Effect of Environmental Factors on *Plectranthus Neochilus* Volatile Composition: A GC-MS-Based Metabolomics Approach

Authors
Maria Isabel Galbiatti[1], Guilherme Perez Pinheiro[1], Elisa Ribeiro Miranda Antunes[1], Vinicius Verri Hernandes[2], Alexandra Christine Helena Frankland Sawaya[3]

Affiliations
1 Department of Plant Biology, Institute of Biology, State University of Campinas, Campinas, São Paulo, Brazil
2 ThoMSon Mass Spectrometry Laboratory, Institute of Chemistry, State University of Campinas, Campinas, São Paulo, Brazil
3 Faculty of Pharmaceutical Sciences, State University of Campinas, Campinas, São Paulo, Brazil

Correspondence
Alexandra Christine Helena Frankland Sawaya
Universidade Estadual de Campinas
Instituto de Biologia - Departamento de Biologia Vegetal
Rua Monteiro Lobato
255 - Cidade Universitária CEP
13083-862 - Campinas-SP
Brazil
Tel.: +55/19/3521 6212
achfsawa@unicamp.br

ABSTRACT

Plectranthus neochilus Schltr. is an aromatic species, commonly used for digestive, antispasmodic, and analgesic purposes. Although many studies have reported the chemical composition of its essential oil, variations in the volatile profile were observed, which may be due to multiple factors linked to growth and field conditions. In order to detect metabolic variations in this species, we employed a GC-MS-based untargeted metabolomics approach analyzing samples of four *P. neochilus* individuals collected over a year. From all analyses, 24 mass features were detected and 21 were identified according to their respective chromatographic peaks. All features varied among samples, particularly *(2E)*-hexenal, 3-octanone and *δ-3*-carene, which showed the highest coefficient of variation percentage in our study. Although the four individuals presented the same peaks in the chromatograms, significant differences in the intensity of specific mass features were detected between individuals throughout the year. Time of sampling did not affect *P. neochilus* volatile composition; the chemical profile remained constant throughout the day. Seasonal trends were observed for the species. Winter months coincided with a drop in the intensity of most components. Air temperature showed a positive correlation with some feature intensities, while myrcene and *α*-thujene resulted in a positive and a negative correlation with rainfall, respectively. This study was the first attempt to correlate metabolic variation and environmental factors in *P. neochilus*. Our approach was successful in identifying the composition and variation of the headspace volatiles of *P. neochilus* leaves.

Introduction

Plants have been used for medicinal purposes since the beginning of civilization [1, 2] and many species are known for their therapeutic properties. Some of the most used medicinal plants are members of the Lamiaceae family, such as *Melissa* spp., *Thymus* spp., *Salvia* spp., and *Plectranthus* spp. [3, 4]. From the *Plectranthus* genus, commonly used in Brazilian folk medicine, *Plectranthus neochilus* Schltr. is a succulent and aromatic herb, used for digestive, antispasmodic, and analgesic purposes [5, 6]. In addition to its traditional use, antioxidant, antimicrobial, and antiparasitic activities of *P. neochilus* essential oil were also described in the literature [7, 8], which are directly related to the volatile composition.

Studies of *P. neochilus* essential oil have reported diverse compositions, although α-thujene, α-pinene, and caryophyllene were frequently three of the main compounds reported in terms of percentage of chromatographic peak area [7, 9–12]. These variations in secondary metabolite contents may be due to multiple factors, including environmental changes linked to growth and field conditions (e. g., temperature, rainfall, and seasonality) [13]. Few studies have reported the influence of environmental factors on the chemical composition of certain medicinal species of the Lamiaceae family [14–16]. However, the correlation between these factors and *P. neochilus* volatile composition remains unknown and should be investigated to promote the safe and effective use of this species.

In order to evaluate these variations, untargeted metabolomics has recently emerged as a powerful tool to assess how an organism's metabolism varies in different situations, which can be applied to plants, animals, and microbes [17]. This approach relies on the fact that no preexisting knowledge of how a biological system behaves is necessary. Therefore, it aims to acquire the largest amount of information from a certain system of interest for further hypothesis generation [18]. In other words, untargeted metabolomics focuses on the analysis and detection of as many metabolites as possible within a targeted system in order to evaluate how these metabolites change according to a predefined factor [19] (e. g., environmental conditions). Different analytical techniques can be employed for that purpose, including NMR [20], LC-MS [21], and GC-MS [22], with the last one being more suitable for volatile compounds.

In this context, GC-MS-based untargeted metabolomics was employed to identify possible variations in the volatile composition of *P. neochilus* throughout a year. Thus, a complete annual profile of volatile composition under different conditions was assessed, indicating how these factors affect the composition of this medicinal plant.

Results

The processing and manual filtration of raw GC-MS data generated 24 mass features of which 21 were identified. ▶ **Table 1** describes the identification of the associated chromatographic peak from each mass feature and the coefficient of variation (CV) among all samples. As the CV is a measure of the variability, the mass features that showed the highest variation percentage in our study were *(2E)*-hexenal, 3-octanone, δ-3-carene, α-ocimene, *(3Z)*-hexenyl acetate, myrcene, and α-cubebene, respectively, with a CV over 60.0 %. After the mass feature identification, univariate and multi-

▶ **Table 1** Detected mass features from the volatile composition of *P. neochilus* leaves analyzed via HS-SPME/GC-MS. The mass feature values reflect the fragment ion *m/z* followed by the retention time (min). The coefficient of variation (CV) reflects the percentage of variation among all samples.

Number	Mass feature	Compound	RI[a]	CV (%)
1	41_3.21	*(2E)*-Hexenal	846	104.7
2	93_4.48	α-Thujene	924	33.3
3	77_4.55	α-Pinene	932	34.76
4	91_4.76	Thuja-2,4(10)-diene	953	38.97
5	136_5.44	Sabinene	969	33.01
6	93_5.50	UF1	–	43.67
7	57_5.56	1-Octen-3-ol	974	31.35
8	57_5.70	3-Octanone[b]	979	93.67
9	93_5.77	Myrcene	988	62.11
10	59_5.89	3-Octanol	988	33.18
11	67_6.17	*(3Z)*-Hexenyl acetate[b]	1004	70.76
12	93_6.32	δ-3-Carene	1008	93.48
13	119_6.71	o-Cymene	1022	59.93
14	109_6.76	UF2	–	45.52
15	68_6.83	Limonene	1024	35.62
16	93_7.06	*(E)*-β-Ocimene	1044	40.49
17	93_7.38	α-Ocimene	1052	85.19
18	105_18.73	α-Cubebene	1348	60.07
19	119_19.76	α-Copaene	1374	41.93
20	81_20.11	β-Bourbonene	1387	55.59
21	161_20.32	β-Cubebene	1387	43.39
22	93_21.54	*(E)*-Caryophyllene	1417	40.69
23	161_23.95	Germacrene D	1480	39.69
24	119_24.92	UF3	–	46.68

UF = unidentified mass feature.; [a]Retention indexes described in Adams [23], with the exception of α-ocimene RI, which was described in Eom et al. [24] and Özel et al. [25].; [b]Identification only by comparison with the NIST11 library.

variate analyses were performed to evaluate variations between individuals and identify which mass features varied in relation to environmental factors (month and time of collection).

Although the four individuals used in this study were growing side by side on the field after being initially collected from different sites, genetic differences may induce variation in the volatile profile of the species. Therefore, the differences between individuals were evaluated. Out of the 24 mass features, 8 varied significantly between individuals (▶ **Table 2**). Tukey's HSD post hoc test showed that the volatile composition of individuals from Atibaia (**U**) differed the most from the other three individuals. The difference between the composition of Nova Odessa (**N**) and Barão Geraldo individuals (**B**) was statistically significant for thuja-2,4(10)-diene, *(3Z)*-hexenyl acetate, and the unidentified mass feature UF2. The compound *(3Z)*-hexenyl acetate also differed between individuals **N** and Paulínia (**C**). Finally, none of the volatile components varied significantly between individuals **B** and **C**, according to this analysis. Although the four individuals presented the same peaks in the chromatograms (**Fig. 1S, Supporting Information**), that is,

Table 2 One-way ANOVA with Tukey's HSD post hoc test of *P. neochilus* Schltr. volatile components that varied significantly (p<0.05) between individuals obtained from: **N** - Nova Odessa-SP, **B** - Barão Geraldo District, Campinas-SP, **U** - Atibaia-SP, and **C** - CPQBA, Paulínia-SP.

Mass Feature	Compound	P value	Tukey's HSD
91_4.76	Thuja-2,4(10)-diene	1.13×10^{-06}	N-B; U-B; U-C; U-N
109_6.76	UF2	6.32×10^{-05}	N-B; U-N
67_6.17	*(3Z)*-Hexenyl acetate	6.45×10^{-05}	N-B; N-C; U-N
161_23.95	Germacrene D	7.89×10^{-05}	U-B; U-C; U-N
119_6.71	o-Cymene	2.87×10^{-04}	U-B; U-C; U-N
41_3.21	*(2E)*-Hexenal	3.19×10^{-04}	U-B; U-C; U-N
93_21.54	*(E)*-Caryophyllene	1.99×10^{-03}	U-B; U-C; U-N
93_7.38	α-Ocimene	4.45×10^{-03}	U-N
UF = unidentified mass feature.			

roughly the same qualitative volatile composition, significant differences in the intensity of specific mass features were detected in our study between individuals throughout the year.

In order to evaluate if the time of day when samples were collected affected their volatile composition, all samples were labeled according to the period of harvest and analyzed using MetaboAnalyst software. Statistical analyses were performed considering the average results of morning and afternoon samples per individual and for all the individuals together. No separation of groups was observed in the principal component analysis (PCA), nor a significant difference for the t-test in both cases. Therefore, all four individuals of *P. neochilus* showed a similar volatile composition in both periods of the day.

Although the average intensity of individual compounds varied between individuals, it was imperative to evaluate if there was a general seasonal variation for this species. Thus, all the samples collected from the four individuals on the same day were grouped by the mean, except for **N** July 2017 sample data, which was removed from the analysis due to sample degradation. ▶ **Figure 1a** shows a heatmap of the monthly average variation in *P. neochilus* volatile composition, whilst ▶ **Fig. 1b** shows the local air temperature and rainfall, two environmental factors associated with seasonality. The winter months in the Southeast of Brazil are generally dry and cold, which coincided with a drop in the intensity of most components, mainly in July, August, and September, whilst an increase in intensity of most mass features was observed in hotter months (▶ **Fig. 1**). There was also an increase in the intensity of five compounds in October, namely, α-thujene, α-pinene, sabinene, limonene, and the unidentified mass feature UF1.

As indicated in ▶ **Fig. 1**, there was a change in the volatile profile of *P. neochilus* throughout a year. Therefore, in order to measure the correlation between the environmental factors and specific mass features, Spearman's rank correlation was performed (▶ **Fig. 2**). Correlations were observed between five mass features and air tempera-ture; the lower the temperature, the lower the intensity of 3-octanone, *(3Z)*-hexenyl acetate, *(E)-β*-ocimene, α-ocimene, and *(E)*-caryophyllene. This positive correlation was mainly detected for the minimum air temperature. Lastly, rainfall correlated positively with myrcene but resulted in a negative correlation coefficient with α-thujene, indicating that as rainfall increased, this mass feature showed a decrease in intensity (▶ **Table 3**).

Discussion

Comparing the results of the present study with previously published studies of *P. neochilus* headspace volatiles, El-Sakhawy et al. [26] reported the presence of over 100 compounds in the aerial parts of this species, 13 of which were similar to the ones identified in our study, including α-thujene, α-pinene, o-cymene, and *(E)*-caryophyllene. Qualitative and quantitative differences in a volatile profile may be due to the use of a different headspace solid-phase microextraction (HS-SPME) fiber for extraction, the plant materials, season of collection, or the difference in approaches used in each study. In this regard, El-Sakhawy et al. [26] reported a descriptive study of the species volatile composition, while in the present study, we used an untargeted metabolomics approach as a tool for detecting the variations in the volatile composition. This was the first study to use an untargeted metabolomics approach to evaluate this specie's volatile composition and the first attempt to correlate metabolic variation and environmental factors in *P. neochilus*.

Regarding *P. neochilus* essential oil, an important source of the species bioactive molecules, three of the main components reported in the literature [7, 8, 12] were also identified in our study: α-thujene, α-pinene, and caryophyllene. Two of these compounds, *(E)*-caryophyllene and α-thujene, may be partially responsible for the therapeutic properties of this species. It was reported that *(E)*-caryophyllene, for example, presented anti-inflammatory activity [27] and α-thujene-rich essential oils showed antimicrobial and antioxidant activities [28]. The mass features associated with α-thujene, α-pinene, and *(E)*-caryophyllene showed a CV of 33.30, 34.76, and 40.69 %, respectively. Most of the mass features varied more than these three compounds. Furthermore, these were the three most intense peaks in our chromatograms (**Fig. 1S, Supporting Information**), indicating that they may be used as markers of the volatile composition of *P. neochilus*.

Although *P. neochilus* is popularly cultivated in household gardens as a medicinal plant, it is not a domesticated species. Therefore, a degree of variability in its genes and metabolism is to be expected. The individuals were collected from different sites, which collectively allowed observation of trends in *P. neochilus* metabolism along a year. The intensity of *(E)*-caryophyllene was statistically different between **U** and the other three individuals. As it is one of the major volatile components found in this study, with reported bioactivity [27], this could eventually lead to a difference in activity between individuals. The other compounds that varied significantly between individuals were generally less intense. As chemotypes have been detected for other medicinal species of Lamiaceae [29–31], further investigation of the *P. neochilus* volatile profile through a population-based study could be undertaken to define if there are also different chemotypes in this species.
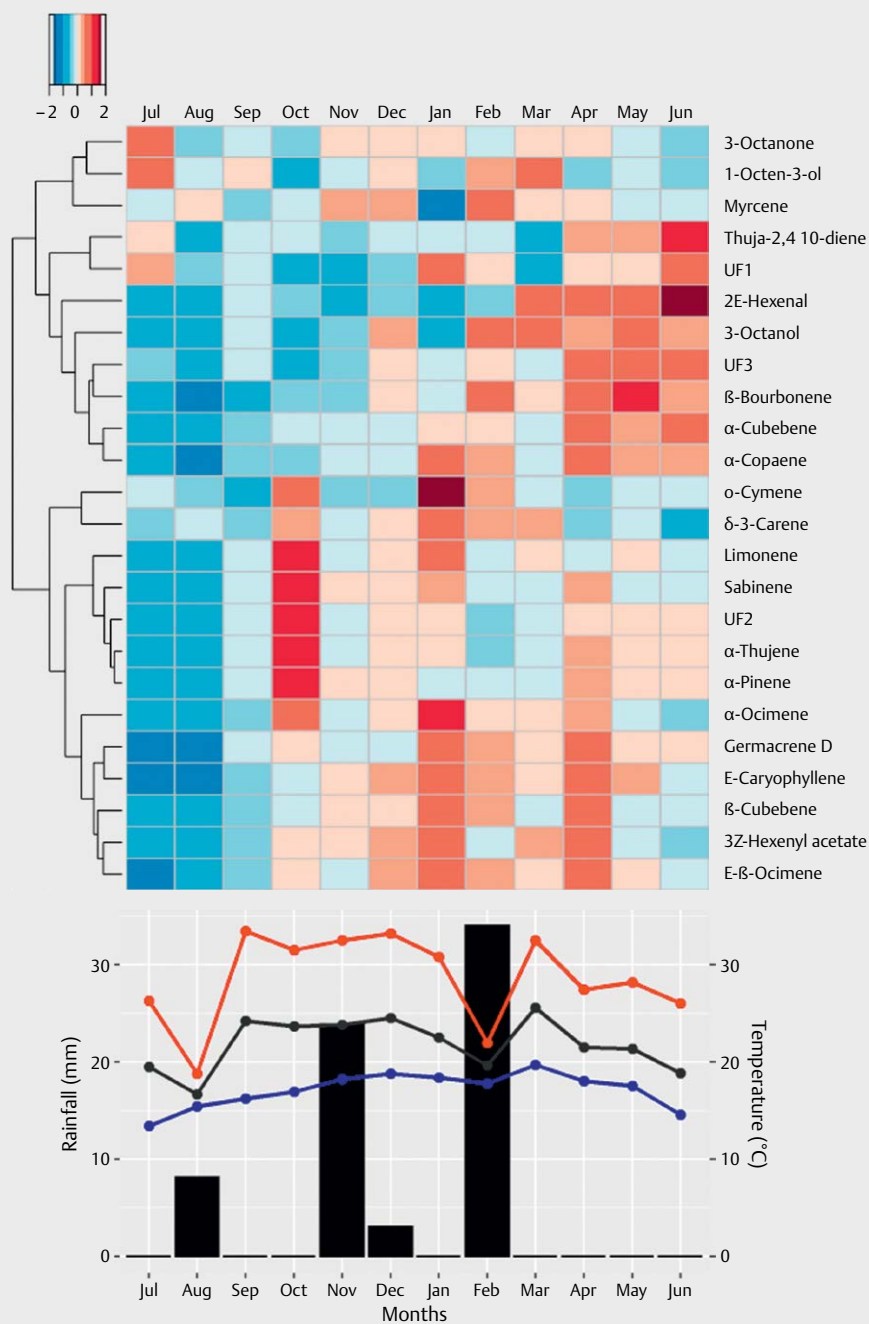
▶ **Fig. 1** **a** Heatmap of the relative intensity of the detected mass features (rows) in different months throughout a year (columns) - red and blue indicate increase and decrease in mass feature intensity, respectively, (autoscaling was performed as a pretreatment of the data). **b** Plot of the rainfall (bars) and maximum, minimum, and mean air temperatures (red, blue, and black lines, respectively) 1 day before sampling.

In addition, the effects of environmental factors on the *P. neochilus* volatile composition were also evaluated, correlating both the metabolic and climate data. The first environmental factor evaluated in our study was the time of the day when samples were collected, which did not affect the *P. neochilus* volatile composition despite changes in temperature, humidity, and luminosity throughout the day. Daily variation in volatile composition was detected for other Lamiaceae species, such as mint (*Mentha suaveolens* - La-

miaceae*)*, which showed higher levels of volatile compounds in the morning [32]. Our results indicate that the volatile composition of *P. neochilus* remains similar throughout the day, thus leaves of this species can be collected at any time without compromising the bioactivity.

Besides time of sampling, seasonality may induce changes in plant volatile composition. Seasonal variation of the composition of plants growing in a field is due to the combined effect of many
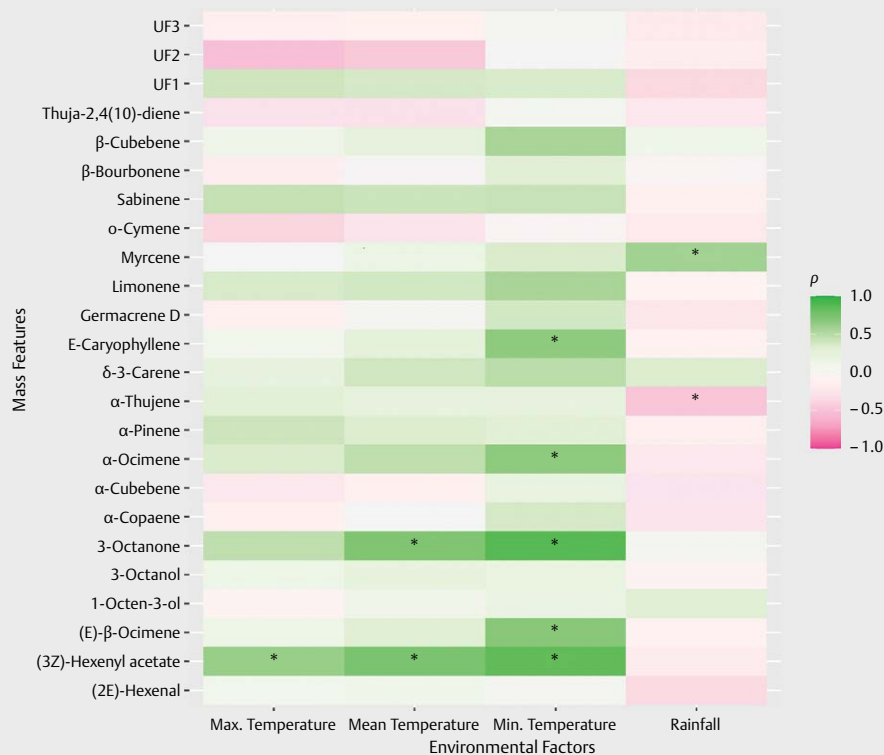
▶ **Fig. 2** Heatmap of the Spearman's rank correlation coefficient (ρ) between the detected mass features (rows) and environmental factors (columns) throughout a year (green and pink indicate positive and negative correlation, respectively). *Correlations with p<0.05.

▶ **Table 3** Correlation between the monthly average intensity of the detected mass features and environmental factors throughout a year via Spearman's rank correlation coefficient (ρ - p<0.005).

Minimum air temperature			
Mass feature	**Compound**	**p**	**ρ**
57_5.70	3-Octanone	0.0004	0.8741
67_6.17	(3Z)-Hexenyl acetate	0.0009	0.8462
93_7.06	(E)-β-Ocimene	0.0155	0.6923
93_7.38	α-Ocimene	0.0219	0.6643
93_21.54	(E)-Caryophyllene	0.0238	0.6573
Maximum air temperature			
Mass feature	**Compound**	**p**	**ρ**
67_6.17	(3Z)-Hexenyl acetate	0.0347	0.6224
Mean Air Temperature			
Mass feature	**Compound**	**p**	**ρ**
57_5.70	3-Octanone	0.0106	0.7203
67_6.17	(3Z)-Hexenyl acetate	0.0078	0.7413
Rainfall			
Mass feature	**Compound**	**p**	**ρ**
93_4.48	α-Thujene	0.0241	− 0.4785
93_5.77	Myrcene	0.0483	0.5866

factors, such as air temperature and rainfall (which were evaluated), as well as luminosity, wind, damage, flowering, etc. [13]. The winter months, mainly July and August, resulted in a general de-

crease in mass feature intensity. The main components previously discussed, α-thujene, α-pinene, and (E)-caryophyllene, followed this pattern.

As the winter months are generally cold and dry, Spearman's rank correlation between air temperature and rainfall with the intensity of each detected mass feature indicated some significant correlations. (E)-Caryophyllene along with some minor components (i. e., mass features No. 8, 11, 16, and 17 in ▶ **Table 1**) (**Fig. 1S, Supporting Information**) showed a positive correlation with air temperature; thus, the lower the air temperature, the lower the intensity of these features. Similarly, Romero et al. [33] showed a positive correlation between (3Z)-hexenyl acetate (mass feature No. 11 in ▶ **Table 1**) with maximum, mean, and minimum air temperature in olive oil (*Olea europaea* - Oleaceae), as compounds generated via the lipoxygenase pathway, such as (3Z)-hexenyl acetate [34], were affected by both temperature and evapotranspiration, which is higher during periods with a higher temperature.

A positive correlation was also established between rainfall and myrcene, a compound that showed analgesic activity in mice [35]. This compound may also be partially responsible for the popular use of *P. neochilus* for analgesic purposes, as well as other *Plectranthus* spp. popularly known as "boldo" [6]. Furthermore, a negative correlation between α-thujene and rainfall was detected. These results are partially similar to Aboukhalid et al. [36], who observed an increase in α-thujene, myrcene, carvacrol, and α-terpinene content in the essential oil of *Origanum compactum* (Lamiaceae) plants from areas with a semiarid climate. The combined effect of multi-

ple environmental factors as well as species-specific mechanisms of response to the environment may partially explain the discrepancy with the literature.

We also found that α-thujene and α-pinene as well as sabinene, limonene, and the unidentified mass feature UF1 showed an increase in October that could not be correlated to temperature. As rainfall and α-thujene resulted in a negative correlation, leading to a higher concentration in dry months, this may partially explain its increase in October (▶ **Fig. 1**). As this is the first attempt to evaluate the effects of environmental factors on *P. neochilus* volatile composition, the correlation analyses were performed with each environmental factor separately and some results detected herein have not yet been investigated in the literature.

Ultimately, the GC-MS-based untargeted metabolomics approach was successful in identifying the composition and variation of the headspace volatiles in leaves of *P. neochilus*. No changes in the volatile profile were observed between samples collected in the morning and afternoon, indicating that leaves of this species can be collected at any time during the day without compromising their activity. Moreover, individual metabolic variation, seasonal trends, and correlations between environmental factors and mass features intensities were detected. Our findings suggest that these environmental factors affect *P. neochilus* volatile composition and may ultimately result in variation in the bioactivity of this species. Therefore, further investigation on the effect of each environmental factor on *P. neochilus* composition and activity is needed to fully elucidate these interactions.

Materials and Methods

Plant material

Stem cuttings were collected from *P. neochilus* plants growing in four different cities in São Paulo (SP), Brazil, and rooted in vermiculite in early 2017. Subsequently, after 45–60 days of rooting, the individuals were cultivated in beds in the Experimental Field of the Institute of Biology, State University of Campinas (Unicamp - São Paulo, Brazil). The substrate used was potting soil, sand, and topsoil in the same proportion. Plant species was identified by Dr. Juliana Lischka Sampaio Mayer (Department of Plant Biology, Unicamp) and voucher specimens were deposited in the Unicamp Herbarium (UEC) under the following access numbers according to the place of collection: Atibaia-SP (individual **U** - UEC150953), Barão Geraldo District, Campinas-SP (individual **B** - UEC193819), Chemical, Biological and Agricultural Pluridisciplinary Research Center (CPQBA), Paulínia-SP (individual **C** - UEC1938817), and Nova Odessa-SP (individual **N** - UEC193818).

Sampling and climate data

Fresh leaves of each individual were collected at 8:00 a.m. and 2:00 p.m. on the same day during the third week of each month, from July 2017 up to June 2018. After harvesting, the leaves were immediately frozen in liquid nitrogen and stored at −80 °C until the end of sampling. The climate data were obtained from the Center for Meteorological and Climatic Research Applied to Agriculture (CEPAGRI, Unicamp) [37] for the period of the experiment.

Sample preparation and headspace solid-phase microextraction

The leaves were ground while frozen and 0.5 g of each sample was placed in 20 mL SPME glass vials, in duplicate. An *n*-alkane standard solution (C8 - C20; Sigma-Aldrich) was also used for the measurement of the retention indexes. Four different SPME fiber assemblies obtained from Supelco were initially evaluated using pooled samples and the GC-MS method adapted from Adams, RP (2007) [23]: polydimethylsiloxane (PDMS), carboxen/PDMS (CAR/PDMS), PDMS/divinylbenzene (PDMS/DVB), and DVB/CAR/PDMS). The PDMS/DVB fiber assembly was selected for the metabolomic analyses, as it adsorbed both monoterpenes and sesquiterpenes in similar proportions to the essential oil in comparison to the other fiber assemblies that were more selective for either class of terpenes. The optimized sampling temperature and extraction time were defined as 50 °C for 5 min. Quality control (QC) samples were also prepared using equal parts of each sample as a quality assurance procedure. **Fig. 2S, Supporting Information**, shows the repeatability of the optimized method, as QC samples were closely related and grouped in the middle of the PCA graph.

Chromatographic analyses

The analyses were performed using an Agilent 7890 A gas chromatograph (Agilent Technologies) coupled to an Agilent Model 5975 C inert MSD with triple-axis detector (Agilent Technologies) and a Gerstel MPS2 Autosampler (Gerstel). Separation of the metabolites was performed in an HP-5ms fused silica capillary column (30 m × 0.25 mm × 0.25 µm film thickness; Agilent J&W), with high purity helium as the carrier gas at a flow rate of 1 mL/min. The chromatographic method was modified from Adams to shorten the analysis time due to the large number of samples. The injector was maintained at a temperature of 240 °C with a 1:3 split ratio and the interface at 220 °C. The mass spectrometer was operated in full scan mode (m/z 40–500). The temperature ramp started at 65 °C increasing to 150 °C at 3 °C/min, totalizing 28.33 min per analysis. The chromatographic peaks were identified by both mass spectra comparison to NIST11 mass spectral library (similarity ≥ 90 %) and comparison of the calculated retention indexes to the retention indexes described in Adams [23] (variation<10). If these criteria were not met, the compound was considered unidentified.

Data processing and statistical analysis

For processing of the raw GC-MS data, XCMS online software was used. The parameters applied are described in **Table 1S, Supporting Information**. For normalization, the support vector regression method was applied using the MetNormalizer R package [38]. After normalization, data was submitted to manual filtration in order to remove redundant information in Microsoft Excel (version 2010) software.

As each chromatographic peak resulted in several mass features, a filtration process was called for in order to remove redundant information. On this basis, the detected mass features were compared to the original mass spectra and manually filtered selecting the features that were base peak ions. If this condition was not met, mass features with the highest abundance in each chromatographic peak were selected, resulting in a single mass feature per chro-

matographic peak. Microsoft Excel (version 2010) software was used for calculation of the CV and MetaboAnalyst online software as well as R software were used for data autoscaling (mdatools package for R), statistical analysis (i. e., ANOVA and t-tests), and chemometric methods (i. e., PCA and mass feature heatmaps) [39, 40].

The correlation between metabolomic and climate data was also performed via Spearman's correlation test, applied using Graph-Pad Prism (version 6.01) software. The climate data from 1 day prior to sampling were selected.

Data Availability

Raw mass feature data can be directly accessed using the following link:

https://xcmsonline.scripps.edu/share/view_job_overview. php?jobid = 1410946

Supporting Information

A typical chromatogram of *P. neochilus* leaf volatiles, PCA, and XCMS online GC/single quad parameters are available as Supporting Information.

Conflict of Interest

The authors declare they have no conflict of interest.

References

[1] Solecki RS. Shanidar IV, a Neanderthal flower burial in Northern Iraq. Science 1975; 190: 880–881

[2] Petrovska BB. Historical review of medicinal plants' usage. Pharmacogn Rev 2012; 6: 1–5

[3] Milevskaya VV, Prasad S, Temerdashev ZA. Extraction and chromatographic determination of phenolic compounds from medicinal herbs in the Lamiaceae and Hypericaceae families: A review. Microchem J 2019; 145: 1036–1049

[4] Michel J, Abd Rani NZ, Husain K. A Review on the Potential Use of Medicinal Plants from Asteraceae and Lamiaceae Plant Family in Cardiovascular Diseases. Front Pharmacol 2020; 11: 1–26

[5] Codd L. Plectranthus (Labiatae) and allied genera in Southern Africa. Bothalia 1975; 11: 371–442

[6] Lorenzi H, Matos FJ, de A. Plantas medicinais no Brasil: Ncativas e exóticas. 2. ed. Nova Odessa: Instituto Plantarum; 2008

[7] Caixeta SC, Magalhães LG, de Melo NI, Wakabayashi KA, Aguiar Gde P, Aguiar Dde P, Mantovani ALL, Alves JM, Oliveira PF, Tavares DC, Groppo M, Rodrigues V, Cunha WR, Veneziani RC, da Silva Filho AA, Crotti AE. Chemical composition and in vitro schistosomicidal activity of the essential oil of Plectranthus neochilus grown in Southeast Brazil. Chem Biodivers 2011; 8: 2149–2157

[8] Mota L, Figueiredo AC, Pedro LG, Barroso JG, Miguel MG, Faleiro ML, Ascensão L. Volatile-oils composition, and bioactivity of the essential oils of Plectranthus barbatus, P. neochilus, and P. ornatus grown in Portugal. Chem Biodivers 2014; 11: 719–732

[9] Lawal OA, Hutchings AH, Oyedeji O. Chemical Composition of the Leaf Oil of Plectranthus neochilus Schltr. J Essent Oil Res 2010; 22: 546–547

[10] Rosal LF, Pinto JEBP, Bertolucci SKV, Brant R, da S, Nicolau E, dos S, Alves PB. Produção vegetal e de óleo essencial de boldo pequeno em função de fontes de adubos orgânicos. Rev Ceres 2011; 58: 670–678

[11] Baldin ELL, Crotti AEM, Wakabayashi KAL, Silva JPGF, Aguiar GP, Souza ES, Veneziani RCS, Groppo M. Plant-derived essential oils affecting settlement and oviposition of Bemisia tabaci (Genn.) biotype B on tomato. J Pest Sci 2013; 86: 301–308

[12] Crevelin EJ, Caixeta SC, Dias HJ, Groppo M, Cunha WR, Martins CHG, Crotti AEM. Antimicrobial Activity of the Essential Oil of Plectranthus neochilus against Cariogenic Bacteria. Evid Based Complement Alternat Med 2015; 2015: 1–7

[13] Gobbo-Neto L, Lopes NP. Plantas medicinais: Fatores de influência no conteúdo de metabólitos secundários. Quim 2007; 30: 374–381

[14] Carneiro FB, Júnior ID, Lopes PQ, Macêdo RO. Variação da quantidade de β-cariofileno em óleo essencial de Plectranthus amboinicus (Lour.) Spreng., Lamiaceae, sob diferentes condições de cultivo. Rev Bras Farmacogn 2010; 20: 600–606

[15] Novak J, Lukas B, Franz C. Temperature Influences Thymol and Carvacrol Differentially in Origanum spp. (Lamiaceae). J Essent Oil Res 2010; 22: 412–415

[16] Mehalaine S, Chenchouni H. Effect of climatic factors on essential oil accumulation in two Lamiaceae species from Algerian semiarid lands. In: Chenchouni H, Errami E, Rocha F, Sabato L, Hrsg. Exploring the nexus of geoecology, geography, geoarcheology and geotourism: advances and applications for sustainable development in environmental sciences and agroforestry research. Cham: Springer International Publishing; 2019: 57–60

[17] Cajka T, Fiehn O. Toward Merging Untargeted and Targeted Methods in Mass Spectrometry-Based Metabolomics and Lipidomics. Anal Chem 2016; 88: 524–545

[18] Schrimpe-Rutledge AC, Codreanu SG, Sherrod SD, McLean JA. Untargeted Metabolomics Strategies-Challenges and Emerging Directions. J Am Soc Mass Spectrom 2016; 27: 1897–1905

[19] Dudzik D, Barbas-Bernardos C, García A, Barbas C. Quality assurance procedures for mass spectrometry untargeted metabolomics. a review. J Pharm Biomed Anal 2018; 147: 149–173

[20] Kim HK, Choi YH, Verpoorte R. NMR-based metabolomic analysis of plants. Nat Protoc 2010; 5: 536–549

[21] Wang G. LC-MS in plant metabolomics. In: Plant Metabolomics. Dordrecht: Springer Netherlands; 2015: 45–61

[22] Jousse C, Pujos-Guillot E. Chapter six - exploring metabolome with GC/MS. In: Rolin D, Hrsg. Metabolomics coming of age with its technological diversity. Elsevier Science & Technology; 2013: 303–329

[23] Adams RP. Identification of essential oil components by gas chromatography/mass spectrometry. 4th edition. Carol Stream: Allured Publishing Corporation; 2007

[24] Eom SH, Yang HS, Weston LA. An evaluation of the allelopathic potential of selected perennial groundcovers: foliar volatiles of catmint (Nepeta×faassenii) inhibit seedling growth. J Chem Ecol 2006; 32: 1835–1848

[25] Özel MZ, Göğüş F, Lewis AC. Determination of Teucrium chamaedrys volatiles by using direct thermal desorption–comprehensive two-dimensional gas chromatography–time-of-flight mass spectrometry. J Chromatogr A 2006; 1114: 164–169

[26] El-Sakhawy FS, Kassem HA, El-Gayed SH, Mostafa MM. Headspace Solid Phase Microextraction Analysis of Volatile Compounds of the Aerial Parts and Flowers of Plectranthus neochilus Schltr. and Salvia farinacea Benth. J Essent Oil Bear Plants 2018; 21: 674–686

[27] Fernandes ES, Passos GF, Medeiros R, da Cunha FM, Ferreira J, Campos MM, Pianowski LF, Calixto JB. Anti-inflammatory effects of compounds alpha-humulene and (−)-trans-caryophyllene isolated from the essential oil of Cordia verbenacea. Eur J Pharmacol 2007; 569: 228–236

[28] Gupta M, Rout PK, Misra LN, Gupta P, Singh N, Darokar MP, Saikia D, Singh SC, Bhakuni RS. Chemical composition and bioactivity of Boswellia serrata Roxb. essential oil in relation to geographical variation. Plant Biosyst 2017; 151: 623–629

[29] Pourhosseini SH, Hadian J, Sonboli A, Ebrahimi SN, Mirjalili MH. Genetic and Chemical Diversity in Perovskia abrotanoides Kar. (Lamiaceae) Populations Based on ISSRs Markers and Essential Oils Profile. Chem Biodivers 2018; 15: 1–14

[30] Muñoz-Acevedo A, González MC, Rodríguez JD, De Moya YS. New Chemovariety of Lippia alba From Colombia: Compositional Analysis of the Volatile Secondary Metabolites and Some in vitro Biological Activities of the Essential Oil From Plant Leaves. Nat Prod Commun 2019; 14: 1–7

[31] Bakha M, El Mtili N, Machon N, Aboukhalid K, Amchra FZ, Khiraoui A, Gibernau M, Tomie F, Al Faiz C. Intraspecific chemical variability of the essential oils of Moroccan endemic Origanum elongatum L. (Lamiaceae) from its whole natural habitats. Arab J Chem 2020; 13: 3070–3081

[32] Llorens-Molina JA, Rivera Seclén CF, Gonzalez SV, Tortajada HB. Mentha suaveolens Ehrh. Chemotypes in Eastern Iberian Peninsula: Essential Oil Variation and Relation with Ecological Factors. Chem Biodivers 2017; 14: 1–9

[33] Romero N, Saavedra J, Tapia F, Sepúlveda B, Aparicio R. Influence of agroclimatic parameters on phenolic and volatile compounds of Chilean virgin olive oils and characterization based on geographical origin, cultivar and ripening stage. J Sci Food Agric 2016; 96: 583–592

[34] Vincenti S, Mariani M, Alberti J-C, Jacopini S, Brunini-Bronzini de Caraffa V, Berti L, Maury J. Biocatalytic Synthesis of Natural Green Leaf Volatiles Using the Lipoxygenase Metabolic Pathway. Catalysts 2019; 9: 1–35

[35] Paula-Freire LIG, Molska GR, Andersen ML, Carlini EL. Ocimum gratissimum Essential Oil and Its Isolated Compounds (Eugenol and Myrcene) Reduce Neuropathic Pain in Mice. Planta Med 2016; 82: 211–216

[36] Aboukhalid K, Al Faiz C, Douaik A, Bakha M, Kursa K, Agacka-Mołdoch M, Machon N, Tomi F, Lamiri A. Influence of Environmental Factors on Essential Oil Variability in Origanum compactum Benth. Growing Wild in Morocco. Chem Biodivers 2017; 14: 1–17

[37] CEPAGRI. Centro de Pesquisas Meteorológicas e Climáticas aplicadas à Agricultura. 2018. Available at: https://www.cpa.unicamp.br/

[38] Shen X, Gong X, Cai Y, Guo Y, Tu J, Li H, Zhang T, Wang J, Xue F, Zhu ZJ. Normalization and integration of large-scale metabolomics data using support vector regression. Metabolomics 2016; 12: 1–12

[39] MetaboAnalyst 5.0. Available at https://www.metaboanalyst.ca/

[40] Xia J, Wishart DS. Metabolomic data processing, analysis, and interpretation using MetaboAnalyst. Curr Protoc Bioinforma 2011; 34: 1–48