.39±0.14	18.07±0.04	47.71±0.14	15.24±0.08	41.20±0.27	17.21±0.17	38.58±0.11
Myrcene	988	990	1.84±0.06	1.78±0.08	2.79±0.12	2.06±0.02	2.84±0.01	1.93±0.03	2.40±0.43	2.05±0.11	3.10±0.02	2.78±0.02	2.97±0.02	2.35±0.12	2.73±0.04	2.46±0.06	2.37± 0.37	2.40±0.18
□- Phellandrene	1002	1002	-	-	0.05±0.01	0.18±0.02	-	-	0.52±0.02	0.31±0.02	0.09±0.01	0.17±0.01	0.35±0.01	-	0.41±0.21	-	0.25±0.02	0.21±0.02
δ-3-Carene	1008	1010	-	-	-	0.11±0.15	0.42±0.01	0.25±0.02	-	-	0.40±0,01	-	-	-	0.55±0.04	-	-	-
□-Terpinene	1014	1015	-	-	-	0.10±0.01	0.17±0.01	-	-	0.14±0.01	0.10±0.01	0.18±0.01	-	-	-	-	-	0.10±0.01
ρ-Cymene	1020	1022	-	-	-	0.13±0.05	0.18±0.02	-	0.38±0.08	-	0.33±0.02	0.28±0.02	0.10±0.01	-		-	0.09±0.01	0.23±0.24
Limonene	1024	1024	7.08±0.01	9.09±0.04	11.81±0.01	2.32±0.01	12.49±0.01	2.88±0.06	14.05±0.21	3.33±0.08	13.58±0.04	3.07±0.07	13.36±0.04	3.22±0.05	11.14±0.23	3.52±0.04	11.40±0.06	2.51±0.01
□-Phellandrene	1025	1026	-	-	-	-	-	-	-	-	0.21±0.01	0.24±0.01	-	-	-	-	-	0.93±0.01
□□□-Ocimene	1032	1029	0.17±0.00	-	0.03±0.01	0.05±0.02	-	0.25±0.19	-	-	-	-	0.14±0.01	0.63±0.02	-	0.47±0.01	-	-
□-Terpinene	1054	1059	-	-	-	-	0.23±0.03	0.02±0.01	0.06±0.00	0.28±0.02	0.25±0.02	0.21±0.02	0.13±0.02	-	-	0.33±0.02	-	0.48±0.01
Terpinolene	1086	1088	-	-	0.08±0.01	0.35±0.05	0.21±0.02	-	0.19±0.07	0.12±0.01	0.14±0.02	0.30±0.02	-	-	-	-	-	-
Oxygenated Monoterpenes			***0.00***	***0.00***	***1.52***	***1.61***	***1.65***	***1.28***	***1.53***	***1.75***	***1.54***	***1.54***	**1.04**	**1.05**	***1.12***	***0.00***	***1.99***	***2.55***
Linalool	1095	1096	-	-	1.08±0.04	0.93±0.05	0.96±0.02	0.97±0.02	1.18±0.03	1.11±0.09	1.22±0.12	1.14±0.12	1.04±0.01	1.05±0.01	1.12	-	1.90±0.08	2.55±0.07
Borneol	1165	1170	-	-	0.08±0.01	-	0.11±0.01	-	-	-	0.13±0.03	-	-	-	-	-	-	-
Terpinen-4-ol	1174	1172	-	-	0.10±0.01	0.32±0.15	0.13±0.01	-	0.10±0.02	0.24±0.04	-	-	-	-	-	-		-
□□erpineol	1186	1188	-	-	0.08±0.01	0.22±0.09	0.24±0.01	0.17±0.02	0.14±0.01	0.24±0.03	-	0.25±0.01	-	-	-	-	0.09±0.01	-
E-Anethole	1282	1280	-	-	0.10±0.01	0.14±0.25	0.13±0.01	0.14±0.02	0.11±0.02	0.16±0.02	0.11±0.01	0.15±0.01	-	-	-	-	-	-
E-Sabinyl acetate	1289	1296	-	-	0.08±0.01		0.08±0.01	-		-	-	-	-	-	-	-	-	-
Hydrocarbon Sesquiterpenes			***8.54***	***9.99***	***9.45***	***11.35***	***8.45***	***13.06***	***6.88***	***10.57***	***2.83***	***2.83***	***4.17***	***3.8***	***10.09***	***8.36***	***11.5***	***11.32***
□-Elemene	1335	1335	-	0.98±0.34	1.50±0.08	2.41±0.18	1.31±0.02	2.20±0.02	1.85±0.09	1.49±0.26	0.23±0.01	0.20±0.01	0.97±0.02	-	3.53±0.04	2.85±0.09	1.69±0.31	0.39±0.44
Longipinene	1400	1401	-	-	0.08±0.01	0.03±0.01	-	-	-	-	-	-	-	-	-	-	-	-
□-Gurjunene	1409	1416	-	-	0.23±0.01	0.41±0.02	0.17±0.01	-	-	0.05±0.01	0.13±0.02	-	-	-	-	-	0.72±0.04	-
E-Caryophyllene	1417	1420	1.70±0.02	0.99±0.07	1.18±0.01	2.03±0.02	1.09±0.02	2.89±0.01	1.13± 0.06	2.87±0.24	0.27±0.02	0.82±0.02	0.49±0.04	0.81±0.10	1.02±0.01	2.40±0.05	2.67±0.17	4.28±0.16
□-Cedrene	1419	1023	-	-	0.06±0.05	0.08±0.01	0.11±0.01	0.12±0.01	-	-	-	-	-	-	-	-	-	-
□-Duprezianene	1421	1426	-	-	0.28±0.36	0.11±0.09	-	0.06±0.01	-	-	-	-	-	-	-	-	-	-
Aromadendrene	1439	1440	2.04±0.02	2.22±0.01	1.48±0.10	1.62±0.04	1.48±0.01	2.38±0.05	1.06±0.05	2.33±0.12	0.18±0.03	0.53±0.03	1.26±0.03	2.02±0.04	1.12±0.11	0.86±0.03	1.29±0.06	2.84±0.19
□-Barbatene	1440	1440	-	-	0.19±0.01	-	-	0.13± 0.02	-	0.15±0.02	-	-	-	-	-	-	-	-
□-Humulene	1452	1455	-	-	0.35±0.47	0.16 ±0.05	0.08±0.01	0.36±0.47	-	0.20±0.26	-	-	-	-	-	-	-	-
allo-Aromadendrene	1458	1460	-	0. 59 ±0.03	0.11±0.02	0.02 ±0.05	0.15±0.01	-	0.07±0.02	0.27±0.06	1.25±0.01	0.32±0.01	0.37±0.06	-	0.60±0.06	0.43±0.32	0.99±0.02	0.63±0.03
□□-Gurjunene	1475	1485	-	-	-	0.08±0.01	-	0.10±0.01	-	-	-	-	-	-	-	-	-	-
□ -Muurolene	1478	1486	-	-	0.32±0.41	0.06±0.01	0.16±0.21	0.08±0.01	-	-	-	-	-	-	-	-	-	-
□-Selinene	1492	1495	0.31±0.01		0.03±0.01	-		-	-	-	-	-	-	-	-	-	-	-
Bicyclogermacrene	1500	1506	4.49±0.01	5.21±0.04	3.08±0.08	3.06±0.03	3.00±0.01	3.82±0.02	2.50±0.10	2.82±0.15	0.95±0.05	0.96±0.05	1.08±0.08	0.97±0.01	3.82±0.03	2.60±0.16	2.33±0.05	2.96±0.02
□-Bisabolene	1505	1508	-	-	0.23±0.06	0.42±0.02	0.27±0.02	0.15±0.01		0.08±0.01	0.20±0.03	-	-	-	-	-	-	0.13±0.01
□-Amorphene	1511	1514	-	-	0.02±0.00	0.71±0.01	0.30±0.01	-	0.16±0.01	0.20±0.01	-	-	-	-	-	-	-	-
□-Curcumene	1514	1419	-	-	-	-	-	-	026±0.03	-	-	-	-	-	-	-	-	-

Table 1. Contd.

7-*epi*-α-Selinene	1520	1525	-	-	-	-	0.21±0.01	0.77±0.57	-	0.11±0.02	-	-	-	-	-	-	-	-
δ-Cadinene	1522	1528	-	-	0.05±0.01	0.15±0.21		-	-	-	-	-	-	-	-	-	0.75±0.05	0.09±0.01
E-γ-Bisabolene	1528	1533	-	-	0.25±0.01	-	0.12±0.01	-	-	-	-	-	-	-	-	-	1.06±0.03	-
Oxygenated Sesquiterpenes			1.17	1.91	5.43	2.98	3.64	2.99	2.96	3.14	2.35	2.35	0.77	1.09	4.23	2.8	2.93	1.76
Spathulenol	1577	1582		0.65 ±0.09	1.34±0.02	1.10±0.05	1.12±0.01	1.34±0.01	1.09±0.02	1.05±0.08	0.98±0.05	0.96±0.05	0.77±0.03	0.44±0.01	1.88±0.02	1.55±0.32	1.60±0.05	0.81±0.05
Viridiflorol	1592	1595	1.17±0.05	1.26 ±0.07	1.90±0.08	1.60±0.16	1.64±0.02	1.48±0.01	1.26±0.05	1.53±0.30	0.90±0.01	1.05±0.01	-	0.65±0.01	2.35±0.09	1.25±0.07	1.10±0.05	0.87±0.05
Carotol	1594	1598	-	-	0.02±0.00	0.03±0.05	0.24±0.03	0.06±0.01	0.17±0.02	0.12±0.01	-	-	-	-	-	-	0.23±0.05	0.08±0.01
Rosifoliol	1600	1605	-	-	0.49±0.02	0.03±0.01	0.37±0.01	0.03±0.01	0.28±0.02	0.14±0.03	0.17±0.02	-	-	-	-	-	-	-
β-Atlantol	1608	1614	-	-	0.82±0.06	0.10±0.01	-	-	-	-	0.28±0.05	0.34±0.05	-	-	-	-	-	-
10-*epi*-γ-Eudesmol	1622	1628	-	-	0.26±0.01	-	0.27±0.03	-	0.16±0.02	-	-	-	-	-	-	-	-	-
1-*epi*-Cubenol	1627	1631	-	-	0.60±0.44	-	-	-	-	-	-	-	-	-	-	-	-	-
γ-Eudesmol	1630	1634	-	-	-	0.12±0.05	-	0.08±0.02	-	0.3±0.03	-	-	-	-	-	-	-	-
Number of compounds identified			11	13	41	38	37	30	27	30	24	24	17	14	16	16	21	22
Total quantified compounds			98.74	96.6	98.59	98.12	97.22	98.59	97.56	98.54	97.58	99.8	96.32	99.21	98.59	96.06	96.08	98.72
Yield of EO %			0.16	0.17	0.19	0.16	0.16	0.19	0.24	0.48	0.63	0.55	0.77	0.72	1.36	1.18	0.98	0.87
Phenological Activity	Vegetative phase						X	X	X	X	X	X	X	X				
	Reproductive phase		X	X	X	X									X	X	X	X
Season of year			Summer		Summer		Autumn		Autumn		Winter		Winter		Spring		Spring	

IRlit - Literature retention index (Adams, 2017); IRcalc - Calculated retention rates (variation); *The content is in average (%) ± standard deviation (SD); X – Presence; Main constituents in bold; SD =Standard Deviation.
Source: Authors 2022

Increase in the relative percent content of β-pinene in January (44.30%) and July (44.39%) and decrease in the percent content of limonene in the same months (7.08% and 3.07%, respectively) were highlighted in the Serra da Tiririca-Niterói/RJ collecting area (PRN). This variation was not observed in the specimens from the Tijuca/RJ collecting area (PRR), which even showed greater uniformity in the content of α-and β-pinene and greater variation in the content of limonene (2.32% in February to 9.09% in January).

Chemical analysis in the different seasons showed that the intraspecific variation in the EO of leaves of *P. rivinoides* collected in the two study areas was very low (Figure 3A-B). PCA analysis showed that it is not possible to distinguish the two areas or the four seasons with respect to the biosynthesis of compounds present in EO. This is confirmed by the Euclidean distance formed in the dendrograms (Figure 4A and B), where clusters can only separate samples with a higher content of α-pinene from samples with a higher content of β-pinene and/or limonene, without separating the two study areas. This implies that *P. rivinoides* exhibits some stability of chemical phenotype in the two different collection areas. Although many studies have reported variation in the chemical profile of EO from the same plant species in different collection areas (Marques et al., 2019; Queiroz et al., 2017; Morshedloo et al., 2018), it is also possible that the variation (chemotype) is small, implying that intrinsic biotic factors are more influential than abiotic factors (Elechosa et al., 2017; Souza et al., 2017).

The results of the present study differ slightly from other results available in the literature, which describe a volatile fraction for *P. rivinoides* composed exclusively of monoterpenes, with a α-pinene (73.2%) and a lower β-pinene (5.2%) for samples collected in the state of São Paulo/Brazil (Perigo et al., 2016). In another study with wild-collected *P. rivinoides* in the state of Paraná/ Brazil, the sesquiterpenes bicyclogermacrene (11.8%) and α-humulene (10.0%) were registered as the maincomponents, with a lower relative proportion of α-pinene (4.4%) and and β-pinene (3.7%) (Bernuci et al., 2016). On the other hand, this study agrees with the studies of Souza et al. (2014), who describe as main constituents the monoterpenes α-pinene(32.9%) and β-pinene (20.9%), besides limonene (3.22%). It is worth noting that the collection sites of the present study are fragments of the Atlantic Forest, as well as the specimen cultivated by Souza et al. (2014), whose

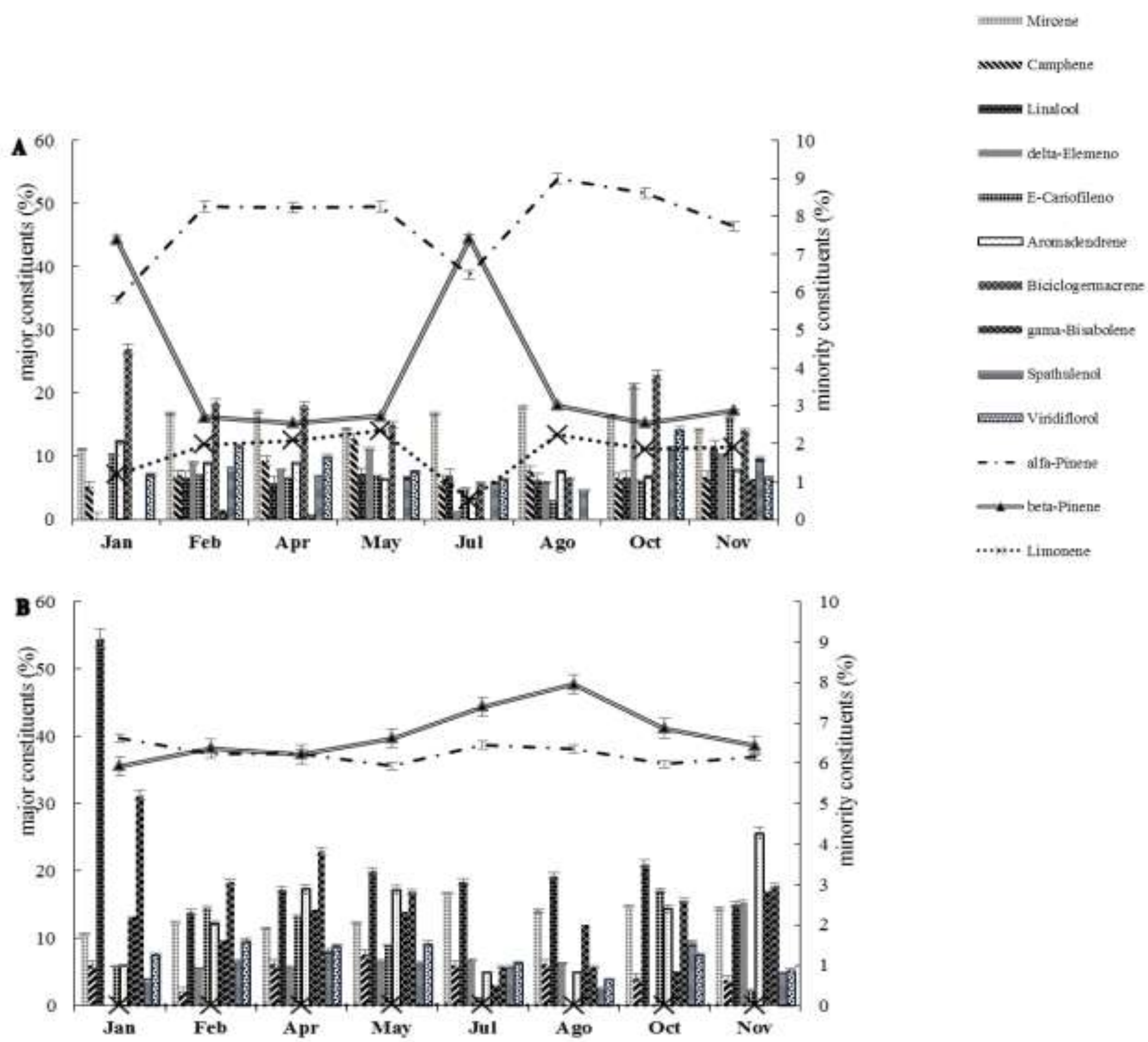

Figure 3. Variation rate of the EO constituents of wild *Piper rivinoides* from the state of Rio de Janeiro. (A) Serra da Tiririca - Niterói/RJ; (B) Rainforest of Tijuca/RJ. Compounds represented by lines are aligned to the primary Y axis, and compounds represented by bars are aligned to the second Y-axis.
Source: Authors 2022

specimen originated from the Atlantic Forest, suggesting a possible ecotype or chemotype for this species.

It is also notable that metabolites from the acetate-mevalonate or methylerythritol phosphate pathway in the EO are exclusive to both plants studied in the present study. Secondary metabolites from, for example, shikimate was not detected, although they are common in Piperaceae and have been identified in other EO samples of *P. rivinoides*. The EO of this plant, collected from the Bom Jesus Biological Reserve in the Brazilian state of Paraná, had a chemical composition completely different from the others previously described. The arylpropanoid E-isoelemicin was the main constituent (40.81%), followed by non-oxygenated monoterpenes (16.88%) and non-oxygenated sesquiterpenes (12.45%). In this study, the compound α-pinene, β-pinene and limonene were not detected (Leal et al., 2019).

The presence of α-pinene and β-pinene in a high relative percentage in EO of P. rivinoides from Rio de Janeiro state is significant because studies conducted with these terpenes have shown various biological activities, including activity against infectious bronchitis virus (IBV) with IC50 of 0.98 ± 0.25 and 1.32 ± 0.11 mM for α-pinene and β-pinene, respectively (Yang et al., 2011).

In addition α-pinene showed a dose-dependent growth inhibitory effect on hepatocellular carcinoma cell lines

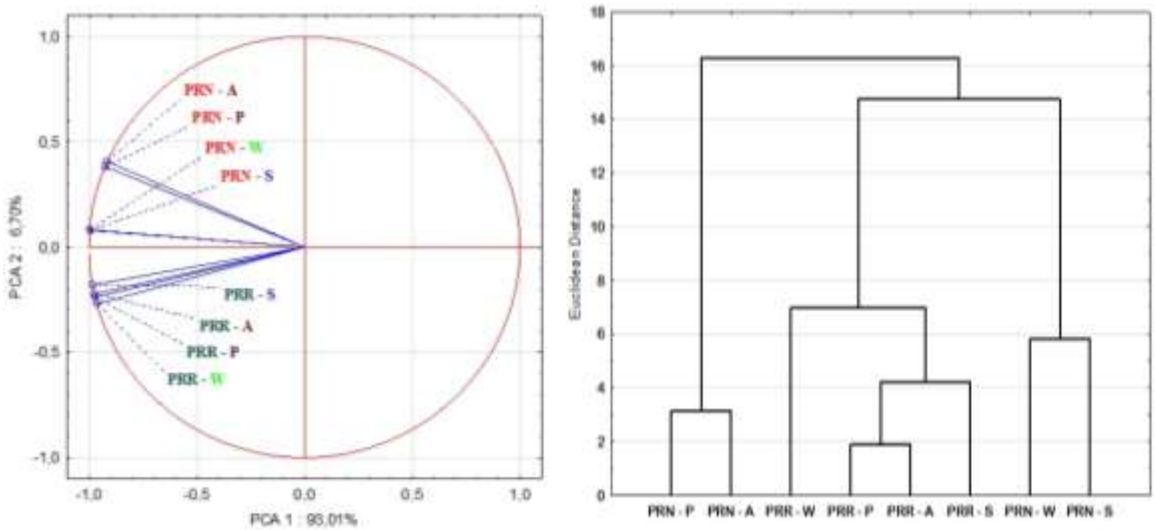

Figure 4. (A) Score graph and (B) dendrogram of the 4 variables (winter, spring, autumn, summer) from the compounds obtained from the essential oil of *Piper rivinoides* Kunth from the accessions of Rainforest of Tijuca/RJ (PRR) and Serra da Tiririca-Niterói/RJ (PRN), based on principal component analysis (PCA). W= Winter, S= Summer, A= Autumn, P= Spring.
Source; Authors 2022

(BEL -7402) with an inhibition rate greater than 79% for all concentrations tested (Chen et al., 2015). A large apoptotic potential or cytotoxic effect against tumor cell line has been reported in the literature for β-pinene (Fonseca et al., 2020; Machado et al., 2021). An assay evaluating nematocidal activity against Bursaphelenchus xylophilus described that among 97 compounds evaluated, the monoterpenes (+)-α-pinene and (-)-α-pinene showed the highest inhibition with 83% and 80%, respectively (IC_{50} 0.64 and 18.03 mM, respectively). For (+)-β-pinene and (-)-β-pinene, the inhibitory effect was moderate at 39% and 35%, respectively (IC_{50} was not described) (Kang et al., 2013).

Studies with EO of Baccharis reticulata Ruiz & Pav. α-pinene, dillapiole and β-pinene, showed excellent antibacterial potential against multidrug-resistant strains of Staphylococcus aureus (MICs ≥256 μg/mL), Pseudomonas aeruginosa (MICs ≥1024 μg/mL), and Escherichia coli (MICs ≥ 1024 μg/mL). The authors concluded that the effect was related to the presence of α-pinene in high concentrations in the sample (Freitas et al., 2020). Other studies also highlight the antimicrobial potential of terpinene and β- pinene monoterpenes (Freitas et al., 2020; Nguyen et al., 2022; Huong et al., 2019; Leite et al., 2007).

There are numerous other studies in the literature describing the biological potential of α and β-pinene monoterpenes (Medeiros et al., 2017; Pereira et al., 2017; Da França et al., 2015; Rivera-Yañez et al., 2017).

All in all, it is evident that the EO medicinal plant P. rivinoides from fragments of the Atlantic Forest in Rio de Janeiro State has shown potential for biological activities, such as the previously published apoptotic effect of β-pinene on oral squamous cell carcinoma (Machado et al., 2021). However, the activity of an essential oil must be evaluated rather than the activity of some of its constituents to avoid antagonisms or synergies between them. For this reason, the overall relationship between the potential uses of this plant and its bioactivity is still very relative and further studies are needed to confirm its bioactivity.

Furthermore, it is also worth highlighting the significant increase in the levels of limonene in PRN (3.07 - 14.05%) and linalool in PRR (2.32 - 9.09%). This is very important because pure limonene and high levels EO of this compound are bradycardia and antiarrhythmic (Nascimento et al., 2019), antifungal (Viriato, 2014; Cai et al., 2019), molluscicidal (Barros et al., 2020), antibacterial and antibactericidal (Yatoo et al., 2021; Hazrati et al., 2020), antioxidant (Yatoo et al., 2021; Hazrati et al., 2020), antiparasitic activities, among others (Vieira et al., 2018). Antimicrobial (Herman et al., 2016), insecticidal (Estrada et al., 2019), anti-inflammatory (Wu et al., 2014), cytotoxic (Chen et al., 2019), antidepressant (Dos Santos et al., 2018), larvicidal (Fujiwara et al., 2017), anesthetic, and sedative (Pereira et al., 2018; Mirghaed et al., 2016) effects have been reported for linalool.

Pearson analyses were also performed to evaluate the correlation of the main compounds and chemical classes with temperature (°C), humidity (%), and precipitation (mm). The results are described in Table 2.

The monoterpens α-pinene and β-pinene, and limonene do not appear to be affected by climatic factors at either collection site. The biosynthesis of monoterpenes and

Table 2. Result of Pearson's correlation analysis between environmental variables and yields of major compounds and chemical classes of essential oils of wild *Piper rivinoides* Kunth collected in Rio de Janeiro and Niteroi.

Compound	Temperature (°C)		Relative humidity (%)		Precipitation (mm)	
	PRN	PRR	PRN	PRR	PRN	PRR
α-Pinene	-0.4082	0.0532	0.4566	-0.3737	0.2609	-0.0644
β-Pinene	0.1717	-0.7721	-0.6098	-0.3624	-0.4593	-0.3989
Myrcene	-0.3450	-0.6338	-0.2948	-0.2856	0.0590	-0.4073
Limonene	-0.0607	0.4137	0.5280	-0.2444	0.4302	-0.2045
Linalool	-0.6786	-0.2358	0.6943	0.3036	0.0435	0.0626
Bicyclogermacrene	**0.6774***	**0.8376***	0.2034	0.1022	0.2848	0.4033
Monoterpenes	-0.3497	**-0.7813***	-0.5401	-0.3918	-0.4906	-0.4985
Sesquiterpenes	0.4794	**0.7236***	0.3670	0.4176	0.4149	0.6055

* P= > 0.05
Source: Authors 2022

sesquiterpenes also does not seem to be affected by climatic abiotic factors evaluated for Serra da Tiririca State Park (PRN). Other results were found for the specimen from Tijuca National Park (PRR), where the percentage content of monoterpenes showed a negative correlation with temperature (r = -0.7813; $p < 0.05$), while the content of sesquiterpenes was positively correlated with temperature (r = 0.7236; $p < 0.05$) and precipitation (r = 0.6055; $p < 0.05$). For the other components of EO, there was a moderate positive correlation between bicyclogermacrene content and temperature for both sites. However, it is known that interactions resulting from biotic factors with this species at this site may be an important factor in this analysis (Salazar et al., 2016; Whitehead et al., 2021).

Conclusion

The results presented here highlight the biological potential of EO, which was extracted from *P. rivinoides* being suitable for medicinal use in the treatment of various problems due to its chemical composition, which is consistent with its traditional medicinal use. It is suggested that *P. rivinoides* EO has low chemical phenotypic variability in both areas, despite environmental and seasonal differences. This chemical stability of EO is little observed in Piperaceae species that show great phenotypical chemistry variability. This is a good thing because it means that the EO's biological activities will continue to be active even in different months with different abiotic influences, making *P. rivinoides* a potential source of bioactive compounds. The seasonality of chemical variations suggests that the phenological cycle has a greater influence on biosynthesis than abiotic factors. Based on the results presented and the literature, we suggest possible chemotypes or ecotypes for *P. rivinoides*. However, a more in-depth study is needed to confirm them by examining EO samples from different regions of Brazil in the field and in cultivation.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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