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Article

Analysis of the Seasonal Variation in Chemical Profile of *Piper glabratum* Kunth Essential Oils Using GC×GC/qMS and Their Antioxidant and Antifungal Activities

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Piper glabratum is a plant native from South America, which has been little studied, despite its use in folk medicine. In this study, chemical composition, antioxidant and antifungal activities of essential oils (EOs) from leaves of *P. glabratum* were investigated. The influence of seasonality on its chemical composition was also evaluated. The chemical characterization was performed by two-dimensional gas chromatography coupled with quadrupole mass spectrometry (GC×GC/qMS). Altogether, 199 compounds were identified in the EOs and their composition varied during the seasonal cycle. The main compounds found were sesquiterpenes and their oxygenated analogues. EOs showed antioxidant activity, measured by 2,2-diphenyl-1-picrylhydrazyl-DPPH (from 28.1 to 33.4 μg mL^{-1}) and β-carotene assays (from 57.4 to 66.4%), and inhibitory action against *C. albicans*. The oils provided expressive properties; however, the yield of EO from winter was not enough for activities assays, remarking the influence of the seasonality on the biological activities of this plant.

Keywords: essential oil, *Piper glabratum*, GC×GC/qMS, antifungal activity, antioxidant activity

Introduction

Essential oils are complex mixtures of compounds which vary at different concentrations with commercially important especially for the pharmaceutical, food, sanitary, and cosmetic industries. The main compounds of these oils are terpenes and terpenoids and the other aromatic and aliphatic constituents, all characterized by relatively low molecular weight.[1] Interactions between these compounds may lead to antagonistic, additive, or synergistic effects, inducing higher or lower bioactivity when compared to the oil's isolated components.[2]

The genus *Piper* contains over 700 species, distributed in tropical and subtropical region of the world.[3] Many species, mainly from India, Asia, and Africa, are economically important; they have been used for centuries in folk medicine, as well as a food additive.[4] The essential oils from different *Piper* species commonly found in Brazil have been studied, and have showed a rich chemical composition, as well as biocidal, cytotoxic, antifungal, antioxidant, and anti-cholinesterase activities.[5-7] Among the *Piper* species native from Brazil, *Piper glabratum* Kunth has been little studied, despite its long use in folk medicine, and the studies that have pointed to its prominent diuretic proprieties,[8] as well as its antiparasitic activity against *Leishmania* and *Trypanosoma cruzi*.[9] Additionally, it has been showed that the essential oils possess anti-inflammatory potential and absence of toxicity.[10]

Comprehensive two-dimensional gas chromatography (GC×GC)[11] is now the most powerful separation technique

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for the analysis of volatile compounds.[12] Since the first application of GC×GC for essential oil characterization,[13] much attention has been devoted to this field, due to its superiority over one-dimensional gas chromatography (1D-GC) for analysis of this matrix.

In GC×GC, fast detectors with high data acquisition rate are mandatory. This feature is required for the correct assignment and quantification of the very fast second-dimension peaks (100-600 ms). A relation exists between the acquisition rate and the number of data points which define a peak. The higher the acquisition rate, the greater the number of data points that is available to define the chromatographic profile.[14,15] Furthermore, there is a compromise between the identification of compounds with a high level of confidence by mass spectrometry, and the mass spectra quality generated by these systems. It can be studied by evaluating spectral skewing and similarity match (mass spectral similarity, %MS), with the MS library compounds, at each data point of the modulated peak. Scanning of fast-eluting peaks can cause skewing of the mass spectra, i.e., variation of the instantaneous mass flux in the ion source over a single scan cycle and can be described by plotting mass-to-charge ratio (*m/z*) of abundant ions in a spectrum across the peak elution.[16]

The goal of the present study was to investigate the chemical profile and seasonal variation of the essential oils from *Piper glabratum* Kunth by rapid-scanning two-dimensional gas chromatography coupled with quadrupole mass spectrometry (GC×GC/qMS). Initially, the detector's performance (in terms of number of data points *per* peak, average spectra quality match, and peak skewing) was evaluated against representative essential oil compounds. The antioxidant and antifungal activity of the oils were also evaluated.

Experimental

Samples and reagents

The leaves of *Piper glabratum* were collected during 2012, according the four stations: March (autumn), June (winter), September (spring) and December (summer), in Dourados-MS (22º14'55"S; 54º53'57"W), Brazil. A voucher specimen was identified (DDMS 4412) by Dra Elsie Franklin Guimarães and deposited in the herbarium of the Federal University of Grande Dourados (UFGD).

Standard and chemicals compounds: *n*-alkane (C_6-C_{30}), β-carotene (≥ 95%), linoleic acid (≥ 98%), 2,2-diphenyl-1-picrylhydrazyl (DPPH), nystatin, and butylated hydroxytoluene (BHT) (≥ 99%), were purchased from Sigma Aldrich (Saint Louis, MO, USA). Solutions of *n*-alkane (100 ppm) were prepared in *n*-hexane. Sabouraud broth and polyoxyethylene sorbitan monopalmitate (Tween®-40) were purchased from Sigma Aldrich (Saint Louis, MO, USA), and anhydrous sodium sulfate p.a. grade from Merck (Darmstadt, HE, Germany). Methanol, chloroform and *n*-hexane (chromatographic grade) were obtained from JT Baker (Phillipsburg, NJ, USA).

Essential oil standards: (–)-β-pinene (99%), α-terpineol (≥ 96 %), limonene (97%), linalool (97%), (*E*)-caryophyllene (≥ 98.5%), carvone (≥ 97%), α-humulene (≥ 96%), 1,8-cineole (99%), camphor (≥ 96%), (*E*)-nerolidol (≥ 85%) and α-bisabolol (≥ 93%) were purchased from Sigma Aldrich (Saint Louis, MO, USA). Standard solutions (1 ppm) were prepared by weight appropriate amounts of standard, dilution in hexane and stored at –4 °C.

Sample preparation

The leaves were collected, chopped, and immediately hydro-distilled for 4 h using a Clevenger-type apparatus. The essential oil was dried using anhydrous sodium sulfate, transferred to dark vials, weight, and stored at –4 °C for further analysis. The mass yields (weight to weight) were calculated on fresh weight basis. Each essential oil (50 mg) was diluted in *n*-hexane (1 mL) before gas chromatographic analysis. The experiments were made in triplicate.

GC×GC/qMS analysis

GC×GC/qMS analysis was carried out on a Shimadzu GC×GC/qMS system consisting of a GC2010 gas chromatograph and a QP2010 Ultra quadrupole mass spectrometer (Shimadzu Corp., TYO, Japan). The mass spectrometer was operated at 70 eV in fast scan mode (20000 Daltons *per* s) over a 40-250 *m/z* range, giving a 65 Hz acquisition rate. The system was provided with a loop-type modulator (Zoex Corp., TX, USA) cooled with liquid nitrogen and with the hot jet pulse time set at 375 ms (300 °C) with modulation times of 5 s. The separations were achieved on a DB-5 (5% phenyl methyl-polysiloxane, Agilent Technologies, CA, USA) analytical column (60 m × 0.25 mm × 0.25 µm) as a first dimension (1D). The second dimension (2D) column was a DB-17 (50% phenyl methyl-polysiloxane, Agilent Technologies, CA, USA) analytical column (2.15 m × 0.18 mm × 0.18 µm). Temperatures were set as follows: 40 °C (hold 5 min), raised to 115 °C (3 °C min^{-1}), and then to 175 ºC (2 ºC min^{-1}) and finally 246 °C (3 ºC min^{-1}) (hold 10 min). The injector, ion source and interface temperature were held at 250 °C. The samples were injected in the split mode (1:20) and the carrier gas flow rate (helium) was 0.91 mL min^{-1}. In

order to calculate the linear-temperature-programmed retention indices (LTPRI) a linear C_6-C_{30} alkane mixture was analyzed using identical GC×GC/qMS conditions.[17] Data were acquired by GCMS Solution software[18] and processed using GCImage software.[19] Compounds were tentatively identified by comparing their electron ionization mass spectral fragmentation (EI-MS) similarity with commercial database (NIST) and by comparing their LTPRI to those reported in a database.[20] An identity spectrum match factor above 800 resulting from NIST and a LTPRI with a match window of ± 20 were determined to be acceptable for tentatively identified compound. The relative amounts of individual compounds were calculated based on GC×GC/qMS peak area without using correction factor.

Antioxidant activity

The evaluation of the antioxidant capacity was carried out by two different methods: DPPH and β-carotene bleaching.

DPPH: free radical scavenging assay

In order to estimate the DPPH scavenging capacity, the essential oil samples at different concentration ranges (1-100 μg mL^{-1}) were mixed in the freshly DPPH solution (0.1 mmol L^{-1} in methanol, 3.0 mL), according to previously described.[21] The mixture was shaken vigorously and left to stand at room temperature in the dark. After 30 min, absorbance (517 nm) was measured against a blank containing all reagents except the test samples, using an ultraviolet-visible (UV-Vis) spectrophotometer (Femto, model 700 plus). BHT was used as positive control.

The scavenging activity was calculated as equation 1:

$$\%\text{DPPH scavenging activity} = \left(\frac{A0 - A}{A0}\right) \times 100 \quad (1)$$

where: A0 is the absorbance of the blank solution and A is the absorbance of the essential oil. The percentage of scavenging activity was plotted against the sample concentration to obtain effective concentration (IC_{50}) defined as the concentration of the sample necessary to scavenge 50% of the DPPH radicals. Determinations were performed in triplicate.

β-Carotene bleaching (BCB) assay

The antioxidant activity of the essential oils were measured on the basis of the β-carotene bleaching system according to the previously reported.[22] Briefly, β-carotene was dissolved in chloroform (0.2 mg mL^{-1}) and an aliquot (1 mL) of this solution was mixed with linoleic acid (20 mg) and Tween-40® (200 mg). Subsequently, chloroform was removed under vacuum using a rotary evaporator (Fisatom, model 801) and then distilled water saturated with oxygen (50 mL) was slowly added with vigorous agitation to form an emulsion. Emulsion aliquots (5 mL) were mixed with each essential oil sample (0.2 mL). Control samples were prepared with methanol (0.2 mL) without sample. BHT was used for comparative propose. As soon as the emulsion was added to each tube, the absorbance (470 nm) was measured (zero time) using a UV-Vis spectrophotometer (Femto, model 700 plus). Tubes were placed in a water bath (50 °C) and oxidation was monitored by absorbance measurements at 15 min intervals until the color of β-carotene had disappeared in the control sample (approximately 105 min). The experiments were carried out in triplicate.

Antioxidant activity (AA) was calculated as percent inhibition relative to the control (equation 2):

$$AA = 100 \times \left(\frac{1 - (Ai - At)}{(A'i - A't)}\right) \quad (2)$$

where Ai: absorbance values (0 min) for the tested sample, At: absorbance values (0 min) for the control, A'i: absorbance value (105 min, 50 °C) for the tested sample, and A't: absorbance value (105 min, 50 °C) for the control. The experiments were performed in triplicate.

Antifungal activity

In order to evaluate the minimum inhibitory concentration (MIC) of the essential oils against the yeast *Candida albicans* (ATCC10231), a broth microdilution assay was performed according to the method previously described.[23] Briefly, serial dilutions of each essential oil (2.0-50 μg mL^{-1}) and a volume of Sabouraud broth, inoculated with the microorganism (10^5 colony-forming units (CFU) mL^{-1}), were added in 96-well plates. The plates were incubated for 72 h at 25 °C. Experiments were performed in triplicate, nystatin was used as positive control and proper blanks were carried out simultaneously.

Results and Discussion

Chemical constituents

There was a variation in the yield of the essential oil according to the harvest time. The yield from spring, summer, and autumn leaves was 0.89, 0.81 and 0.70% respectively, while winter had the poorest yield by far, at only 0.05%. This is probably due to Dourado's climate at that time of year; while summers tend to be extremely hot

and sunny, on winter the weather is milder, with an extreme low amount of rainfall. As a consequence, the essential oil from winter was only used for chemical characterization; the amount obtained was not enough for antioxidant and antifungal assays.

In the beginning of the chemical composition study, the performance of the rapid-scanning qMS for identification of essential oil compounds with a high level of confidence was evaluated. To assess the performance, a set of essential oil standard compounds of different classes-namely monoterpenes hydrocarbons (β-pinene and limonene), oxygenated monoterpenes (1,8-cineole, linalool, camphor, α-terpineol, and carvone), sesquiterpenes hydrocarbons ((*E*)-caryophyllene and α-humulene), and oxygenated sesquiterpenes ((*E*)-nerolidol and α-bisabolol)-were used to evaluated important qMS parameters (number of data points *per* peak, the average spectra quality match, and the peak skewing). Therefore, the mass spectrometer was operated in fast scan mode (20000 amu s^{-1}) over a 40-250 *m/z* range, giving a 65 Hz acquisition rate.

Table 1 reports the number of modulations *per* peak, data points, peak weight, average spectra quality match (MS%) and the selected ions (target ion (T), and qualifier ions (Q1 and Q2)) used to evaluate the skewing of the mass spectra, for each investigated compound.

The system employed generated at least 7 data points *per* peak, for all analyzed compounds. These results are considered sufficient amount of data points.[24] Even for the group of compounds that showed the lowest number of data points (β-pinene, α-terpineol and (*E*)-nerolidol), the results were satisfactory. The majority of evaluated compounds showed peak width of 180 ms with number of scans *per* peak ranging from 9 to 11.

The quality of the mass spectra was measured by the similarity match (%MS). It was obtained by the comparison of experimental mass spectra and mass spectra from commercial library (NIST), and was evaluated at each scan of the main modulated peak, for each investigated compound. According to the results, good similarity match was obtained with an average from 92 to 94%. Furthermore, the low percent relative standard deviation (%RSD) values for this parameter showed that %MS were constant across the evaluated peaks.

The compounds showed some mass spectral distortion across their chromatographic peak. As can be seen in Table 1, the %RSD values for the ratios T/Q_1 and T/Q_2 ranged from 4.8 to 9.8%. It means that mass ratios (or mass spectral) were not even across the peak elution. However, these %RSD values are considered synonymous of relative stability for this parameter.[16]

The high qMS performance observed with the standard compounds (high similarity match and acceptable stability of the mass ratio) allowed us to adopt this experimental setting to characterize complex samples as *P. glabratum* essential oil with high level of confidence. Figure 1 illustrates a total ion chromatogram from the GC×GC/qMS presented as a contour plot for the essential oil samples.

Considering the four essential oils, a total of 199 compounds were identified, with satisfactory library matches and LTPRIs. The experimental LTPRIs showed a good concordance with those reported by Adams[20] for 1D-GC. The identified compounds are listed in Table 2 with their LTPRI from the literature, LTPRI differences (experimental LTPRI – literature LTPRI), and relative percentage of the chromatographic area.

Table 1. Number of modulations *per* peak; number of points *per* peak for the main modulated peak; peak width at the baseline; average spectral similarity (%MS); target ion (T), and qualifier ions (Q_1 and Q_2) ratios, with their percent relative standard deviation (%RSD) for each 2D peak

No.	Compound	Modulations *per* peak	Number of points in main modulation peak	Peak width at baseline / ms	MS (RSD) / %	T/Q_1 (RSD) / %	T/Q_2 (RSD) / %
1	β-pinene	4	7	120	92.0 (1.9)	93/69 (9.4)	93/79 (9.1)
2	limonene	3	8	120	92.6 (1.1)	68/93 (8.4)	68/67 (8.2)
3	1,8-cineole	3	10	180	92.2 (1.6)	81/93 (9.8)	81/108 (8.1)
4	linalool	5	10	180	92.7 (2.0)	71/93 (9.3)	71/55 (8.1)
5	camphor	5	11	180	93.0 (3.0)	95/81 (4.8)	95/108 (8.9)
6	α-terpineol	3	7	120	93.5 (1.8)	59/93 (9.8)	59/121 (9.1)
7	carvone	5	9	180	92.4 (2.0)	82/54 (8.9)	82/108 (8.8)
8	(*E*)-caryophyllene	4	9	180	93.0 (1.2)	93/69 (7.9)	93/91 (5.6)
9	α-humulene	5	9	180	94.0 (0.7)	93/80 (8.8)	93/121 (7.8)
10	(*E*)-nerolidol	5	7	120	93.0 (1.1)	69/93 (5.5)	69/107 (9.3)
11	α-bisabolol	5	10	180	92.2 (1.9)	69/109 (9.5)	69/119 (9.7)

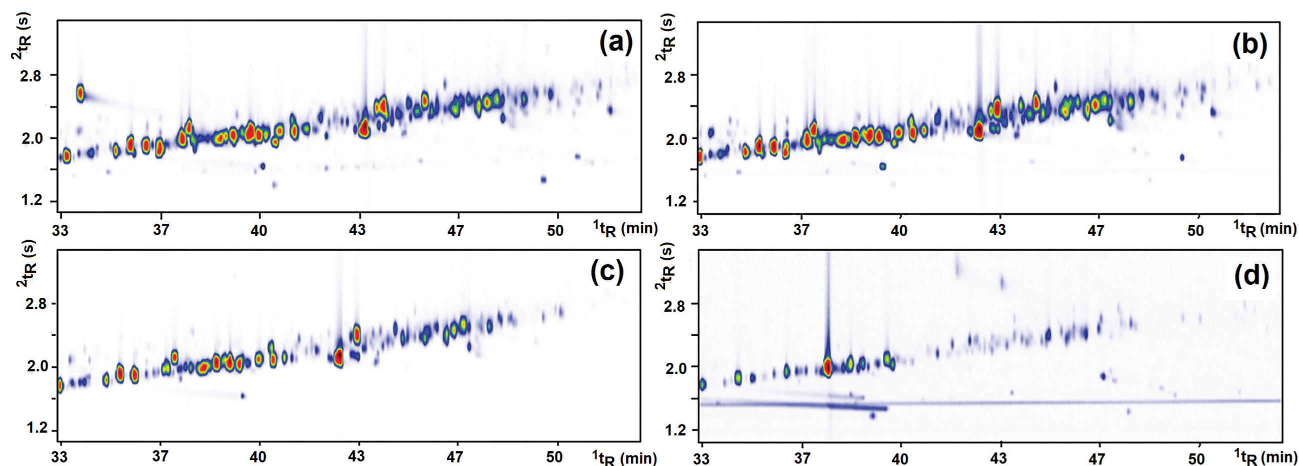

Figure 1. Total ion chromatogram presented as contour plot from essential oils of summer (a), spring (b), autumn (c), and winter (d) leaves of *P. glabratum*.

Table 2. The list of identified compounds, literature LTPRI values, LTPRI differences and their percentage composition in the essential oils from *Piper glabratum* analyzed by GC×GC/qMS

No.	Compound	LTPRI$_{lit}$[a]	Spring		Summer		Autumn		Winter	
			Δ_{LTPRI}[b]	V[c] / %	Δ_{LTPRI}[b]	V[c] / %	Δ_{LTPRI}[b]	V[c] / %	Δ_{LTPRI}[b]	V[c] / %
1	isocitronellene	918	5	0.01	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
2	tricyclene	921	6	0.05	2	0.13	2	0.31	n.i.	n.i.
3	**artemisia triene**[d]	923	5	0.01	7	8.47	7	6.40	n.i.	n.i.
4	camphene	946	n.i.	n.i.	−4	0.07	−4	0.13	n.i.	n.i.
5	*m*-ethyltoluene	958	−1	0.02	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
6	*p*-ethyltoluene	960	−2	0.02	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
7	heptan-2-ol 6-methyl	958	n.i.	n.i.	5	0.01	n.i.	n.i.	n.i.	n.i.
8	sabinene	969	0	0.14	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
9	β-pinene	974	−4	0.01	−2	9.66	−4	6.75	n.i.	n.i.
10	1-octen-3-ol	974	8	0.02	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
11	6-methyl-5-hepten-2-one	981	n.i.	n.i.	7	0.97	4	0.78	n.i.	n.i.
12	**Ψ-cumene**[d,e]	988	0	0.03	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
13	**myrcene**[d]	988	2	0.02	2	1.26	0	0.88	n.i.	n.i.
14	3-octanol	988	10	0.02	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
15	decane	1000	n.i.	n.i.	0	0.05	n.i.	n.i.	n.i.	n.i.
16	**α-phellandrene**[d]	1002	3	0.02	−2	0.02	−4	0.07	n.i.	n.i.
17	**α-terpinene**[d]	1014	n.i.	n.i.	−1	0.29	−4	0.19	n.i.	n.i.
18	***p*-cymene**[d,e]	1020	n.i.	n.i.	2	0.11	−2	0.07	n.i.	n.i.
19	limonene	1024	1	1.14	1	2.80	−2	2.52	n.i.	n.i.
20	1,8-cineole	1026	0	0.85	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
21	indane	1035	n.i.	n.i.	−5	0.01	n.i.	n.i.	n.i.	n.i.
22	**(*Z*)-β-ocimene**[d]	1032	6	0.06	6	0.62	3	1.08	n.i.	n.i.
23	**(*E*)-β-ocimene**[d]	1044	4	0.15	4	0.21	1	0.34	n.i.	n.i.
24	γ-terpinene	1054	3	0.02	3	0.68	−1	0.49	n.i.	n.i.
25	*cis*-sabinene hydrate	1065	0	0.08	2	0.05	−3	0.06	n.i.	n.i.
26	**2-ethyl-*p*-xylene**[d,e]	1085	−10	0.02	−10	0.02	n.i.	n.i.	n.i.	n.i.
27	**4-ethyl-*m*-xylene**[d,e]	1086	−4	0.01	−4	0.01	n.i.	n.i.	n.i.	n.i.
28	terpinolene	1086	−1	0.02	−1	0.28	−4	0.19	n.i.	n.i.
29	**4-ethyl-*o*-xylene**[d,e]	1092	−4	0.01	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
30	*trans*-sabinene hydrate	1098	n.i.	n.i.	−1	0.04	−5	0.05	n.i.	n.i.
31	linalool	1095	7	0.10	5	0.07	2	0.05	n.i.	n.i.
32	*exo*-fenchol	1118	n.i.	n.i.	−8	0.04	n.i.	n.i.	n.i.	n.i.
33	**perillene**[d]	1102	n.i.	n.i.	15	0.40	12	0.76	n.i.	n.i.

Table 2. The list of identified compounds, literature LTPRI values, LTPRI differences and their percentage composition in the essential oils from *Piper glabratum* analyzed by GC×GC/qMS (cont.)

No.	Compound	$LTPRI_{lit}$[a]	Spring		Summer		Autumn		Winter	
			Δ_{LTPRI}[b]	V[c] / %	Δ_{LTPRI}[b]	V[c] / %	Δ_{LTPRI}[b]	V[c] / %	Δ_{LTPRI}[b]	V[c] / %
34	**1,3,8-*p*-menthatriene**[d]	1108	4	0.01	n.i.	n.i.	8	0.05	n.i.	n.i.
35	**isodurene**[d,e]	1129	–12	0.01	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
36	*cis-p*-menth-2-en-1-ol	1118	3	0.02	3	0.06	n.i.	n.i.	n.i.	n.i.
37	methyl octanoate	1123	5	0.02	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
38	(*E*)-limonene oxide	1132	n.i.	n.i.	4	0.03	n.i.	n.i.	n.i.	n.i.
39	*trans-p*-menth-2-em-1-ol	1136	4	0.01	4	0.03	0	0.02	n.i.	n.i.
40	**(*Z*)-myroxide**[d]	1131	n.i.	n.i.	n.i.	n.i.	9	0.02	n.i.	n.i.
41	**(*E*)-myroxide**[d]	1140	5	0.04	n.i.	n.i.	2	0.03	n.i.	n.i.
42	camphor	1141	n.i.	n.i.	1	0.03	n.i.	n.i.	n.i.	n.i.
43	camphene hydrate	1145	n.i.	n.i.	0	0.05	n.i.	n.i.	n.i.	n.i.
44	**prehnitol**[d,e]	1148	0	0.02	0	0.01	n.i.	n.i.	n.i.	n.i.
45	pinocarvone	1160	n.i.	n.i.	1	0.13	–3	0.06	n.i.	n.i.
46	isoborneol	1155	n.i.	n.i.	9	0.03	n.i.	n.i.	n.i.	n.i.
47	δ-terpineol	1162	5	0.08	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
48	(2*E*)-nonen-1-al	1157	n.i.	n.i.	12	0.02	n.i.	n.i.	n.i.	n.i.
49	*cis*-pinocamphone	1172	1	0.00	1	0.05	n.i.	n.i.	n.i.	n.i.
50	thuj-3-en-10-al	1181	n.i.	n.i.	–5	0.03	n.i.	n.i.	n.i.	n.i.
51	terpinen-4-ol	1174	2	0.23	2	0.77	–1	0.65	n.i.	n.i.
52	**naphthalene**[d,e]	1178	0	0.01	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
53	myrtanal	1198	n.i.	n.i.	–19	0.01	n.i.	n.i.	n.i.	n.i.
54	α-terpineol	1186	6	0.43	4	0.43	0	0.29	n.i.	n.i.
55	myrtenol	1219	n.i.	n.i.	–20	0.04	–20	0.03	n.i.	n.i.
56	dodecane	1200	n.i.	n.i.	2	0.02	n.i.	n.i.	n.i.	n.i.
57	*trans*-piperitol	1207	2	0.01	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
58	(*E*)-2-dodecene	1205	4	0.01	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
59	verbenone	1204	n.i.	n.i.	5	0.01	n.i.	n.i.	n.i.	n.i.
60	*trans*-carveol	1215	5	0.01	5	0.02	n.i.	n.i.	n.i.	n.i.
61	carvone	1243	3	0.04	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
62	*cis*-myrtanol	1250	5	0.01	3	0.01	n.i.	n.i.	n.i.	n.i.
63	piperitone	1249	n.i.	n.i.	6	0.01	n.i.	n.i.	n.i.	n.i.
64	(4*Z*)-decen-1-ol	1255	n.i.	n.i.	1	0.02	n.i.	n.i.	n.i.	n.i.
65	*trans*-myrtanol	1258	n.i.	n.i.	2	0.02	n.i.	n.i.	n.i.	n.i.
66	(4*E*)-decen-1-ol	1259	n.i.	n.i.	3	0.02	n.i.	n.i.	n.i.	n.i.
67	***p*-propylphenol**[d,e]	1260	6	0.05	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
68	***o*-*tert*-butylphenol**[d,e]	1274	6	0.01	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
69	***p*-*sec*-butylphenol**[d,e]	1279	7	0.02	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
70	isobornyl acetate	1285	2	0.02	1	0.06	–1	0.05	n.i.	n.i.
71	**(*E*)-anethole**[d,e]	1282	6	0.01	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
72	lavandulyl acetate	1288	n.i.	n.i.	n.i.	n.i.	7	0.05	n.i.	n.i.
73	3-thujyl acetate	1290	n.i.	n.i.	6	0.06	n.i.	n.i.	n.i.	n.i.
74	*trans*-pinocarvyl acetate	1298	4	0.02	0	0.03	-1	0.04	n.i.	n.i.
75	(2*E*,4*Z*)-decadienal	1292	n.i.	n.i.	n.i.	n.i.	6	0.01	n.i.	n.i.
76	tridecane	1300	n.i.	n.i.	2	0.02	n.i.	n.i.	n.i.	n.i.
77	**(*Z*)-methyl cinnamate**[d,e]	1299	9	1.14	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
78	*cis*-pinocarvyl acetate	1311	4	0.05	0	0.13	–2	0.12	n.i.	n.i.
79	myrtenyl acetate	1324	n.i.	n.i.	0	0.35	–2	0.14	n.i.	n.i.
80	methyl decanoate	1323	4	0.72	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
81	methyl geranate	1322	5	0.16	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
82	**(*Z*)-isosafrole**[d,e]	1336	-8	0.02	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
83	α-terpinyl acetate	1346	n.i.	n.i.	–11	0.27	–13	0.17	n.i.	n.i.

Table 2. The list of identified compounds, literature LTPRI values, LTPRI differences and their percentage composition in the essential oils from *Piper glabratum* analyzed by GC×GC/qMS (cont.)

No.	Compound	$LTPRI_{lit}$[a]	Spring		Summer		Autumn		Winter	
			Δ_{LTPRI}[b]	V[c] / %	Δ_{LTPRI}[b]	V[c] / %	Δ_{LTPRI}[b]	V[c] / %	Δ_{LTPRI}[b]	V[c] / %
84	7-*epi*-silphiperfol-5-ene	1345	n.i.	n.i.	−9	0.03	−10	0.03	n.i.	n.i.
85	δ-elemene	1335	1	0.13	n.i.	n.i.	n.i.	n.i.	−2	4.53
86	α-cubebene	1345	4	0.54	2	0.76	1	0.75	n.i.	n.i.
87	*trans*-carvyl acetate	1339	12	0.03	10	0.06	8	0.06	n.i.	n.i.
88	cyclosativene	1369	−7	0.90	−9	1.08	−11	0.93	n.i.	n.i.
89	*neoiso*-dihydro carveol acetate	1356	8	0.01	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
90	*cis*-carvyl acetate	1365	1	0.03	−3	0.05	−5	0.05	n.i.	n.i.
91	α-ylangene	1373	−2	0.12	−6	0.14	−7	0.21	n.i.	n.i.
92	linalool isobutanoate	1373	−2	0.02	−6	0.04	−7	0.02	n.i.	n.i.
93	α-copaene	1374	1	2.81	−1	2.59	−3	2.21	−5	0.53
94	isoledene	1374	6	0.02	2	0.02	n.i.	n.i.	n.i.	n.i.
95	β-bourbonene	1387	−3	0.11	−7	0.10	−7	0.25	−9	1.04
96	*trans*-myrtanol acetate	1385	−1	0.10	−5	0.17	n.i.	n.i.	n.i.	n.i.
97	**(*E*)-methyl cinnamate**[d,e]	1376	10	3.31	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
98	β-cubebene	1387	n.i.	n.i.	0	0.29	−1	0.15	n.i.	n.i.
99	α-duprezianene	1387	4	0.01	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
100	β-elemene	1389	4	0.53	n.i.	n.i.	0	0.46	−2	1.19
101	(2*E*)-decenyl acetate	1408	−12	0.05	−15	0.10	n.i.	n.i.	n.i.	n.i.
102	7-*epi*-sesquithujene	1390	7	0.02	5	0.02	3	0.04	n.i.	n.i.
103	tetradecane	1400	n.i.	n.i.	0	0.02	n.i.	n.i.	n.i.	n.i.
104	α-gurjunene	1409	0	1.48	−4	1.40	0	1.46	−5	0.10
105	α-cedrene	1410	−1	0.01	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
106	(*E*)-caryophyllene	1417	2	4.89	−1	4.16	−3	4.90	−3	2.48
107	β-copaene	1430	−1	3.51	−4	3.35	−6	3.98	−7	0.38
108	γ-elemene	1434	−5	0.04	−8	0.04	n.i.	n.i.	n.i.	n.i.
109	α-*trans*-bergamotene	1432	4	4.31	1	2.04	n.i.	n.i.	n.i.	n.i.
110	neryl acetone	1434	n.i.	n.i.	1	0.04	n.i.	n.i.	n.i.	n.i.
111	aromadendrene	1439	n.i.	n.i.	n.i.	n.i.	−6	0.13	−1	0.15
112	α-guaiene	1437	n.i.	n.i.	3	0.12	1	0.12	n.i.	n.i.
113	***cis*-muurola-3,5-diene**[d]	1448	n.i.	n.i.	n.i.	n.i.	−7	0.07	n.i.	n.i.
114	***trans*-muurola-3,5-diene**[d]	1451	n.i.	n.i.	n.i.	n.i.	−6	0.61	n.i.	n.i.
115	α-humulene	1452	0	4.41	−4	3.13	−4	1.65	−5	1.27
116	geranyl acetone	1453	4	3.15	1	2.89	−1	2.74	n.i.	n.i.
117	β-santalene	1457	n.i.	n.i.	0	0.33	n.i.	n.i.	n.i.	n.i.
118	allo-aromadendrene	1458	1	1.11	n.i.	n.i.	n.i.	n.i.	−4	0.27
119	**(*E*)-β-farnesene**[d]	1454	n.i.	n.i.	n.i.	n.i.	3	0.07	n.i.	n.i.
120	9-*epi*-(*E*)-caryophyllene	1464	2	0.01	n.i.	n.i.	n.i.	n.i.	−4	0.18
121	**drima-7,9(11)-diene**[d]	1469	0	0.29	−3	0.31	−5	0.70	n.i.	n.i.
122	***trans*-cadina-1(6),4-diene**[d]	1475	3	3.30	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
123	**germacrene D**[d]	1484	−3	1.03	−10	3.38	−11	5.47	−8	77.74
124	β-selinene	1489	−3	3.46	−8	3.31	−8	4.84	n.i.	n.i.
125	**(*E*)-β-ionone**[d]	1487	1	0.08	−2	0.04	n.i.	n.i.	n.i.	n.i.
126	***trans*-muurola-4(14),5-diene**[d]	1493	−1	0.62	n.i.	n.i.	n.i.	n.i.	−7	0.39
127	α-selinene	1498	n.i.	n.i.	−6	4.29	−6	6.19	n.i.	n.i.
128	bicyclogermacrene	1500	−3	6.93	n.i.	n.i.	n.i.	n.i.	−8	3.49
129	α-muurolene	1500	2	3.88	−2	3.08	−2	3.50	−3	0.18
130	pentadecane	1500	n.i.	n.i.	0	0.19	0	0.12	n.i.	n.i.
131	δ-amorphene	1511	n.i.	n.i.	−6	0.26	−8	0.45	n.i.	n.i.
132	*trans*-cycloisolongifol-5-ol	1513	−8	0.04	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
133	α-bulnesene	1509	−2	1.11	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.

Table 2. The list of identified compounds, literature LTPRI values, LTPRI differences and their percentage composition in the essential oils from *Piper glabratum* analyzed by GC×GC/qMS (cont.)

No.	Compound	$LTPRI_{lit}$[a]	Spring		Summer		Autumn		Winter	
			Δ_{LTPRI}[b]	V[c] / %	Δ_{LTPRI}[b]	V[c] / %	Δ_{LTPRI}[b]	V[c] / %	Δ_{LTPRI}[b]	V[c] / %
134	(*E*,*E*)-α-farnesene[d]	1505	8	1.50	3	0.42	n.i.	n.i.	n.i.	n.i.
135	γ-cadinene	1513	2	2.45	−1	1.81	−3	2.42	−3	0.29
136	elixene	1511	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	6	2.59
137	***trans*-calamenene**[d,e]	1521	3	0.45	−1	0.52	−1	0.99	n.i.	n.i.
138	δ-cadinene	1522	3	2.83	0	2.52	−2	2.87	−2	0.99
139	*trans*-cadina-1,4-diene	1533	0	1.04	−5	0.87	−5	0.98	n.i.	n.i.
140	α-cadinene	1537	1	0.18	−4	0.10	−4	0.22	n.i.	n.i.
141	selina-3,7(11)-diene	1545	n.i.	n.i.	−8	0.02	−8	0.04	n.i.	n.i.
142	**α-calacorene**[d,e]	1544	n.i.	n.i.	n.i.	n.i.	−5	0.15	n.i.	n.i.
143	10-*epi*-cubebol	1533	14	0.10	10	0.11	10	0.07	n.i.	n.i.
144	elemol	1548	n.i.	n.i.	4	0.13	−1	0.09	n.i.	n.i.
145	germacrene B	1559	−4	0.12	n.i.	n.i.	−9	0.29	−9	0.21
146	ledol	1565	7	0.16	10	0.03	n.i.	n.i.	n.i.	n.i.
147	**β-calacorene**[d,e]	1564	n.i.	n.i.	−5	0.03	−5	0.04	n.i.	n.i.
148	germacrene D-4-ol	1574	−9	0.16	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
149	(*E*)-nerolidol	1569	1	8.45	−4	6.76	−2	8.86	n.i.	n.i.
150	spathulenol	1577	0	1.33	−3	0.67	−3	0.23	−5	0.27
151	caryophyllene oxide	1582	0	5.56	−3	4.67	−5	4.40	−5	0.19
152	gleenol	1586	1	0.38	−3	0.37	−4	0.40	n.i.	n.i.
153	viridiflorol	1592	−2	0.75	−5	0.14	−5	0.09	n.i.	n.i.
154	salvial-4(14)-en-1-one	1594	n.i.	n.i.	−5	0.08	−5	0.15	−7	0.09
155	(2*E*)-dodecenyl acetate	1608	−13	0.11	−18	0.07	−18	0.14	n.i.	n.i.
156	1-hexadecene	1593	4	0.01	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
157	guaiol	1600	−1	0.13	−5	0.12	−5	0.15	n.i.	n.i.
158	epiglobulol	1608	n.i.	n.i.	−8	0.56	−9	0.32	−11	0.17
159	humulene epoxide II	1608	−1	2.59	−4	2.38	−4	0.56	n.i.	n.i.
160	humulane-1,6-dien-3-ol	1619	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	−9	0.27
161	1,10-di-*epi*-cubenol	1618	−6	0.72	−9	0.61	−9	0.75	n.i.	n.i.
162	5-*epi*-7-*epi*-α-eudesmol	1607	8	0.48	5	0.37	5	0.32	n.i.	n.i.
163	isolongifolan-7-α-ol	1618	2	0.13	−1	0.05	−1	0.13	n.i.	n.i.
164	*cis*-isolongifolanone	1612	n.i.	n.i.	5	0.09	5	0.03	n.i.	n.i.
165	1-*epi*-cubenol	1627	0	2.49	−3	2.16	−3	1.90	n.i.	n.i.
166	caryophylla-4(12),8(13)-dien-5-α-ol	1639	−4	0.67	−7	0.50	−7	0.40	n.i.	n.i.
167	*epi*-α-muurolol	1640	2	1.95	−1	1.28	−1	1.48	-3	0.41
168	α-muurolol	1644	3	2.55	0	2.06	0	1.82	n.i.	n.i.
169	selin-11-en-4-α-ol	1658	n.i.	n.i.	−8	0.91	−8	2.30	n.i.	n.i.
170	α-cadinol	1652	2	1.78	n.i.	n.i.	n.i.	n.i.	−2	0.60
171	vulgarone B	1649	5	0.04	3	0.03	n.i.	n.i.	n.i.	n.i.
172	14-hydroxy-9-epi-(*E*)-caryophyllene	1668	−11	0.25	−14	0.42	−14	0.27	n.i.	n.i.
173	(*E*)-9-tetradecen-1-ol	1668	−5	0.08	−8	0.06	n.i.	n.i.	n.i.	n.i.
174	intermedeol	1667	−2	0.28	−5	0.22	−5	0.36	n.i.	n.i.
175	(*Z*)-α-santalol	1674	−4	0.96	−7	0.85	−7	0.84	n.i.	n.i.
176	guaia-3,10(14)-dien-11-ol	1676	3	0.29	n.i.	n.i.	−2	0.20	n.i.	n.i.
177	khusinol	1675	n.i.	n.i.	0	0.25	0	0.17	n.i.	n.i.
178	eudesma-4(15),7-dien-1-β-ol	1687	−3	0.06	−5	0.08	−5	0.34	n.i.	n.i.
179	**apiole**[d,e]	1677	8	0.03	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
180	α-bisabolol	1685	2	0.03	−1	0.02	n.i.	n.i.	n.i.	n.i.
181	(*Z*)-α-*trans*-bergamotol	1690	2	0.10	0	0.05	n.i.	n.i.	n.i.	n.i.
182	(*Z*,*E*)-farnesol	1713	−11	0.04	−14	0.02	−14	0.03	n.i.	n.i.

Table 2. The list of identified compounds, literature LTPRI values, LTPRI differences and their percentage composition in the essential oils from *Piper glabratum* analyzed by GC×GC/qMS (cont.)

No.	Compound	$LTPRI_{lit}$[a]	Spring		Summer		Autumn		Winter	
			Δ_{LTPRI}[b]	V[c] / %	Δ_{LTPRI}[b]	V[c] / %	Δ_{LTPRI}[b]	V[c] / %	Δ_{LTPRI}[b]	V[c] / %
183	14-hydroxy-α-humulene	1713	−9	0.07	−13	0.07	−9	0.11	n.i.	n.i.
184	heptadecane	1700	n.i.	n.i.	2	0.07	n.i.	n.i.	n.i.	n.i.
185	longifolol	1713	4	0.07	2	0.05	−1	0.05	n.i.	n.i.
186	(*E*)-nerolidyl acetate	1716	9	0.04	8	0.02	n.i.	n.i.	n.i.	n.i.
187	cedr-8(15)-en-9-α-ol acetate	1741	−1	0.01	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
188	β-acoradienol	1762	−8	0.06	−10	0.04	n.i.	n.i.	n.i.	n.i.
189	14-oxy-α-muurolene	1767	−5	0.03	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
190	14-hydroxy-α-muurolene	1779	−4	0.04	−5	0.03	n.i.	n.i.	n.i.	n.i.
191	ethyl myristate	1793	n.i.	n.i.	4	0.08	n.i.	n.i.	n.i.	n.i.
192	14-hydroxy-δ-cadinene	1803	−4	0.02	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
193	methyl hexadecanoate	1921	7	0.01	9	0.08	n.i.	n.i.	n.i.	n.i.
194	methyl oleate	2087	11	0.01	13	0.03	n.i.	n.i.	n.i.	n.i.
195	methyl stearate	2128	n.i.	n.i.	4	0.12	n.i.	n.i.	n.i.	n.i.
196	tetracosane	2400	n.i.	n.i.	6	0.01	n.i.	n.i.	n.i.	n.i.
197	pentacosane	2500	n.i.	n.i.	3	0.01	n.i.	n.i.	n.i.	n.i.
198	hexacosane	2600	n.i.	n.i.	2	0.01	n.i.	n.i.	n.i.	n.i.
199	heptacosane	2700	n.i.	n.i.	2	0.01	n.i.	n.i.	n.i.	n.i.

[a]$LTPRI_{lit}$: linear-temperature-programmed retention index from literature; [b]Δ_{LTPRI}: LTPRI differences (experimental LTPRI – literature LTPRI); [c]V: percentage of volume of the compounds related to the total of tentatively identified peaks; in bold: [d]compounds with π-conjugated system; [e]compounds with aromatic rings. n.i.: not identified.

The essential oil from summer leaves showed the highest number of identified compounds (142), the main representative being the monoterpenes β-pinene (9.66%) and artemisia triene (8.47%), and oxygenated sesquiterpene (*E*)-nerolidol (6.76%). The sesquiterpenes hydrocarbons showed a high relative concentration (40.50%), and included compounds such as α-selinene (4.29%) and (*E*)-caryophyllene (4.26%). Notable differences were recorded in the essential oils composition during the season. Indeed, 30 compounds were identified only in essential oil from summer leaves, among them β-santalene, 3-thujyl acetate, and neryl acetone.

The essential oil from spring leaves showed 136 identified compounds. The major constituents were found to be (*E*)-nerolidol (8.45%), sesquiterpene bicyclogermacrene (6.93%), and oxygenated sesquiterpene caryophyllene oxide (5.56%). The sesquiterpenes hydrocarbons displayed the highest contribution (54.10%) among which (*E*)-caryophyllene (4.89%), and α-humulene (4.41%) were detected at high relative concentrations. A group of 36 compounds were identified only in this sample, among which (*E*)-methyl cinnamate and *trans*-cadina-1(6),4-diene were the most representative. Phenylpropanoids were also identified exclusively in this oil.

As well as summer, essential oil from autumn leaves presented a high relative concentration of (*E*)-nerolidol (8.86%), β-pinene (6.75%), and artemisia triene (6.40%). Similar to the oils from summer and spring leaves, the essential oil from autumn leaves presented a high relative concentration of sesquiterpenes hydrocarbons (47.20%), among which α-selinene (6.19%), germacrene D (5.47%), (*E*)-caryophyllene (4.90%) were the most representative compounds. This oil showed 101 identified compounds, 7 of which were not identified in the other analyzed oils, such as *trans*-muurola-3,5-diene and α-calacorene.

The essential oil from winter leaves was the poorest analyzed oil, with only 26 compounds identified. It presented germacrene D (77.40%), δ-elemene (4.53%), and bicyclogermacrene (3.49%) as its main constituents. Furthermore, it presented the highest relative content of sesquiterpenes hydrocarbons (98.00%) of all four oils studied. Two compounds (elixene and humulane-1,6-dien-3-ol) were found in the oil obtained in winter, which were absent in the other oil analyzed. The typical dry winter in the Dourados region might explain the significant difference in the yield and chemical composition of the essential oil from winter leaves.

In sum, all of the analyzed oils consisted of sesquiterpenes hydrocarbons as a major fraction (Figure 2). The oils from summer and autumn leaves were characterized by higher relative contents of monoterpene hydrocarbons in comparison with the other two samples. Furthermore, the

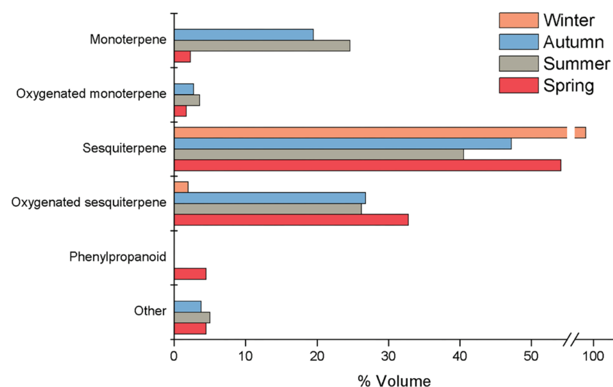

Figure 2. Semi-quantitative distribution of the main classes of compounds in the essential oils of *Piper glabratum*.

lowest relative amount of oxygenated sesquiterpenes was observed in essential oil from winter leaves.

There are few studies available in the literature regarding the chemical profile of essential oils from *P. glabratum*. Assis *et al.*[25] analyzed the oil using 1D-GC and identified 67 compounds, among them phenylpropanoids as eugenol and apiole. The major compounds were β-caryophyllene, longiborneol, and (*E*)-nerolidol. While Branquinho *et al.*[10] used the same approach and found 27 compounds, among them β-pinene, longiborneol, α-pinene, and (*E*)-caryophyllene as the major compounds. The majority of the compounds found in those studies were also found in ours; however, the use we made of GC×GC/qMS has showed the real richness and complexity of these oils, and allowed for its detailed chemical characterization.

Antioxidant and antifungal activities

DPPH scavenging

The half maximal inhibitory concentration (IC_{50}) values of the essential oils from leaves collected in summer, spring and autumn, and BHT were 28.08 ± 0.22, 28.12 ± 0.29, 33.45 ± 0.41, and 17.98 ± 0.33 μg mL^{-1}, respectively. The antioxidant activities of the oils were around 60% of the antioxidant activity of the synthetic antioxidant BHT.

This is the first study related to the antioxidant activity of essential oil from *Piper glabratum*. However, the antioxidant activities of the EOs of other *Piper* species have been documented in the literature.[6,26,27] There are not many studies on the antioxidant activity of isolated compounds of essential oils, and most of them refer to the activity of the whole matrix. However, the isolated monoterpenes 1,8-cineole, β-myrcene, sabinene, α-terpinene, γ-terpinene and terpinolene, besides the sesquiterpenes, bisabolol and farnesol, have been shown antioxidant activity in previous studies.[28] These compounds were identified in the essential oils from *P. glabratum,* suggesting that they might be involved in antioxidant activity observed in the assays.

β-Carotene bleaching (BCB)

The antioxidant activity of the essential oils from leaves collected in summer, spring and autumn was 57.40 ± 0.99, 60.07 ± 0.25, and 66.43 ± 0.38% respectively, expressed as percent inhibition relative to the control. The BCB method works in an aqueous emulsion of linoleic acid and β-carotene, which is discolored by the radicals generated by the spontaneous oxidation of the fatty acid, promoted by thermal induction.[29]

The presence of an antioxidant can be retarded the β-carotene discoloration by competing with it for the radicals. Therefore, the presence of compounds with similar structure to β-carotene in the essential oils, such as π-conjugated molecules, could explain the observed antioxidant activity. As can be seen in Table 2 (compounds in bold), these type of hydrocarbons displayed high contribution-12, 16 and 18% of the chromatographic area in the essential oils from spring, summer and autumn leaves, respectively-among which (*E*)-methyl cinnamate, (*E*,*E*)-α-farnesene and artemisia triene were detected at high relative concentrations.

Antifungal activity

Essential oils from summer, spring and autumn leaves exhibited potent activity against *Candida albicans* yeast (MIC values of 26 ± 0.4, 27 ± 0.7, and 32 ± 0.2 μg mL^{-1}, respectively). The antifungal activity of these essential oils was lower than that of nystatin, the positive control (MIC value of 3.0 ± 0.2 μg mL^{-1}).

The antimicrobial activity of essential oil has been explained by the presence of monoterpenes and sesquiterpenes with aromatic rings and phenolic hydroxyl groups and other active terpenes, as well as alcohols, aldehydes and esters, all of which might contribute to the overall essential oil antimicrobial effect of the essential oil.[30] Several aromatic compounds were identified in the *P. glabratum* essential oils (Table 2). These compounds represented 5.2, 0.7 and 1.3% of the chromatographic area in the oils from spring, summer and autumn leaves, respectively. Compounds among which (*E*) and (*Z*)-methyl cinnamate (spring) and *trans*-calamenene (summer and autumn) were detected at high relative concentrations. Furthermore, some compounds identified in the essential oil from *P. glabratum*, such as β-pinene and (*E*)-caryophyllene, are known to possess antimicrobial activity against *Candida albicans* yeast.[31,32] Thus, these compounds might play an important role in the antifungal findings.

Conclusions

In this study, GC×GC/qMS has proved to be a versatile tool for the analysis of the essential oils from *P. glabratum,* its use allowing for an in-depth knowledge about the chemical composition of the oils. Due to the variability of the environmental factors, the essential oils obtained from leaves collected at different seasons showed important differences in chemical profile. However, in spite of these differences, the essential oils from spring, summer, and autumn leaves showed similar and strong antioxidant and antifungal activities. The oils from winter leaves were poor in yield and number of identified compounds, and it was not possible at the time to evaluate its activities. These findings point to the influence of the seasonality on the biological activities of this plant.

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