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Chemical Diversity and Redox Values Change as a Function of Temporal Variations of the Essential Oil of a Tropical Forest Shrub

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Abstract: This study investigated the chemical phenotypical variability of *Piper lhotzkyanum* Kunth (Piperaceae), a shrub found in Brazilian tropical forests, over time (different periods of the day and seasons) and under natural conditions. For this, essential oils (EOs) from the leaves were collected in different seasons and times of the day and analyzed by gas chromatography coupled with mass spectrometry, and gas chromatography coupled with a flame ionization detector. The indices were applied to evaluate the chemical diversity as well as the dynamics of redox of the mixtures. The results showed that the EOs were dominated by non-oxygenated sesquiterpenes, with β-elemene, *E*-caryophyllene, and α-zingiberene being the main compounds identified in all collections. Temporal and seasonal analyses revealed important fluctuations in the chemical composition, redox, and chemical diversity indices of the species. A correlation between climatic factors and the variation in redox and chemical diversity was observed, highlighting the chemical phenotypic plasticity *P. lhotzkyanum*. This study resolves a previously unanswered question by confirming that natural light does not produce interconversion of major compounds. The adaptation capacity of this species to the environmental changes suggests new cultivation strategies to maximize the quality of EO and promote a more sustainable future in partnership with nature.

Keywords: *Piper lhotzkyanum*; Piperaceae; chemical phenoplasticity; seasonality; circadian

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1. Introduction

Chemical diversity is a crucial aspect of natural products, and studying this aspect is vital for understanding ecological niches and identifying potential pharmacological activities for humans [1]. Several factors may influence chemical diversity, including environmental conditions, genetic makeup, and interactions with other organisms. Particularly in medicinal plants, a variety of secondary compounds can be crucial for both treatment effectiveness and safety [2].

One important concept related to chemical diversity is chemodiversity, which refers to the diversity of chemical compounds found within a given ecosystem [3]. The study of chemodiversity can provide valuable insights into ecological interactions [2]. Moreover, with natural products drawn from these environments acting as an abundant source of novel pharmaceuticals, the chemical variety of tropical rainforests, for instance, has been a propelling factor in drug development efforts [3,4]. Understanding changes in metabolites promotes comprehension of the functioning of ecological niches and holds direct implications for human health [2–4].

Chemical diversity is also critical for the development of herbal medicines and other natural products. The pharmaceutical industry must consider the chemical stability of natural products, and identifying stability-indicating factors is essential for ensuring product quality and safety. A recent study evaluated the photostability of the essential oil (EO) of *Piper lhotzkyanum* Kunth (the object of this study), a popular medicinal plant whose leaves are used for respiratory problems, gastrointestinal problems, rheumatism, and as a sedative for various pains. This study showed that light exposure in laboratory conditions (light providing an overall illumination of not less than 1.2 million lux hours) can significantly alter the composition of the oil and potentially affect its therapeutic efficacy [5].

Given that *P. lhotzkyanum* is often collected in the wild for medicinal purposes, it is worth considering the potential influence of natural environmental factors on its chemical stability, since alterations due to these factors can affect the pharmacological and biological activity of this species for the user community [5]. Specifically, could the instability related to light exposure observed in the laboratory occur over seasons and days in the natural environment and affect harvesting/collection systems carried out by the population that practices agroforestry management of this species?

Previous research has shown that species of the Piperaceae family, to which *P. lhotzkyanum* belongs, exhibit significant chemical phenotypic variability and diversity in relation to volatile constituents, particularly in the qualitative and quantitative aspects of their considerable variation in the qualitative and quantitative diversity of EOs volatile compounds [5]. Depending on the space and time of collection and the season of the year, for example, the chemical composition can change considerably [6,7]. However, the temporal variability (seasonal and daily) of foliar EOs of a natural population of *P. lhotzkyanum* has not been evaluated. Therefore, this manuscript aims to assess this chemical variation (qualitative and quantitative), correlate it with environmental factors, and verify if its chemical composition is affected by light in nature.

2. Materials and Methods

2.1. Plant Material

Leaves of *Piper lhotzkyanum* Kunth without signs of disease or herbivory were collected in the Atlantic Forest region in the Serra dos Órgãos National Park (humid tropical climate all year round), located in the city of Teresópolis in the state of Rio de Janeiro, Brazil, which is 1144 m above sea level (coordinates: 12°11′45″ S, 38°58′05″ W). The botanical material was identified by Doctors Elsie Franklin Guimarães and George Azevedo Queiroz and a voucher RB01426181 was deposited in the Herbarium of the Botanical Garden Research Institute of Rio de Janeiro, Brazil. The authorization to collect plants was granted by the Chico Mendes Institute for Biodiversity Conservation, number 57296-1. This study was registered with the Genetic Heritage Management Council under code AE4E953.

2.2. Experimental Design

The experimental design comprised 24 collections to evaluate temporal variations, with 8 and 16 collections for annual and daily variations, respectively. Each collection was made in triplicate using three specimens from the same individuals under similar conditions in the area. For the evaluation of seasonal (annual) temporal variation, 100 g of fresh leaves were sampled during the autumn (March and May 2019), winter (July and August 2019), spring (October and November 2019), and summer months (January and February 2020). Samples were taken monthly on the 15th day, between 9:00 am and 10:00 am, according to seasonal separation criteria. For the evaluation of circadian (daily) temporal variation, 100 g of fresh leaves were sampled at 6:00 am, 9:00 am, 12:00 pm, 3:00 pm, 6:00 pm, 9:00 pm, 12:00 am, and 3:00 am on 15–16th July 2019 (dry season/high daily solar radiation/vegetative phase) and 15–16th January 2020 (rainy season/low daily solar radiation/reproductive phase). The leaves were stored under refrigeration in dry ice in dark Ziploc® bags until EO extraction, which was performed on the same day of collection (Ramos et al., 2020). Data on abiotic factors, including precipitation (mm), radiation (KJ.m^{-2}), mean temperature (°C), and humidity (%) at the study site and time of collection were obtained from the Brazilian Institute of Metrology and Research (http://www.inmet.gov.br/portal accessed on 1 January 2022) for the weather station (A618–OMM: 86888), which is equipped with a Vaisala system, model MAWS 301 (Vaisala Corporation, Helsinki, Finland), and are presented in the Supplementary Materials (Figure S1).

2.3. Essential Oil Extraction

The collected leaves (100 g) were manually crushed with scissors and placed in a 2 L glass round-bottom flask containing 700 mL of distilled water. The mixture was subjected to hydrodistillation for three hours using a Clevenger-type apparatus (Wasicky, 1963). The resulting EO were dried over anhydrous sodium sulfate (Na_2SO_4, Sigma-Aldrich, São Paulo, Brazil), filtered, and stored at -4 °C until further analysis. Yields were expressed as the percentage of fresh plant material (g/100 g) [8].

2.4. Essential Oil Analysis

The EOs were diluted in dichloromethane (1 mg/mL) (Merck, Brazil) and subjected to gas chromatography coupled with mass spectrometry (GC-MS) for identification, and GC coupled to a flame ionization detector (GC-FID) for compound quantification. GC-MS analysis was carried out using an HP 6890 GC coupled to an Agilent MS 5973N mass spectrometer (Hewlett-Packard, Brazil) operating at 70 eV ionization energy in positive mode, with a mass range of m/z 40–600 atomic mass units (u). The GC conditions were an HP-5MS capillary column (30 m × 0.25 mm ID × 0.25 μm film thickness) with helium (~99.999%) as the carrier gas at a constant flow rate of 1.0 mL/min. Temperature programming was from 60 °C to 240 °C, with an increase of 3 °C/min. Quantitative data on volatile constituents were obtained by normalizing the peak area using an HP-Agilent 6890 gas chromatograph flame ionization detector (Hewlett-Packard, Brazil), operating under conditions of an HP-5MS capillary column (30 m × 0.25 mm ID × 0.25 μm film thickness), with temperature settings from 60 °C to 240 °C, an increase of 3 °C/min, using hydrogen as the carrier gas at a constant flow rate of 1.0 mL/min. The injector and detector temperatures were set at 270 °C, and samples were injected at 1 μL splitless [8]. Retention index (RI) and peak area quantification were obtained based on GC-FID results. The relative percentage of individual components was calculated based on GC peak areas without FID response factor correction. Linear retention indices (LRIs) were calculated for separate compounds relative to *n*-alkanes (C_8–C_{28}, Sigma-Aldrich, Brazil). Constituents were identified by comparison of their calculated LRIs with those in the literature, and by comparison of the mass spectrum with those recorded by the NIST library (National Institute of Standards and Technology) "NIST14" and Wiley (ChemStation data system) "WILEY7*n*." [8]. Additionally, authentic pattern co-injection was performed whenever possible [8].

2.5. Chemical Diversity, Micromolecular Parameters and Chemometric Analysis

To evaluate the chemical diversity (H') of EOs for facilitating temporal comparisons, the Shannon–Wiener Index was used [2], as follows:

$$H' = -\sum P_i ln P_i$$

In these equations, Pi is equivalent to the proportional abundance of the respective compound, obtained by dividing the quantity determined by GC-FID by the total number of compounds identified in the sample, of which i is that number.

To characterize the micromolecular oxidation–reduction of the EO mixture, the Ramos and Moreira Index (R&M) for mixtures was applied [9]. The equation is given below:

$$I_{RM} = \frac{\sum S_{RO}}{N_{CI}}$$

In these equations, the S_{RO} is the Weighted Average Redox Standard of the compound under investigation and is calculated by multiplying the quantitative sum of the oxidation states of the carbon atoms present in the compound identified in the sample, and then dividing the result by the number of carbon atoms in the molecular structure (n). The R&M is then obtained by summing the S_{RO} of all compounds in the mixture, divided by the number of compounds identified (N_{CI}) in the sample. A lower R&M value signifies that the mixture has a lower average oxidation state relative to a sample with a higher R&M value [9], indicating a higher proportion of oxidized compounds.

To evaluate the pattern of redox homeostasis (R_{ho}), a new equation is proposed; the sum of R&M obtained by subtracting the final time (t_f) from the initial time (t_i) of each sample, collected over different time intervals, was calculated from seasonal and circadian temporal variation studies. The equation is given below:

$$R_{ho} = \sum\left(t_f - t_i\right)$$

All data on the percentage of compounds in EO were reported as the mean for three independent experiments (extraction). Statistical significance was evaluated by Tukey's test (ANOVA by Tukey HSD post hoc test) and significance was set at $p < 0.05$. Depending on the normality of the data, Pearson and Spearman correlation coefficients (r) were applied. These were calculated to determine the relationship between the analyzed parameters (climate factors vs. compounds, compounds vs. compounds, chemical diversity vs. components). Principal Component Analysis (PCA), Hierarchical Cluster Analysis (HCA) and Heat Map were applied to verify the interrelation in the composition of the EO collected at different time intervals. All analyses were performed using Statistica® 12 software version 10 (StartSoft Inc., Tulsa, OK, USA) and OriginPRO® 10.0 software (OriginLab, Northampton, MA, USA).

3. Results

3.1. Essential Oil Yields

The EOs presented vibrant yellow colors with a characteristic strong citrus aroma impression yielding between 0.01 to 0.28% (*w*/*w*) (Tables 1 and 2).

Table 1. Results of the seasonal temporal analysis of Essential Oils from *Piper lhotzkyanum* Kunth (Piperaceae) Leaves Collected in 2019–2020: Yields, Diversity Index (H′), Weighted Average Redox Standard (S_{RO}), and Ramos & Moreira Index (R&M).

Compound	RI_{cal}	RI_{lit}	**Percentual Relative Area (%) ***							
			2019						**2020**	
			Autumn		**Winter**		**Spring**		**Summer**	
			Mar	**May**	**Jul**	**Ago**	**Oct**	**Nov**	**Jan**	**Feb**
α-Thujene	920	924							tr	tr
α-Pinene	**929**	**932**	**6.2**	**6.8**	**1.6**	**1.0**	**0.4**	**1.2**	**7.0**	**3.4**
Camphene	942	946							tr	tr
Sabinene	969	969			tr				tr	
β-Pinene	**969**	**974**	**1.8**	**7.9**	**1.9**	**1.1**	**1.1**	**1.3**	**4.5**	**2.1**
6-Methyl-5-hepten-2-one	981	981		0.3	0.1				0.1	tr
Myrcene	987	988		0.3	tr	0.1		0.2	tr	tr
α-Phellandrene	1000	1002	0.1	0.2	tr		0.5		tr	tr
α-Terpinene	1010	1014	0.2		0.3			0.6	0.4	tr
ρ-Cymene	1018	1022			tr				tr	tr
Limonene	1022	1024		1.2	0.3				1.6	tr
β-Phellandrene	1023	1025			0.8					
1,8-Cineole	1027	1026							tr	tr
Z-β-Ocimene	1031	1032	1.1	0.6	tr	0.6	0.5		0.8	tr
***E*-β-Ocimene**	**1042**	**1044**	**4.7**	**5.0**	**3.1**	**5.2**	**2.3**	**3.0**	**12.6**	**3.4**
γ-Terpinene	1052	1054	0.4	0.6	0.5	0.3		0.3	tr	tr
Terpinolene	1081	1086	0.2		0.1				0.2	tr

Table 1. *Cont.*

Compound	**RI_{cal}**	**RI_{lit}**	**Percentual Relative Area (%) ***							
			2019						**2020**	
			Autumn		**Winter**		**Spring**		**Summer**	
			Mar	**May**	**Jul**	**Ago**	**Oct**	**Nov**	**Jan**	**Feb**
Linalool	1090	1095	0.1		0.1		0.4		tr	tr
E-4,8-Dimethyl-1,3,7-nonatriene	1099	-	0.2				0.3	0.1		tr
Perillene	1108	1102			0.2					
Allo-ocimene	1130	1128	1.5		1.0	0.3		0.2		tr
Terpinen-4-ol	1175	1174			tr					
α-Terpineol	1183	1186	0.1							tr
Linalyl formate	1210	1214		1.7						
NI MW194	1265	-			tr	tr	0.3		tr	tr
Bicycloelemene [#]	**1312**	**-**	**4.1**	**1.3**	**3.3**	**1.5**	**4.5**	**4.9**	**1.2**	**5.8**
δ-Elemene	1335	1335							2.7	
α-Cubebene	1349	1348	0.1		0.1			0.3		2.5
α-Copaene	1370	1374			0.6	0.4	0.3			0.5
β-Cubebene	1384	1387			tr					
β-Elemene	**1391**	**1389**	**59.6**	**24.0**	**49.4**	**66.1**	**23.0**	**62.0**	**16.1**	**31.0**
Cyperene	1396	1398		0.3	tr					tr
Italicene	1404	1405	tr							
α-Gurjunene	1407	1409	tr	0.6	0.2					0.8
***E*-Caryophyllene**	**1413**	**1417**	**5.7**	**4.9**	**6.3**	**5.3**	**8.5**	**6.1**	**1.8**	**4.3**
E-α-Ionone	1425	1428							tr	
γ-Elemene	1430	1434	0.4	0.7	0.4	0.2	2.0	0.4	2.9	0.7
NI MW204	1436	-							tr	
Aromadendrene	1438	1439	0.1	0.3	0.3	0.2	1.0	0.3	1.4	1.6
Z-β-Farnesene	1440	1440			1.0	0.2	2.5			1.6
cis-Muurola-3,5-diene	1446	1448								0.3
Neryl propanoate	1449	1452		0.6						
α-Humulene	1450	1452	0.2	1.4	0.1	0.4	0.1	0.6	2.9	
E -β-Farnesene	1455	1454	0.1			tr		0.1	0.9	
Allo-aromadendrene	1457	1458	0.4		0.1			0.1		
4,5-di-*epi*-Aristolochene	1474	1471	tr		0.8			0.1	tr	1.1
β-Chamigrene	1477	1476	0.8	0.5	0.6	0.8	0.9	0.9	4.8	3.5
γ-Muurolene	1479	1478	0.5						tr	
ar-Curcumene	1481	1479		0.4			0.8			0.8
Germacrene D	1483	1484			0.1	0.4		0.03±		1.1
NI MW204	1487	-			tr	tr				
β-Selinene	**1489**	**1489**	**2.6**	**1.7**	**2.8**	**3.0**	**3.9**	**3.0**	**7.5**	**3.6**
α-Zingiberene	**1492**	**1493**		**6.9**	**0.1**		**16.9**			
Viridiflorene	1495	1496		0.6	tr	tr				4.1
α-Selinene	1497	1498							5.7	
Z-Dihydro-apofarnesal	1499	1498		0.3						
Biclogermacrene	**1500**	**1500**	**5.1**		**6.1**	**5.3**	**1.5**	**5.5**		**4.1**
α-Muurolene	1502	1500	0.1							
E,E-α-Farnesene	1504	1505			1.3	tr				
β-Bisabolene	1506	1505			0.3		0.4			
Germacrene A	1510	1508						0.1	1.6	tr
γ-Cadinene	1512	1513	0.1	1.7	0.2	0.1	0.4	0.1	tr	tr
Cubebol	1516	1514						tr		0.6
epi-7-α-Selinene	1519	1520	0.6							
Sesquiphelandrene	1522	1521			0.7		3.2			3.0
δ-Cadinene	1524	1522	0.2	1.6	0.8	0.5	2.1	0.6	tr	0.7
NI MW204	1525	-		tr						
Zonarene	1526	1528					tr			0.4
NI MW204	1528	-						tr		
cis-Calamenene	1530	1528			0.2	tr			tr	
NI MW204	1531	-			0.3	tr				
trans-Cadine-1,4-diene	1534	1533							tr	
α-cadinene	1536	1537		0.5		tr				
Selina-3,7(11)-diene	1542	1545	0.1	0.6			0.1		tr	0.8
Hedycariol	1547	1546								0.4
Germacrene B	1553	1559	0.7				0.8	0.2	tr	0.2
E-Nerolidol	1560	1561	0.1	1.1		0.7		0.6	tr	0.1
3-Hexenyl-*Z* -benzoate	1566	1565	0.3							
Spathulenol	1575	1577	0.2			0.2	0.7	0.1	tr	0.7

Table 1. *Cont.*

Compound	**RI$_{cal}$**	**RI$_{lit}$**	**Percentual Relative Area (%) ***							
			2019						**2020**	
			Autumn		**Winter**		**Spring**		**Summer**	
			Mar	**May**	**Jul**	**Ago**	**Oct**	**Nov**	**Jan**	**Feb**
Caryophyllene oxide	1580	1582		2.5		0.2	1.2	0.3	tr	0.6
NI MW204	1581	-				tr				
NI MW220	1588	-	tr	tr						tr
Globulol	1591	1590		1.2	0.1	0.4	0.4	0.2	tr	0.1
Viridiflorol	1594	1592	0.1		0.1	0.2	0.1		tr	
Longiborneol	1597	1599	tr		tr	tr	0.2	0.4		
Guayol	1600	1600		1.3						
Rosifoliol	1605	1600							tr	
Humulene II epoxide	1607	1608							tr	tr
NI MW220	1612	-			0.6	tr				
NI MW220	1617	-	0.1							
10-*epi*-γ-Eudesmol	1620	1622						tr		
Isolongifolanone	1623	1625								0.2
1-*epi*-α-Cubenol	1625	1627	0.1		0.1		0.8		0.4	0.3
γ-Eudesmol	1629	1630							tr	
NI MW220	1633	-					1.0			
α-Acorenol	1635	1632	0.1							
Selina-1,3,7(11)-trien-8-one	1637	1632		tr						
epi-α-Cadinol	1637	1638			0.1	0.1	1.2	0.1		
Caryophyll-4(12),8(13)-dien-5-ol	1636	1639							tr	0.1
Hinesol	1638	1640		1.2						
epi-α-Muurolol	1639	1640			tr	0.2	2.2	0.1		
Selina-3,11-dien-6α-ol	1641	1642	0.1	1.0					0.8	
α-Muurolol	1643	1644		1.9	2.3					0.6
NI MW220	1645	-								0.1
Cubenol	1646	1645							tr	
NI MW222	1647	-	tr							
NI MW218	1648	-	tr							
NI MW220	1649	-			1.9					
NI MW222	1650	-		tr	0.1	tr	0.1			
Pogostol	1652	1651								0.1
Selin-11-en-4-α-ol	1656	1658	2.2		1.2	2.6	0.4	2.3	1.7	0.1
Neo-Intermedeol	1659	1658						tr	tr	
Intermedeol	1662	1665		1.4	tr	tr		tr	1.0	
NI MW220	1687	-		tr						
NI MW220	1688	-	tr							
NI MW220	1690	-	tr							
Eudesm-7(11)-en-4-ol	1698	1700		1.3	tr		0.1			0.1
NI MW220	1725	-								0.1
7,14-anyhdro-Amorpha-4,9-diene	1750	1755			0.1	0.2				
Phytol	1941	1942								0.1
E -Phytyl acetate	2222	2218				0.3				
Tricosane	2297	2300			0.1					
Non-oxygenated monoterpenes			11.6	22.8	10.0	8.5	4.9	6.7	22.5	9.0
Oxygenated monoterpenes			0.1	1.7	0.1	0.0	0.4	0.0	1.3	0.0
Non-oxygenated sesquiterpenes			81.3	46.7	75.9	82.8	72.9	85.1	57.3	72.3
Oxygenated sesquiterpenes			3.0	13.7	6.5	4.9	8.3	4.0	0.6	4.2
Other chemical classes			0.1	0.1	0.1	0.1	0.3	0.1	0.2	0.2
Total identified (%)			96.5	84.8	92.7	96.5	86.9	95.8	81.7	85.6
Essential oil yeld (%) (*w*/*w*)			0.14	0.10	0.12	0.14	0.28	0.10	0.15	0.22
H′			2.0	2.9	2.1	1.5	3.2	1.6	2.7	2.6
S$_{RO}$			−150.4	−136.4	−146.5	−156.0	−138.6	−153.1	−129.3	−135.6
R&M			−3.8	−3.4	−2.3	−3.6	−3.5	−4.1	−2.4	−2.2

RI$_{calc}$ = Calculated Retention Index (HP-5MS column); RI$_{lit}$ = Literature Retention index (Adams [10]); Main constituents in bold. * Quantities are averaged out of three replicates. All compounds were identified by MS and RI in accordance with experimental; $^{\#}$ Identified by [11]; NI: Not identified; MW = molecular weight; H′: Diversity Index; S$_{RO}$: Weighted Average Redox Standard; R&M: Ramos & Moreira Index; tr = Trace (relative percentage value less than 0.05%). Autumn–Mar: March and May: May; winter–Jul: July and Aug: August; spring–Oct: October and Nov: November; and summer–Jan: January and Feb–February.

Table 2. Results of the circadian temporal analysis of Essential Oils from *Piper lhotzkyanum* Kunth (Piperaceae) Leaves Collected in 2019–2020: Yields, Diversity Index (H′), Weighted Average Redox Standard (S_{RO}), and Ramos & Moreira Index (R&M).

Compounds	RI_{calc}	RI_{lit}	**Percentual Relative Area (%) ***															
			July 2019 (Dry Season)								**January 2020 (Rainy Season)**							
			12 pm	**03 am**	**06 am**	**09 am**	**12 am**	**03 pm**	**06 pm**	**09 pm**	**12 pm**	**03 am**	**06 am**	**09 am**	**12 am**	**03 pm**	**06 pm**	**09 pm**
α-Thujene	920	924									0.3							
α-Pinene	**929**	932	0.7			0.8				0.5	3.4	1.3	0.5		1.3	3.1	1.3	1.5
β-Pinene	970	974	1.2		1.2	1.0				0.5	2.7	4.8	1.3		1.2	2.3	1.0	1.1
6-Methyl-5-hepten-2-one	978	981									3.9							
Myrcene	987	988									0.4							tr
α-Phellandrene	1000	1002	0.6		0.4	1.7				1.8	tr							
α-Terpinene	1010	1014									1.7							
Limonene	1022	1024	0.8		0.6	1.3				0.9	tr				tr	3.7		1.2
Z-β-Ocimene	1031	1032	0.3			0.4				0.2	tr		0.2	0.5	3.3	tr	2.2	3.3
E-β-Ocimene	1042	1044									3.7			0.3	0.4	0.4		0.5
γ-Terpinene	1052	1054									0.4					0.7		
Terpinolene	1081	1086									1.3							
Linalool	1090	1095	0.1			0.1				0.1	tr							
trans-Sabinene hydrate	1098	1098									tr							
Allo-Ocimene	1130	1128	0.2			0.2				0.1						tr	tr	
2-(1*Z*)-Propenyl phenol	1145	1146	0.2			0.1		0.1		1.1								
NI MW194	1265		0.1								0.2		0.4	0.5		0.3		
Bicycloelemene #	**1312**		**2.5**	**1.1**	**0.7**	**1.2**	**0.6**	**1.1**	**0.9**	**1.3**	**3.0**	**3.2**	**6.7**	**3.0**	**5.8**	**4.0**	**3.9**	**3.3**
δ-Elemene	1335	1335			0.3						tr		3.0	tr		tr		3.5
α-Cubebene	1349	1348	0.5	0.4	0.2	0.7			0.1	0.2	tr	0.4	tr		tr	tr		tr
α-Copaene	1370	1374	0.7	0.8	0.5	0.8	0.6	0.7	0.5	0.7	tr			tr		1.4		
β-Elemene	**1391**	**1389**	**7.7**	**11.6**	**9.8**	**8.4**	**9.7**	**8.0**	**9.2**	**11.7**	**19.5**	**22.1**	**28.4**	**29.2**	**29.2**	**19.7**	**20.2**	**35.1**
Cyperene	1396	1398	0.9		0.4	0.9			0.5	0.4	0.5					tr		
α-Gurjunene	1407	1409			0.2					0.1			4.3		1.1	1.1	1.0	
***E*-Caryophyllene**	**1413**	**1417**	**9.8**	**10.8**	**9.0**	**8.1**	**9.4**	**7.4**	**7.2**	**5.9**	**4.8**	**5.6**	**5.2**	**4.8**	**5.8**	**6.0**	**7.9**	**4.5**
γ-Elemene	1430	1434	3.2		1.7				1.2	1.1	2.5	0.9	3.2	1.7	1.7	2.6	3.2	2.8
α-Guaiene	1436	1437	0.4			0.5	0.2	0.3	0.3	0.3	tr					tr		
Aromadendrene	1438	1439	0.8		0.7	0.9	0.6	0.9	0.8	0.7	1.5	3.9	4.7	tr	3.8	1.6	1.2	2.5
6,9-Guaiadiene	1441	1442	0.2			0.2										tr		
α-Humulene	1450	1452			0.9				0.8									
E-β-Farnesene	1543	1454	3.3	4.0	3.4	2.7	1.4	1.7	3.3	3.1	3.3	2.0	2.1	3.7	2.7	4.4	2.3	2.3
Rotundene	1456	1457	1.0		0.8	0.8	0.7	0.6	0.5	0.5	tr					1.0		
Allo-Aromadendrene	1459	1458			0.4			0.3								0.9		
NI MW220	1463	-							1.0									
γ-Gurjunene	1474	1475			1.3				1.3							3.1		
β-Chamigrene	1477	1476									3.6	3.9	2.1	4.5	2.5		2.3	1.6
γ-Muurolene	1479	1478														1.9		
ar-Curcumene	1481	1479		1.4	1.2				1.7	1.3	tr	5.0	2.9	4.2	1.3	4.4	2.7	tr
Germacrene D	1483	1440									tr	4.5	2.6	3.0	3.5		2.9	4.0
γ-Curcumene	1485	-		2.0	1.1			1.1	1.1	2.1								
β-Selinene	**1489**	**1489**	**3.4**	**4.9**	**3.2**	**6.5**	**5.7**	**3.4**	**4.4**	**2.4**	**3.5**	**4.0**	**2.9**	**5.3**	**4.8**	**4.4**	**4.3**	**5.5**
α-Zingiberene	**1492**	**1493**	**18.9**	**26.1**	**21.0**	**18.8**	**23.0**	**20.6**	**21.1**	**20.6**	**17.9**	**2.8**	**4.3**	**15.1**	**7.7**	**10.4**	**11.0**	**9.5**
Viridiflorene	1495	1496											1.0	1.9	tr			
α-Selinene	1497	1498					4.0											
α-Muurolene	1502	1500							0.5									
β-Bisabolene	1506	1505	2.3	3.2	2.2	2.2	2.0	1.9	2.3	2.0	tr	3.2	2.3	4.4	7.9	2.8	2.3	5.4
α-Bulnesene	1508	1509							0.5									
δ-Amorphene	1510	1511	1.3		1.0					1.0	3.3		3.4	4.5		2.0	1.0	
γ-Cadinene	**1512**	**1513**	**3.2**	**4.6**	**3.4**	**6.1**	**6.5**	**4.1**	**1.1**	**3.2**	**2.5**	**4.6**			**4.1**	**0.6**	**1.3**	**tr**
Cubebol	1516	1514									0.9							
NI MW222	1518								1.5							0.7		tr
β-Sesquiphellandrene	**1522**	**1521**	**6.6**	**8.0**	**4.5**	**2.2**	**3.5**	**3.8**	**4.0**	**5.3**	**6.3**	**8.0**	**6.0**	**1.0**	**1.0**	**0.8**	**3.4**	**4.5**
δ-Cadinene	1524	1522		1.0			0.9				tr	8.1		3.1	0.8	4.0	2.2	
Zonarene	1527	1528	0.5	tr	0.3	0.4	0.7		0.4	0.5	tr	tr				tr		
NI MW220	1532	-	0.8		0.6	0.7		0.7	0.7	0.7								
α-Cadinene	1536	1537									tr			0.7	1.2	0.7	0.7	1.0
Selina-3,7(11)-diene	1542	1545	0.9	0.3	0.6	1.1		0.9	0.7	0.7	0.4	0.3		1.3	1.0	0.7	0.7	0.5
β-Vetivenene	1552	1554			0.2													
Germacrene B	1558	1559		5.1							0.4	5.1	0.7	0.8	1.1	0.3	0.9	1.0
NI MW222	1559	-									0.3					0.3		
E-Nerolidol	1560	1561	1.1		0.7	0.7	0.8	0.9	0.9	0.9	0.2		0.5	1.1	0.4	0.3	0.3	0.5
Maaliol	1565	1566														0.4		
NI MW220	1570	-	0.2			0.2		0.2	0.2	0.3								
Spathulenol	1578	1577	2.7	0.2	1.1	2.9	1.2	1.1	1.3	1.4	0.3	0.2		0.3	0.5	tr	0.5	0.6
Caryophyllene oxide	1580	1582	1.1	1.3	2.9	1.5	1.7	1.9	3.5	3.9	0.4	1.3	1.5	2.3	1.8	1.3	1.2	1.9
Globulol	1591	1590	1.0	0.5	1.3	0.9	0.9	0.9	1.1	0.9	1.5	0.5	0.4	0.6	0.4	0.3	0.4	0.3
Viridiflorol	1594	1592	0.5								0.3		0.2	0.2	0.2	0.3		
NI MW222	1596	-			0.2		0.2	0.2			0.2					0.1		
Longiborneol	1597	1599											0.2	0.2	0.2			0.1
Guaiol	1600	1600									tr					0.1		
Rosifoliol	1601	1600			0.6	0.6	0.7	0.7	0.6	0.5								
Ledol	1603	1602	0.4		0.4	0.4	0.4	0.4	0.2	0.5							0.2	
5-*epi*-7-*epi*-α-Eudesmol	1606	1607				0.2			0.3	0.4								
Humulene epoxide II	1609	1608			0.4	0.4	0.2	0.3	0.4	0.5	0.2					0.2		
epi-Cedrol	1612	1618	0.7		0.7	1.4		1.6	1.7	1.4	0.1					0.1		
1,10-di-*epi*-Cubenol	1619	1618	0.4		0.8	0.4		0.3	0.4	0.5							0.2	
NI MW222	1620	-									0.2							
10-*epi*-γ-Eudesmol	1621	1622	4.3		4.8	4.3	1.2	3.6	6.0	4.7							0.4	

Table 2. *Cont.*

Compounds	RI_{calc}	RI_{lit}	Percentual Relative Area (%) *															
			July 2019 (Dry Season)								January 2020 (Rainy Season)							
			12 pm	03 am	06 am	09 am	12 am	03 pm	06 pm	09 pm	12 pm	03 am	06 am	09 am	12 am	03 pm	06 pm	09 pm
1-*epi*-Cubenol	1625	1627	1.6		1.1	1.6	1.2	1.6	1.9	1.4	0.7				0.7	0.5	1.1	0.1
NI MW220	1628	-			0.5													
epi-α-Cadinol	1633	1638	0.8	0.7		1.0		1.0	0.9	0.9	tr	0.7		0.3	0.7	0.6	0.3	
Allo-Aromadendrene epoxide	1635	1639	0.4		0.3	0.4			0.4	0.5	0.3					0.2		
Caryophylla-4(12),8(13)-dien-5β-ol	1637	1639			0.6			0.6	0.7	0.6								
Allo-Aromadendrene epoxide	1638	1639									tr					0.1		
epi-α-Muurolol	1641	1640		0.1							0.1	0.1		0.1	0.2			tr
α- Muurolol	1642	1644		0.3	0.3	0.4				0.2	0.6	0.3		tr		0.7	1.0	0.2
NI MW222	1644		1.1			1.0			1.4	1.1								
Cubenol	1645	1645	4.6		4.4	5.7	3.5	3.9	3.0	3.1								
NI MW220	1646				0.4													
Agarospirol	1647	1646									0.1				0.2	0.2	0.3	
Pogostol	1652	1651					0.1			0.2			0.5			0.1		
α-Cadinol	1653	1652														0.1		
Selin-11-en-4-α-ol	1656	1658		0.2			tr	0.2			0.2	0.2		0.1	0.2		tr	0.3
E-Bisabol-11-ol	1666	1667						tr			0.1					tr		
14-Hydroxy-9-*epi-E*-caryophyllene	1669	1668	1.8		1.1	1.3	1.2	1.2	1.4	1.0	0.2			0.1				0.1
NI MW220	1672		0.4		0.5	0.3			0.4	0.2	0.1							
α-Bisabolol	1684	1685				0.4			0.2	0.3						tr	tr	
Germacra-4(15),5,10(14)-trien-1-α-ol	1686	1685	0.2			0.2				0.2						tr		
NI MW220	1687					0.4												
NI MW220	1688				0.4	0.2		0.3	0.4									
Shyobunol	1689	1688		0.2							tr	0.2		tr	tr			tr
2*Z*,6*Z*-Farnesol	1699	1698	0.3			0.4		0.3		0.5								
Eudesm-7(11)-en-4-ol	1700	1700	0.6		0.6	0.6		0.6	0.7	0.6	0.1							
Amorpha-4,9-dien-2-ol	1702	1700	0.2		0.2	0.2		0.2	0.2	0.2	tr					tr		
Nootkatol	1715	1714	0.3		0.2	0.2		0.3	0.3							tr		
2*E*,6*E*-Farnesal	1742	1740				0.1			0.1	0.1								
γ-Costol	1746	1745	0.4		0.3	0.3		0.2	0.3	0.3	tr							
6*S*,7*R*-Bisabolone	1750	1749				0.1			0.1		tr							
Xanthorrhizol	1752	1751	0.1			0.2		0.2	0.1	0.2								
α-Costol	1774	1773	0.4		0.3	0.3			0.3	0.2								
2-α-Hydroxy-amorpha-4,7(11)-diene	1776	1775	0.1		0.2	0.3												
Benzyl benzoate	1760	1759	0.1			0.1		0.3	0.1	0.1								
β-Vetivone	1823	1822			0.2	0.2												
Phytol	1946	1942	0.5			0.7					tr							
Non-oxygenated monoterpenes			1.9	0.0	1.2	1.8	0.0	0.0	0.0	1.0	17.8	6.1	1.7	0.0	2.5	5.3	2.3	2.5
Oxygenated monoterpenes			0.3	0.0	0.0	0.2	0.0	0.1	0.0	1.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Non-oxygenated sesquiterpenes			62.2	77.3	65.1	60.9	65.8	53.6	62.2	60.5	79.5	87.3	82.7	92.0	86.9	78.7	75.5	82.4
Oxygenated sesquiterpenes			25.0	3.6	24.6	28.5	13.2	22.2	28.9	27.4	6.9	3.6	3.1	5.1	5.4	5.5	6.1	4.0
Total identified (%)			98.8	88.8	97.0	98.8	82.4	80.0	97.1	98.9	98.4	97.0	91.2	98.5	98.5	95.7	86.2	98.5
Essential oil yeld (%) (*w*/*w*)			0.22	0.20	0.08	0.14	0.11	0.03	0.11	0.15	0.20	0.20	0.06	0.12	0.01	0.19	0.06	0.19
H′			2.54	2.93	2.97	2.65	2.59	2.74	3.10	2.94	2.40	3.25	2.40	3.21	3.29	2.57	2.92	3.24
S_{RO}			−156.69	−157.35	−141.78	−154.32	−157.44	−131.60	−127.32	−151.88	−157.35	−156.47	−154.43	−144.80	−156.03	−157.18	−150.76	−137.37
R&M			−2.49	−2.71	−5.67	−2.66	−2.46	−4.39	−2.83	−2.62	−4.25	−2.24	−5.33	−4.99	−4.22	−4.03	−2.43	−3.71

RIcalc = Calculated Retention Index (HP-5MS column); RI_{lit} = Literature Retention index (Adams [10]); Main constituents in bold. * Quantities are averaged out of three replicates. All compounds were identified by MS and RI in accordance with experimental; # Identified by [11]; NI: Not identified; MW = molecular weight; H′: Diversity Index; S_{RO}: Weighted Average Redox Standard; R&M: Ramos & Moreira Index; tr = Trace (relative percentage value less than 0.05%).

For the seasonal temporal study, the highest productivities were recorded for the EOs obtained in January 2020 (0.22%) and August 2019 (0.28%) (Table 1). The circadian temporal study showed that the highest contents of EOs during the rainy (R) and dry (D) seasons were registered at 00:00 pm (R: 0.20; D:0.22) and 03:00 am (R: 0.20; D: 0.20), respectively. In both seasons, the nighttime period (9:00 pm to 6:00 am) provided the highest average content of EO production (Table 2). There was a statistical difference between the means throughout the representative months of each season ($p < 0.0001$), as well as throughout the circadian evaluation (R: $p = 0.0021$; D: $p = 0.0005$). However, when comparing the average productivity between the dry and rainy seasons, there was no significant difference ($p = 0.5825$). Only the average difference between day and night during the dry season showed significant differences ($p = 0.0120$).

Considering the circadian temporal study, Pearson's analyses (Figure 1) showed an inversely proportional correlation in the dry season with temperature ($r = -0.904$; $p = 0.016$) and in the rainy season with radiation ($r = -0.810$; $p = 0.006$).

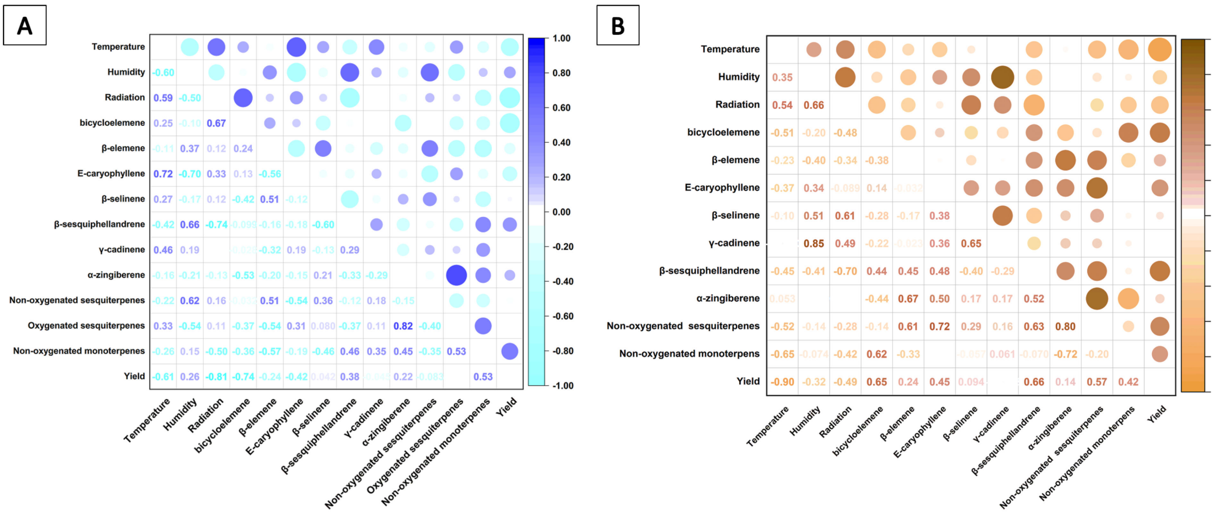

Figure 1. Correlation Analysis of Chemical Composition, Yield of Essential Oil, and Environmental Factors in *Piper lhotzkyanum* Samples during Rainy (**A**) and Dry (**B**) Seasons.

3.2. Chemical Profile of the Essential Oil

GC-MS and GC-FID were used to identify and quantify the constituents of *P. lhotzkyanum* leaf EO, respectively, in seasonal (S) and circadian (C) studies. A total of 114 (C) and 123 (S) volatile compounds were identified, representing 99.3% and 99.6% of the total oil composition, respectively (Table 1). Non-oxygenated sesquiterpenes (S: 46.7–85.1%; R: 0–17.8%, D: 0–1.9%) were identified as the predominant constituents, followed by a small relative percentage of oxygenated sesquiterpenes (S: 0.6–13.7%; R: 3.1–6.9%; D: 13.2–28.9%) and non-oxygenated monoterpenes (S: 4.9–22.8%; R: 3.6-9.1%; D: 0.0–1.9%) (Table 2). The rainy season showed higher percentages of non-oxygenated sesquiterpenes compared to the dry season ($p = 0.0003$) (Figure S2).

The non-oxygenated sesquiterpenes β-elemene (S: 16.1–66.1%; R: 19.9–35.1%; D: 7.7–11.7%), *E*-caryophyllene (S: 1.8–6.1%; R: 4.4–7.9%; D: 5.9–10.8%), and α-zingiberene (S: 0–16.9%; R: 2.8–17.9%; D: 18.9–26.0%) were the main compounds identified in all samples.

3.3. Seasonal Temporal Variation of Essential Oil Components

The data for the present analysis demonstrated that the contents of the major components β-elemene (p = 0.0006) and *E*-caryophyllene (p = 0.00009) vary significantly in relation to seasonal changes. β-elemene remained the major component throughout the seasons, presenting some fluctuations, thus demonstrating significant differences between its percentages ($p < 0.001$) (Figure S2). It should be noted that the highest contents were found in the months of August (66.1%) and November (62.0%) of 2019, at the peaks of winter and spring, respectively. Other components that changed the most with the seasons were α-pinene (highest contribution in autumn at 6.8%—March, 6.2%—May, and in the summer at 7.0%—January); α-zingiberene and *E*-caryophyllene (highest contribution in spring at 16.9% and 8.5%—October, respectively); and *E*-β-ocimene and β-selinene (highest contribution in summer at 12.4 and 7.4%—January, respectively) (Table 1).

Principal component analysis (PCA) was applied to model the data set of compound abundance present in the EOs (above 5%) for the seasonal period (Figure S4). The aim of the analysis was to identify the main variables that influence the variation of the compounds present in EOs. The PCA model was responsible for 95.00% of the total data variance. Based on the results, the first component, which has 86.14% of the total variance, was marked by the negative load of β-elemene (−7.87). Additionally, the separations in the second component for the months of January and October were obtained due to the loads of *E*-β-ocimene (−1.31) and α-zingiberene (+1.80), respectively. These results suggest that the variation in the compounds present in EOs is mainly influenced by the high content of β-elemene, and that there are significant differences between the months of January and October due to the presence of high percentages of *E*-β-ocimene and α-zingiberene, respectively.

Figure 2A shows the results of the hierarchical cluster analysis (HCA) that corroborates with the PCA. The HCA separated the months of the year into two groups based on Euclidean distance. The highest distance of 33.29 was observed for the months of January, October, and May, while a distance of 17.93 was observed for the months of February, August, November, June, and March, due to their low and high percentages of β-elemene, respectively. At the smallest distance of 17.93, a great chemical similarity is noted in the composition.

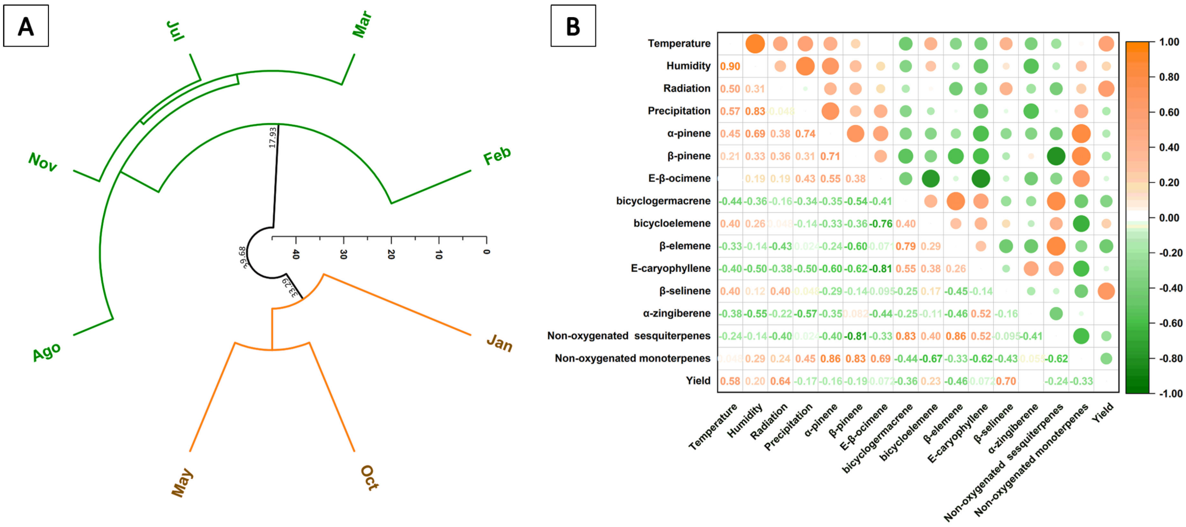

Figure 2. Temporal Seasonal Study of *Piper lhotzkyanum* Samples: Principal Component Analysis (**A**) and Correlation Analysis (**B**) of Active Compound Content, Chemical Classes, Essential Oil Yield, and Environmental Factors.

The results regarding Pearson's analysis showed moderate correlations between humidity ($r = 0.69$; $p = 0.04$) and precipitation ($r = 0.74$; $p = 0.02$) with α-pinene (Figure 2B). It was possible to observe some values that exhibit positive correlations with biosynthetic productions between non-oxygenated monoterpenes α- and β-pinene ($r = 0.71$; $p = 0.008$) and non-oxygenated sesquiterpenes β-elemene and bicyclogermacrene ($r = 0.79$; $p = 0.007$).

3.4. Circadian Temporal Variation of Essential Oil Components

The non-oxygenated sesquiterpenes β-elemene and α-zingiberene were the main compounds identified in the samples. The highest content of β-elemene (35.1%) was at 9:00 pm during the rainy season and of α-zingiberene (26.0%) was at 3:00 am during the dry season in the circadian study. The content of β-elemene between the dry and rainy seasons differed significantly ($p = 0.002$) (Figure S3). Although the fluctuations in α-zingiberene percentage were high during the dry season, these increments did not show significant differences with the contents of β-elemene and *E*-caryophyllene ($p = 0.001$) (Figure S3).

Hierarchical Cluster Analysis (HCA, Figure 3A) revealed the presence of two distinct groups for circadian analysis. The first group, with a Euclidean distance of 22.47, included all samples collected during the rainy season. The second group, with a distance of 13.09, comprised all samples collected during the dry season. This separation was determined by the presence of high percentages of β-elemene and α-zingiberene, respectively. In the Correlation Heat Map of Figure 3B, it was also possible to highlight the relationship between percentages of these two major compounds, making it possible to define the best collection time, which was 3:00 am during the dry season and 9:00 pm during the rainy season for β-elemene and α-zingiberene, respectively.

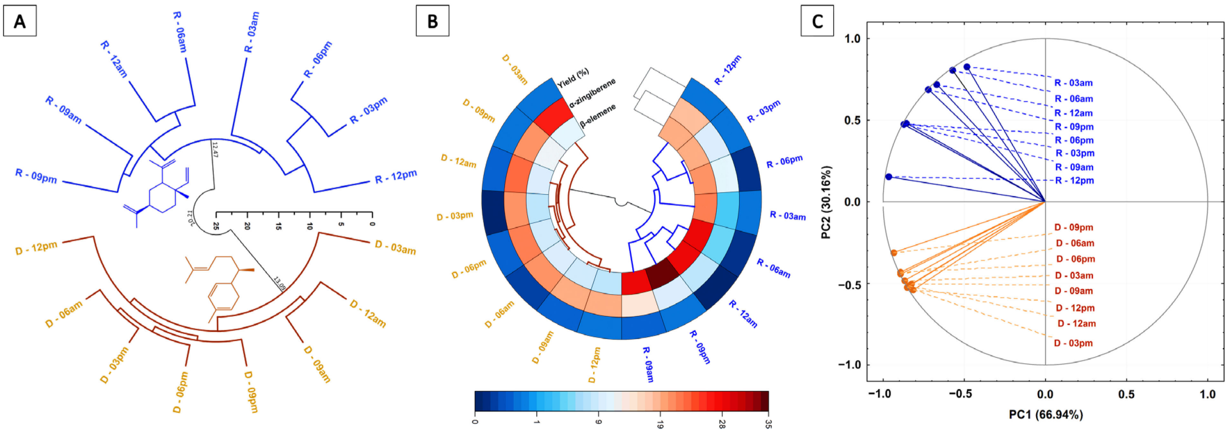

Figure 3. Temporal Circadian Rhythm Study of *Piper lhotzkyanum* Samples: Hierarchical Analysis (**A**), Correlation Heat Map (**B**), and Principal Component Analysis (**C**).

The PCA analysis performed was able to explain 97.10% of the total variance of the data described by PC1 and PC2. The scores graph (Figure 3C) generated from this model showed a clear separation between samples collected in the dry and rainy seasons, confirming the results obtained by HCA. The most significant contribution to the separation of these samples was observed with the presence of positive β-elemene (+4.01) and negative α-zingiberene (−3.36) charges.

The results of the correlation between climatic variables and the primary constituents of *Piper lhotzkyanum* EOs were presented for the rainy and dry seasons. In Figure 1A, for the rainy season, a strong and significant negative correlation was observed between radiation and the components β-sequilphellandrene ($r = -0.74$; $p = 0.01$) and bicycloelemene ($r = 0.67$; $p = 0.03$). In addition, there was a negative correlation between humidity and *E*-caryophyllene ($r = -0.70$; $p = 0.02$) and a positive correlation between humidity and non-oxygenated sesquiterpenes ($r = 0.62$; $p = 0.02$) and β-sequilphellandrene ($r = 0.66$; $p = 0.02$).

In the dry season, Figure 1B shows a moderate negative correlation between temperature and non-oxygenated monoterpenes ($r = -0.65$; $p = 0.02$). In addition, radiation showed a significantly positive correlation with non-oxygenated sesquiterpenes β-selinene and β-sequilphellandrene and with humidity and γ-candinene ($r = 0.85$; $p = 0.001$).

3.5. Temporal Variation of Chemical Diversity and Redox Patterns in Essential Oils

Tables 1 and 2 present the values of indices for each sample, while Table 3 shows the mean values, standard deviation, coefficient of variation, and Spearman correlations between abiotic factors and indices (S_{RO} and R&M). The values of chemical diversity were calculated using the Shannon–Wiener Index, ranging from 1.51 to 3.28 (S: 3.17–1.50; R: 3.28–2.39; D: 3.09–2.53), as presented in Tables 1 and 2. The seasonal study revealed that October showed the highest chemical diversity (3.17), while the winter average (July and August) presented the lowest (1.82). The circadian study identified that at 9:00 am (3.28) and 3:00 pm (3.09) the highest chemical diversity in the rainy and dry seasons was observed, respectively. There were significant differences in means (p>0.01) over the year (seasonal study) and during the day in the dry and rainy seasons, but there were no significant differences in chemical diversity among the analyzed seasons or between day and night ($p > 0.05$).

Table 3. Analysis of the trend of Redox Homeostasis (R_{ho}), descriptive statistics, Environmental Factors Correlation, and Diversity Index (H′), Weighted Average Redox Standard (S_{RO}), and Ramos & Moreira Index (R&M) of the Essential Oils from Circadian and Seasonal Temporal Studies of *Piper lhotzkyanum* Kunth (Piperaceae).

Analyzed Variable	Descriptive Statistics			Spearman Coefficient					R_{ho}
	μ	SD	RSD	T(°C)	RH(%)	R(KJ.m^{-2})	P(mm)	H'	
H'—S	2.32	0.52	22.62	0.24	0.02	0.24	-0.14	-	-
H'—R	2.91	0.34	11.66	0.47	−0.29	0.10	-	-	-
H'—D	2.81	0.18	6.30	−0.18	0.14	0.20	-	-	-
S_{RO}—S	147.30	10.30	6.99	0.29	0.12	0.48	−0.05	0.71 *	−14.75
S_{RO}—R	151.80	5.61	3.70	−0.01	−0.05	0.20	-	0.52 *	0.88
S_{RO}—D	147.30	0.90	0.61	0.69 *	0.29	0.46	-	−0.52 *	−0.66
R&M—S	−3.16	0.66	−20.97	0.02	−0.17	0.67 *	−0.45	0.45	−1.65
R&M—R	−3.90	0.83	−21.25	−0.43	−0.05	−0.17	-	0.48	0.20
R&M—D	−3.23	0.90	−27.89	0.23	0.26	0.32	-	−0.69 *	−0.22

* Quantities are averaged out of three replicates; μ—Mean; SD—Standard deviation; RSD—Relative standard deviation; T(°C)—Temperature; RH(%)—Relative humidity; R(KJ.m^{-2})—Radiation; P(mm)—precipitation; H'—chemical diversity; S_{RO}—Weighted Average Redox Standard; R&M—Ramos & Moreira Index; S—Seasonal; R—Rainy season; D—Dry season.

The values of Weighted Average Redox Standard (S_{RO}) ranged from −157.35 to −127.32 (S: −155.97–−129.29; R: −157.34–−137.37; D: −157.44–−127.32).

When we applied the R&M index to evaluate the redox of the EO mixture, we observed variations between −2.15 and −5.67 (S: −4.13–−2.15; R: −5.33–−2.24; D: −5.67–−2.46). It is interesting to note that the coefficient of variation for the indices (R&M and S_{RO}) was less than 30%, indicating data homogeneity (Table 3).

It was found that seasonal variations showed that the mean values of R&M of EOs were more oxidized in the summer (January and February: −2.25) and more reduced in spring (October and November: −3.80). The circadian study revealed that, in both dry and rainy seasons, EOs are more reduced at 3:00 am. In the rainy season, the most oxidized EO was found at 12:00 am, and in the dry season, at 9:00 am. Over the years (seasonal) and days (circadian), the means of R&M showed significant differences ($p > 0.01$).

A redox cycle was identified: EOs with more reduced compounds were found between 3:00 am and 12:00 pm (mean of R: −4.64; D: −3.79), and EOs with more oxidized compounds were found between 3:00 pm and 12:00 am (R: −3.16; D: −2.66). The trend of redox homeostasis (R_{ho}) for R&M and S_{RO} tended to zero in 24 h for circadian samples.

Spearman correlation results showed significant positive correlations between the S_{RO} of the dry season and temperature ($r = 0.69$; $p = 0.01$) and seasonal R&M with radiation ($r = 0.67$; $p = 0.02$). In both seasonal and circadian seasons, there were correlations between chemical diversity (H′) and oxidation–reduction patterns (S_{RO} and R&M). In a directly proportional relationship, the increase in chemical diversity leads to an increase in R&M and S_{RO} in the rainy season, while it occurs inversely proportionally in dry periods (Table 3).

4. Discussion

The circadian and seasonal temporal variations were important factors for the yield of *P. lhotzkyanum* EO. These results correlate with other studies that show the production of EO in plants varies throughout the year and day, with seasonal and circadian peaks [12–14]. The production of EO is affected by many environmental factors, such as ecological niche dynamics, temperature, solar radiation, humidity, water availability, among others [15,16].

The highest EO productivities were observed during the night, between 9:00 pm and 6:00 am, in both seasons. These results suggest that the nighttime period is more favorable for EO production in *P. lhotzkyanum*. This pattern may be related to the plant's circadian cycle, as many metabolic processes, including EO biosynthesis, are influenced by the plant's circadian rhythm [8,17–19].

The circadian clock is a biological oscillator widespread in organisms that allows for timing physiological processes in response to predictable day/night cycles. This endogenous mechanism has a rhythm of approximately 24 h under normal environmental conditions [20–22].

The emission of volatiles is highly regulated and restricted to specific times of day, seasons of the year, and phenology, in many plant species [23]. This phenomenon has also been observed in the *Piper* genus [6–8,24], which suggests that these plants have the ability to regulate metabolic, physiological, and developmental processes through their biological clock [20,21,25].

In addition to regulation by the circadian clock, multiple excitatory factors can also contribute to the emission of these volatiles, which can affect the quality of the produced EO [21–23]. Therefore, understanding the mechanisms that regulate EO production in *P. lhotzkyanum* may be important for the development of chronocultivation strategies that maximize the production and quality of EO.

This study identified and quantified the constituents of the EO from the leaves of *P. lhotzkyanum* in different temporal variations, with a predominant majority of sesquiterpenes. As for the predominance of this class, it is in accordance with other studies carried out with other *Piper* species [5–7,26].

It was observed that the rainy season presented higher percentages of non-oxygenated sesquiterpenes compared to the dry period, indicating an influence of climate on oil composition. The excitatory relationship between the influence of soil and air humidity increased during the rainy period, which can affect the synthesis and release of sesquiterpenes [9,27]. According to studies, plants produce more sesquiterpenes in reaction to water stress. However, the requirement to create these chemicals might diminish under optimum circumstances, like rain [1,28]. Thus, it is possible that plants produce fewer oxygenated sesquiterpenes during the rainy period, favoring the predominance of non-oxygenated ones [1,9].

This strategy may be related to factors that ensure fitness, in which *P. lhotzkyanum* may be investing more resources in other biological processes during the rainy period, such as photosynthesis and vegetative growth [29,30]. This may leave fewer resources available for the production of oxygenated sesquiterpenes, which require more energy and nutrients to be synthesized. As a result, non-oxygenated sesquiterpenes may be produced in greater quantity compared to oxygenated ones [30].

Other factors should be taken into consideration, such as environmental and ecological conditions [31], alterations in population dynamics [32,33], ecological niche structure [34], and evolutionary drivers [9,31] such as intergenerational epigenetic variation and others [7].

Among the identified compounds, the non-oxygenated sesquiterpenes β-elemene, *E*-caryophyllene, and α-zingiberene were the main constituents present in all samples, corroborating with previous works evaluating the chemical profile of this plant [5]. These compounds are known for their therapeutic properties, including anti-inflammatory, antioxidant, and antitumor activity [35,36].

It is noteworthy that the composition of *P. lhotzkyanum* EO varied according to seasonal and circadian periods. This can be explained by the influence of environmental factors, such as water availability and light intensity [25,37]. These results highlight the importance of considering the collection period and environmental conditions when evaluating the quality and composition of EO from *P. lhotzkyanum* and EOs of other Piperaceae species [8,24].

It is important to emphasize that these chemometric analyses enabled a better understanding of the relationship between yields and the compounds in question, which can be extremely useful for optimizing production processes. It should be noted that the chemical stability of bioactives guides and permeates the production of herbal medicines, from their maintenance during cultivation/harvest to the stages of processing and post-harvest, passing through the stages of storage of both the medicinal raw material and the finished product [1,4,22]. Few studies are found in the literature relating the circadian rhythmic and seasonal activities of the plant to chemical stability regarding plant volatiles [2,22].

The standardization and chemical stability of the extract (which would be a process of analyzing the seasonal profile with the aim of defining the optimal harvest time) includes the stability of the products derived from transformations, since *P. lhotzkyanum* in question presents chemical alteration of its EO caused by artificial light (photostability test). This test showed that α-zingiberene during exposure to light interconverts to bicyclogermacrene [5]. However, this study pointed out that natural light does not influence this increase in bicyclogermacrene, making it possible to infer that care must be taken during and post-obtaining process of obtaining the EO. Thus, we were able to answer the question proposed above in the introduction: verify if the EO composition of *P. lhotzkyanum* is affected by light in nature as it is in the laboratory.

The percentage of β-elemene and α-zingiberene allowed the separation of samples through chemometric analyses in circadian temporal variation, thus highlighting the existence of these EO chronotype, also previously described for *Piper gaudichaudianum* [9]. β-elemene is a compound present in the EO of several plants and has been studied due to its medicinal properties and effects on the ecology of plant communities and animal behavior [3,35]. In ecology, it may act as a defense mechanism against herbivores and pathogens, as it is toxic to many species of insects and fungi [38,39]. Studies have also shown that β-elemene has repellent effects on insects, such as mosquitoes and ants, and is able to inhibit the growth of some species of pathogenic fungi, such as *Aspergillus fumigatus* and *Candida albicans* [38–40]. Another possible ecological function of β-elemene is as a chemical signal for intra- and interspecific communication, being released in response to environmental damage or stress, attracting natural enemies of herbivores, such as predators or parasitoids in a tritrophic interaction for plant defense [38,40].

Compound α-zingiberene, in turn, has medicinal properties and impact on ecology [41]. In nature, it plays a crucial role as a floral pheromone, attracting pollinators and increasing pollination efficiency, as well as promoting seed germination and seedling growth [41–43]. It also has a defensive function against herbivores and pathogens, being toxic to many species of insects and fungi, as well as acting as a natural insect repellent and possessing antibacterial and antifungal properties [41,43,44].

During the rainy season, there is a lower incidence of radiation and temperature, resulting in higher EO yields and β-elemene content. Conversely, during the dry season, the opposite occurs: lower qualitative yields and higher α-zingiberene content. These data indicate a relationship between the periods and the volatility of these compounds in the leaves, as there is a proportional change in the equilibrium of the contents in the complex EO mixture.

It is known that β-elemene (C_{15}) exhibits higher volatility compared to α-zingiberene (C_{15}). This difference in volatility constants is mainly due to the differences in their molecular geometries. While α-zingiberene has a more compact molecular structure with a cyclic conformation, which reduces its volatility, β-elemene has a more linear structure, which increases its volatility [45,46]. This difference in compound volatility may have interesting ecological implications. For example, β-elemene may be more suitable for insect control applications, as it is more volatile and can dissipate more easily in the environment, while α-zingiberene may be more suitable for fungal or bacteria control applications, as it is less volatile and can remain on the plant surface for longer [38,40,41]. Other issues that should be considered in this equation include the possibility of differential histolocalization of β-elemene in cells on the surface (upper epidermis and/or parenchyma) and/or in secretory structures (trichomes), genetic factors, and dynamics of the ecological niche in the dry season, which is common in the plant kingdom [47].

Variations in high α-zingiberene and β-elemene percentages have direct implications for technological applications and concerns about the EO production chain. As mentioned earlier, the sensitivity and susceptibility of this EO to light incidence can result in the conversion of α-zingiberene into bicyclogermacrene in high light exposure. This can have implications not only for the quality of the EO but also for possible different biological and pharmacological applications that may occur [48].

The results indicate that temporal changes in environmental conditions, such as temperature and radiation, can affect the chemical diversity and oxidation and reduction characteristics of compounds present in leaves. These findings are important for a better understanding of plant adaptations to environmental changes and may have significant implications for biotechnology and biodiversity conservation [2]. There are several hypotheses that can explain the fluctuation in chemical diversity and redox values over time, such as defense against herbivores [49], plant niche dynamics [34], environmental communication [50], selection by abiotic pressures (abiotic filter) [51], and metabolism regulation (redox theory) [2,52,53].

It is important to note that the Piperaceae family is one of the most diverse in terms of species and presents EO with different components derived from distinct metabolic pathways that lead to the formation of compounds from the chemical classes of terpenoids (monoterpenes, sesquiterpenes, and rarely diterpenes), arylpropanoids, butylbenzenes, chromenes, and other simple phenolics [54,55]. Considering this chemical diversity, it is possible to perceive that the fluctuations in R&M vary to reach an average redox balance throughout the day. The coefficient of variation in the rainy and dry periods presents values below 30%, suggesting homogeneity in the analyzed information. Furthermore, it is possible to perceive that the sums of the differences obtained from the R&M values tend to approach zero, confirming the tendency towards redox homeostasis. The redox theory suggests that plants produce chemical compounds as a means to regulate their metabolism and maintain redox balance. Variation in chemical diversity may be related to changes in environmental conditions that affect the plant redox balance [2,9,52].

Understanding the processes that affect interactions between plants, herbivores, and other organisms is essential for comprehending natural ecology and developing efficient biological control technologies [1,2]. Induction of these metabolites and confirming variations in the response to environmental stresses can have a significant impact on the support capacity for β and γ chemodiversity as a resource, while the use of the same chemical information at multiple trophic levels may have opposing effects on plant fitness [3]. By understanding the relative importance of these processes, it is possible to develop strategies for maximizing crop yields and conserving biodiversity in the face of global environmental changes [1].

5. Conclusions

The circadian and seasonal variations in the production of essential oils in *P. lhotzkyanum* revealed a true symphony of redox fluctuations, chemical diversity, and phenoplasticity, highlighting the incredible ability of the plant to adapt and respond to the environmental changes present in a Brazilian tropical forest. The results showed that the EOs were dominated by non-oxygenated sesquiterpenes, with β-elemene, *E*-caryophyllene, and α-zingiberene being the main compounds identified in all collections. The findings also underscore the importance of factors such as climate and environment in the quality of the produced EOs, describing for the first time that the dry and rainy periods are crucial for the yields of β-elemene and α-zingiberene. With the identification of the EO constituents and their metabolic dynamics, the study paves the way for new chronocultivation strategies that maximize production and quality. It can promote lasting benefits for ecology and chemical diversity and their relationships with chemical diversity and redox profile of the samples. This study provides a solution to a previously unresolved question by confirming that natural light does not produce the interconversion of major compounds, such as that of α-zingiberene to bicyclogermacrene. In addition to providing solutions for quality control of this medicinal plant with a focus on chemical diversity at a temporal scale, a new index was presented to evaluate metabolic homeostasis (R_{ho}) based on EO data. By understanding the underlying mechanisms of EO production, we can move towards a more sustainable and prosperous future, where nature and plants are true partners in the search for more effective and beneficial solutions for all.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d15060715/s1; Figure S1. Climatic data of the Serra dos Órgãos National Park area, Teresopólis City (Brazil), during the collections of *Piper lhotzkyanum* Kunth leaves. Data collected from reference INMET (2019–2021). Monthly averages of temperature, precipitation, and relative humidity from January to December 2019 and January to February 2020 (A). Ombrothermal diagram from January to December 2019 and January to February 2020 (B). Data of temperature, relative humidity, and radiation from the leaves' collection time for the circadian study in July 2019 (D) and January 2020 (C); Figure S2. Variations in the Percentage of Chemical Class Contents in the Essential Oils from *Piper lhotzkyanum* Kunth Leaves.; Figure S3. Variations in the Percentage of Major Components in the Essential Oils from *Piper lhotzkyanum* Kunth Leaves; Figure S4. Biplot (Principal Component Analysis—PCA) Resulting from the Analysis of the Essential Oils Obtained from leaves of *Piper lhotzkyanum* Kunth (Piperaceae) Collected for the Seasonality Study.

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