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Intraspecific Chemical Variability and Antioxidant Capacity of *Siparuna guianensis* Aubl. Essential Oil from Brazil

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Abstract: *Siparuna guianensis* Aubl. is an essential-oil-producing plant with diverse ethnopharmacological uses and bioactive potential. This study aims to evaluate the intraspecific variation in the yield, chemical composition, and antioxidant capacity of *S. guianensis* essential oil (EO). The specimens (SG-1 to SG-6) were collected in June, five in the district of Outeiro and one in the Salvaterra municipality (Brazil). EOs were obtained by hydrodistillation. The chemical compositions were analyzed by gas chromatography coupled to a mass spectrometer (GC-MS). The DPPH radical scavenging tests and inhibition of β -carotene oxidation by linoleic acid were carried out to evaluate the antioxidant capacity of EOs. Principal components analyses were performed to verify the interrelationships between the studied specimens' oil yields, chemical composition, and antioxidant capacity. Regarding chemical constituents, all studied samples showed the occurrence of spathulenol with an average concentration of $25.6 \pm 15.6\%$. The samples that presented the highest amounts of this constituent were SG-5 (43.3%) and SG-1 (41.8%); the spathulenol amounts in other samples were 33.2% (SG-4); 13.8% (SG-2); 11.5% (SG-6) and 9.8% (SG-3). Moreover, there was no significant variability in yield and antioxidant capacity using DPPH and β -carotene/linoleic acid; both tests found insignificant values. This species presents a notable intraspecific chemical variability. Despite notable antitumor activities, the plant presents intraspecific chemical variability in composition, which suggests new studies to evaluate the impacts on bioactive compounds.

Keywords: sesquiterpenes; DPPH; lipid peroxidation; volatiles; chemometrics



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1. Introduction

Siparuna guianensis Aubl. (Siparunaceae) is known by several popular names according to the country and/or region of distribution in Brazil, such as “negramina”, “capitiú”, “limão-bravo” and “cicatrizante-das-guianas”; it is considered an essential oil (EO) producer [1].

This species is native to Brazil and morphologically presents as a shrub or medium-sized tree. Its recurrence covers the Amazon, Caatinga, Cerrado, and Pantanal biomes. It grows in soil with terrestrial substrate and has subglobose fruits varying from 1 to 1.5 cm in diameter when ripe and dark red in color [2].

The *S. guianensis* leaf decoction is used as a drink for stomach ailments. The leaves are used for compresses or poultices against headaches and rheumatism. Moreover, in Panama and Guyana, extracts are used as insecticides. In Guyana, the leaves are also used

to prepare fish traps due to the typical odor of the species that disguises human smell [1], so this popular name means “Plant that smells like fish” (capitiú; Caá, “bush/plant” + pitiú, “fish smell”) in the Tupi-Guarani language [3].

Preliminary reports on *S. guianensis* EO indicated favorable results in controlling bacteria [4]. Thus, Gram-positive bacteria and fungi have been shown to be the most susceptible to the effects of *S. guianensis* EO [4].

Plants belonging to the same genus can exhibit remarkable chemical variability. In other words, this variability may be associated with several factors, such as the extraction method, collection site, plant part, and genetic characteristics, among others [5,6]. In this way, the same species can proliferate in different locations, manifesting qualitative and quantitative variations in the chemical composition of the EOs. Therefore, the amounts and/or major compounds can differ significantly [7].

Surprisingly, there is a dearth of research on the intraspecific chemical variability of *S. guianensis* in the existing literature. This study, therefore, is of utmost importance as it aims to investigate the potential influence of intraspecific variation on the yield, chemical composition, and antioxidant capacity of *S. guianensis* leaf EO. The findings of this research will significantly contribute to our understanding of the composition of their bioactive chemical components and their phytotherapeutic potential.

2. Materials and Methods

2.1. Plant Material

The leaves (100 g) of six specimens were collected in the district of Outeiro and the municipality of Salvaterra, state of Pará, Brazilian Amazon (Table 1) at 21 AMSL (height above mean sea level) altitude (Figure 1). The plant samples were collected for the study in June, transported in plastic bags with aeration, and placed in an air-conditioned room at room temperature for drying for twelve days from the collection date. The botanical identification was performed by Carlos Alberto Santos da Silva by comparing authentic samples, and the exsiccates of the studied specimens were incorporated into the “Marlene Freitas da Silva” herbarium. The collections were registered in the National Genetic Heritage and Associated Traditional Knowledge Management System (SisGen) under number A6689F5.

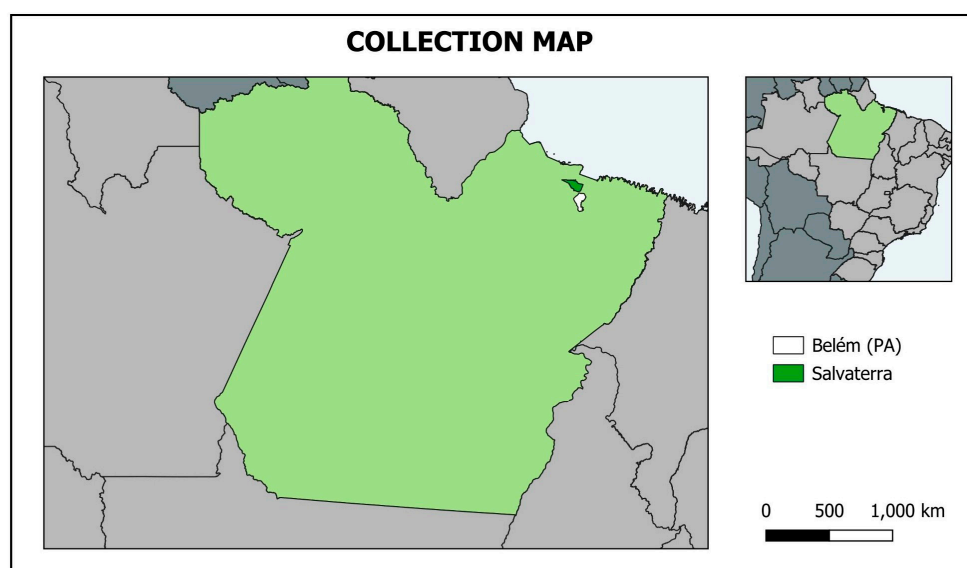


Figure 1. Collection areas of *Siparuna guianensis* specimens.

Table 1. Location data collection and botanical identification.

Sample *	Collection Site	Voucher	Coordinates
SG-1	Outeiro, Pará, Brazil	MFS010318	1°15'42.26" S. 48°28'3.17" W
SG-2	Outeiro, Pará, Brazil	MFS010604	1°15'48.42" S. 48°28'7.98" W
SG-3	Outeiro, Pará, Brazil	MFS010605	1°15'53.73" S. 48°28'11.84" W
SG-4	Outeiro, Pará, Brazil	MFS010606	1°15'52.12" S. 48°28'10.89" W
SG-5	Outeiro, Pará, Brazil	MFS001082	1°15'57.94" S. 48°28'16.49" W
SG-6	Salva terra, Pará, Brazil	MG246279	0°45'45.02" S. 48°30'58.47" W

*SG = *Siparuna guianensis* sample.

2.2. Extraction of Essential Oils and Yield

The extraction method was previously described by us [8]. Leaf samples (about 50 g) were crushed and subjected to EO extraction by hydrodistillation with a modified Clevenger-type apparatus for 3 h in duplicates. EO yields were calculated at 0 moisture-free biomass (BLU). The residual moisture of the material was obtained by drying in a drying oven at 110 °C until a constant weight.

After extraction, to remove residual water, the EOs were centrifuged for 5 min at 3000 rpm with anhydrous sodium sulfate (Na_2SO_4), and again subjected to the centrifuge under the same conditions. The oils were stored in amber ampoules and kept under refrigeration at 5 °C. The EO yields (%) were calculated from moisture-free (BLU) samples using the mass ratio, oil and moisture by the equation below.

$$\text{EO yield\%} = \frac{\text{oil obtained (mL)}}{\text{mass of material (g)} \times \left(\frac{\text{mass of material (g)} \times \text{moisture (\%)}}{100} \right)} \quad (1)$$

2.3. Essential Oils Chemical Composition Analysis

The chemical compositions of the EOs were analyzed by gas chromatography coupled to mass spectrometry (GC/MS) using the Shimadzu QP 2010 ultra system (Shimadzu, Tokyo, Japan). The instrument was equipped with an AOC-20i auto-injector and an Rtx-5MS silica capillary column (30 m long and 0.25 mm diameter; 0.25 μm film thickness) supplied by Restek (Bellefonte, PA, USA). Operating conditions included a temperature program of 60 °C to 240 °C (with a rise rate of 3 °C/min). Injector temperature at 250 °C; the carrier gas used was helium at a rate of 1 mL/min, split-type injection 1:20 (5 μL of EO in 500 μL of hexane). Mass spectra were obtained by electronic impact at 70 eV, and ion source temperature was maintained at 200 °C.

The identification of the chemical components was based on the comparison of the linear retention indices with the retention times of a series of homologous *n*-alkanes and on the fragmentation patterns observed in the mass spectra, using reference data from the Adams [9] and Flavor and Fragrance 2 [10] libraries.

2.4. DPPH Antiradical Capacity

The antiradical capacity was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl; Sigma-Aldrich, St. Louis, MO, USA, production batch STBH5699) free radical method. Stock solutions of the EO were prepared at a concentration of 10 mg/mL in ethanol (Sigma-Aldrich, production batch 459844). Aliquots of these solutions (50 μL) were mixed with 1900 μL of DPPH and 50 μL of 0.5% (m/m) Tween 20 (Dinâmica, production batch 100687). The reaction medium was incubated for 120 min. The control was prepared by replacing the EO solution with ethanol. Absorbances were measured every 30 min over 2 h at 517 nm on a UV-Vis spectrophotometer ULTROSPEC 7000 (Biochrom US, Holliston, MA, USA). The results were calculated using the following equation:

$$\text{IDPPH (\%)} = \left(\frac{\text{AbsB} - \text{AbsA}}{\text{AbsB}} \right) \times 100\% \quad (2)$$

where AbsA and AbsB are the absorbances of the sample and control (blank), respectively.

To identify the antioxidant capacity equivalent to Trolox (Cayman Chemical Company, production batch 0468715-21, Sigma-Aldrich, St. Louis, MO, USA), a calibration curve was constructed with Trolox at concentrations of 30, 60, 150, 200, and 250 µg/mL in ethanol under the same conditions used to determine the inhibition of EO. The capacity equivalent to Trolox was calculated using Equation (3) below:

$$m \frac{ET}{g} = IDPPH \times 100 \times a \times D \quad (3)$$

where “a” is the angular coefficient of the curve and “D” is the dilution factor [11].

2.5. Lipid Peroxidation Capacity

A stock solution of β-carotene (Sigma-Aldrich, production batch MKCP5833)/linoleic acid (Sigma-Aldrich, production batch SLCL0533) mixture was prepared as follows: 10 mg of β-carotene was subsequently solubilized in 500 µL of chloroform (Sigma-Aldrich, production batch 29031300) and reserved (solution A). In an amber vial, chloroform (HPLC grade), 40 µL of linoleic acid, and 530 µL of concentrated Tween were added, and 1 mL of chloroform and then mixed with solution “A”. The chloroform was completely evaporated.

Right away, 70 mL of oxygen-saturated water was added with vigorous stirring. Samples were read with 200 µL diluted EO solution (1 mg/mL), 200 µL (1 mg/mL) of the antioxidant Trolox, and a control group with 200 µL ethanol. The absorbance of the reaction medium was measured at 470 nm and monitored in the interval from zero to 120 min under heating at 30–40 °C. Antioxidant activity (AA%) was calculated in relation to the percentage of inhibition in relation to the control used below. The experiments were carried out in triplicate [12].

$$AA = \frac{(\text{Control}_{0\text{min}} - \text{Control}_{120\text{min}}) - (\text{Sample}_{0\text{min}} - \text{Sample}_{120\text{min}})}{\text{Control}_{0\text{min}} - \text{Control}_{120\text{min}}} \times 100$$

2.6. Statistical Analyses and Bibliographic Search Criteria

To evaluate the intraspecific chemical variability, principal component analysis (PCA) was used to compare the six samples with previously reported samples from the literature (Appendix A). The components of oils with concentrations greater than 4% were used as variables in OriginPro test software version 2024b (OriginLab Corporation, Northampton, MA, USA). Statistical significance was assessed by the ANOVA away test followed by Tukey’s test ($p < 0.05$) using the GraphPad Prism software version 5.0.

The analysis of bibliometric data was carried out by a literature search using keywords of related articles to the theme proposed in this work, using the VOSViewer software (version 1.6.15). The articles were downloaded from the databases in a supported software format. The primary data retrieved from the databases include information related to the article title, author names, keywords, and citation information, including reference lists. These data were then used to generate a network map, a visual representation of the interconnections between the keywords, which provided a comprehensive overview of the research landscape [13].

3. Results

3.1. Yield and Chemical Composition of Essential Oils

The *S. guianensis* EO yields presented an average of 0.97%. The highest yield was found in samples SG-6 (1.2%) and SG-2 (1.1%), displaying no statistical differentiation (see Figure 2 below).

Regarding the chemical composition of the EOs, 82 chemical components were identified by GC-MS analysis (chromatograms are shown in Appendix C), comprising, on average, 88.2% of the total content of the oils. The oxygenated sesquiterpenoid class was

the most representative, with an average of 71.4% in the six studied specimens, as shown in Table 2 below.

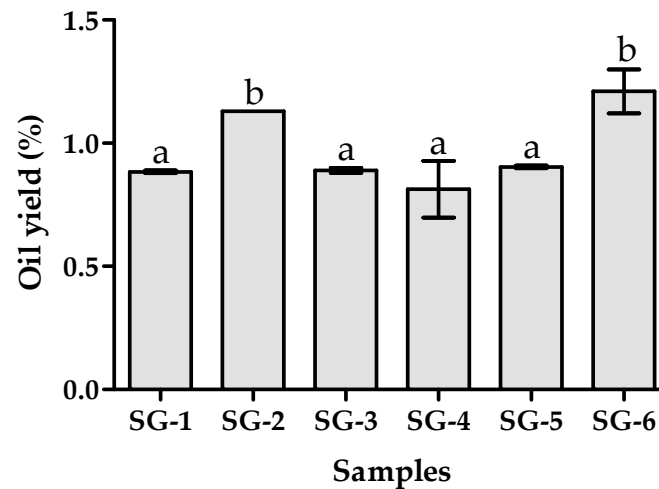


Figure 2. Comparison of essential oils yield of *Siparuna guianensis* samples. Values with the same letters in the bars do not differ statistically in the Tukey test ($p > 0.05$).

Table 2. Chemical composition of essential oils from *Siparuna guianensis* samples.

RI _C	RI _L	Constituents	SG-1	SG-2	SG-3	SG-4	SG-5	SG-6	Compounds Classes
934	924 ^a	α-Thujene			0.1			0.1	MH
934	932 ^a	α-Pinene	0.4		0.1			0.3	MH
973	969 ^a	Sabinene				tr		tr	MH
977	974 ^a	β-Pinene						tr	MH
1028	1024 ^a	Limonene			0.1			tr	MH
1337	1335 ^a	δ-Elemene	0.2	0.2	0.2				SH
1350	1345 ^a	α-Cubebene	1.5	2.4	0.3				SH
1377	1374 ^a	α-Copaene	3.6	2.5	0.2	2.1	0.2	0.1	SH
1381	1387 ^a	β-Bourbonene	3.9	2.6	0.4	3.8	0.4	1.3	SH
1387	1389 ^a	β-Elemene	3.9	4.8	1.1	3.4		0.4	SH
1391	1387 ^a	β-Cubebene	3.3						SH
1417	1410 ^a	β-Longipinene		1.6					SH
1420	1417 ^a	<i>E</i> -Caryophyllene	2.4	2.0	0.4	1.5			SH
1439	1437 ^a	α-Guaiene			0.3				SH
1446	1436 ^a	β-Copaene		0.2					SH
1454	1452 ^a	α-Humulene	0.3	0.4	0.1				SH
1461	1464 ^a	<i>9-epi-E</i> -Caryophyllene	0.3			1.7			SH
1472	1482 ^a	γ-Amorphene	1.0	0.5	0.8		0.8		SH
1478	1488 ^a	Germacrene D		0.9					SH
1487	1486 ^a	β-Selinene						0.3	SH
1477	1478 ^a	γ-Muurolene	0.2				0.2	0.1	SH
1486	1492 ^a	β-Selinene		0.6					SH
1489	1486 ^a	Dauca-5,8-diene		0.6					SH
1491	1494 ^a	Curzerene		2.7				0.9	SO
1495	1501 ^a	Epizonarene			0.4				SH
1496	1486 ^a	α-Amorphene		0.3			0.2		SH
1496	1495 ^a	δ-Amorphene	1.3						SH
1498	1500 ^a	Bicyclogermacrene	1.4		0.4				SH
1500	1500 ^a	α-Muurolene	0.2	0.6				0.1	SH
1513	1514 ^a	Cubebol	1.3	2.7				0.9	SO
1516	1513 ^a	γ-Cadinene	1.3	0.5	0.6				SH
1517	1522 ^a	δ-Cadinene	3.2	2.9	0.3	5.0		1.1	SH
1658	1651 ^a	Pogostol						2.2	SO

Table 2. Cont.

RI _C	RI _L	Constituents	SG-1	SG-2	SG-3	SG-4	SG-5	SG-6	Compounds Classes
1538	1544 ^a	α -Calacorene	0.1	0.5					SH
1547	1548 ^a	Elemol	0.5	4.4	30.3	11.5			SO
1559	1559 ^a	Germacrene B			7.0		1.6		SH
1567	1562 ^a	<i>epi</i> -Longipinanol	2.1				36.1		SO
1573	1582 ^a	Viridiflorol		0.2					SO
1576	1574 ^b	Ledol					0.3		SO
1579	1582 ^a	Caryophyllene oxide	2.8	3.4		8.7	0.9	0.4	SO
1585	1577 ^a	Spathulenol	41.8	13.9	9.8	33.3	43.3	11.5	SO
1588	1596 ^a	Fokienol	4.6				4.4		SO
1592	1592 ^a	Viridiflorol		1.6					SO
1598	1605 ^a	Curzerenone		9.6				23.9	SO
1614	1604 ^a	Khusimone		0.2			1.4		SO
1617	1608 ^a	β -Atlantol						0.3	SO
1630	1627 ^a	<i>epi</i> -Cubenol	0.4	1.5		1.0	0.6		SO
1639	1643 ^a	2- <i>epi</i> - β -Cedren-3-one	4.7			2.1			SO
1640	1646 ^a	Agarospinol						3.7	SO
1641	1645 ^b	τ -Muurolol		0.4					SO
1644	1645 ^a	Cubenol	1.2	3.1		2.4		0.8	SO
1648	1644 ^a	α -Muurolol	2.4	0.3				2.5	SO
1650	1640 ^a	β -Eudesmol			4.3				SO
1652	1649 ^a	α -Eudesmol			5.4				SO
1653	1652 ^a	Himachalol		2.6					SO
1658	1651 ^a	Pogostol						2.2	SH
1652	1644 ^b	Aromadendrene				1.1	0.8		SH
1653	1661 ^a	<i>allo</i> -Himachalol					0.3		SO
1656	1676 ^a	Mustakone	0.3	0.7	16.5	1.2			SO
1657	1659 ^a	Cadin-4-en-10-ol		1.0					SO
1683	1684 ^a	<i>epi</i> - α -Bisabolol		0.3					SO
1683	1688 ^a	Eudesma-4(15)-dien-1 β -ol			7.8				SO
1690	1692 ^a	Junicedranol		0.4					SO
1688	1693 ^a	Germacrone						0.1	SO
1729	1734 ^a	Eremofilone					1.3		SO
1728	1733 ^a	<i>iso</i> -Bicyclogermacrenal		0.1					SO
1765	1766 ^a	Drimenol	6.4	4.3		6.1	5.6	15.7	SO
1775	1773 ^a	α -Costol		0.2					SO
1879	1884 ^b	<i>n</i> -Hexadecanol		0.2					O
1943	1941 ^a	Drimenin						7.0	SO
2019	2026 ^a	<i>E,E</i> -Geranyllinalool		0.5					O
2101	2100 ^a	Heneicosane		0.3					O
2603	2600 ^a	<i>n</i> -Tetracosane		5.6		0.9			O
		Monoterpene hydrocarbons	0.4	0.0	0.2	0.0	0.0	0.5	
		Oxygenated monoterpenoids	0.0	0.0	0.0	0.0	0.0	0.0	
		Sesquiterpene hydrocarbons	27.9	24.0	12.4	18.5	4.1	5.6	
		Oxygenated sesquiterpenoids	68.5	53.4	74.1	66.2	94.1	73.7	
		Others	0.0	6.6	0.0	0.9	0.0	0.0	
		Total identified	96.7	84.1	86.7	85.7	98.2	79.8	

RI_C = calculated retention index; RI_L = literature retention index; ^a, Adams [14]; ^b, Mondello [10]; main constituents in bold; standard deviation was less than 2.0 ($n = 2$).

One of the key findings in our study is the consistent presence of the oxygenated sesquiterpenoid spathulenol in all the samples, with an average concentration of $25.6 \pm 15.6\%$. Notably, the samples SG-5 (43.3%) and SG-1 (41.8%) exhibited the highest amounts of this constituent, while the other samples showed varying levels: 33.2% (SG-4); 13.8% (SG-2); 11.5% (SG-6), and 9.8% (SG-3).

In addition to spathulenol, our analysis revealed the presence of other significant constituents in the EO. For instance, elemol (0.5–30.3%) was found in samples SG-1 (0.5%),

SG-2 (4.3%), SG-4 (11.5%), and SG-3 (30.3%), with an average concentration of $11.6 \pm 13.2\%$. However, it was not detected in samples SG-5 and SG-6, indicating the unique chemical profiles of these samples.

Analogous to elemol, mustakone (0.3–16.5%) was also present in four of the six specimens, with an average of $4.6 \pm 7.9\%$, in SG-3 (16.5%), SG-1 (0.3%), SG-2 (0.7%), SG-4 (1.2%), SG-3 (30.3%), and absent in samples SG-5 and SG-6.

Also noteworthy is the occurrence of drimenol (4.3–15.7%) with an average content of $7.0 \pm 4.6\%$ in sample SG-6 (15.7%), the highest concentration, followed by samples SG-1 (6.4%), SG-2 (4.3%), SG-4 (5.6%), SG-5 (6.0%), and absent in the SG-3 sample.

Curzerenone (9.7–23.9%), with a mean of $16.8\% \pm 4.6$, was identified only in samples SG-6 (23.9%) and SG-2 (9.0%) and absent in the other samples. Likewise, the constituent *epi*-longipinanol, with an average concentration of $19.1\% \pm 24.0\%$, showed the highest concentration in sample SG-5 (36.1%), a low content in sample SG-1 (2.1%), and was not detected in the other samples.

3.2. Intraspecific Chemical Variability and Occurrence

Regarding *S. guianensis* samples from the literature, the occurrence is reported only in Brazil; the geographic distribution of specimens is shown in Figure 3. The 13 specimens were mostly collected in the Brazilian northern region (7 specimens), followed by the southeast region (2 specimens), and 1 specimen in the central-west region.

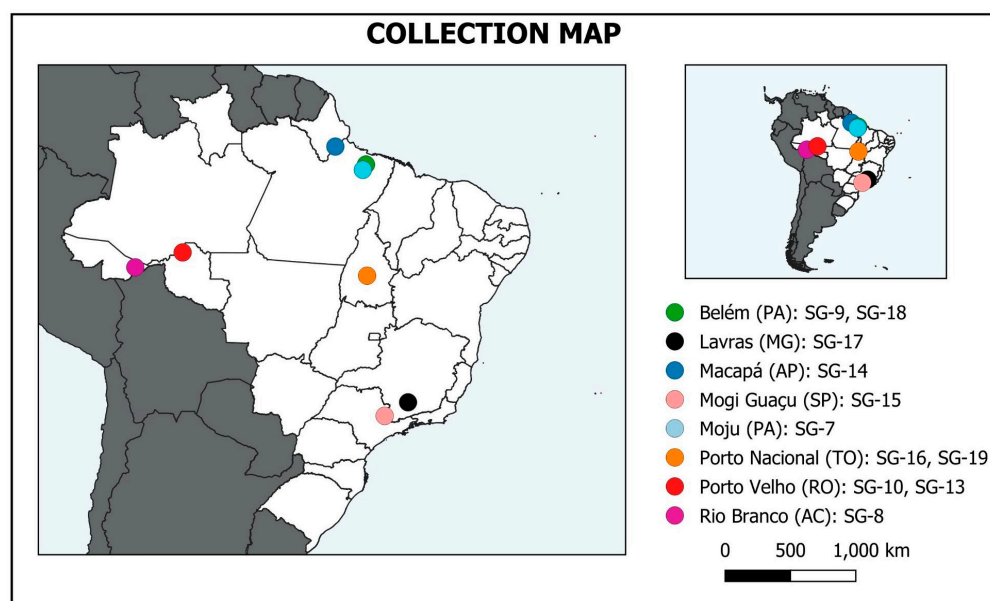


Figure 3. Map of botanical material collection in studies found in the database.

The interrelationship of constituent classes of *S. guianensis* EOs specimens of this work (SG-1 to SG-6) and literature (SG-7 to SG-19) were evaluated using principal components analysis (PCA), based on the following classes: OM: oxygenated monoterpenoids; MH: monoterpene hydrocarbons; SH: sesquiterpene hydrocarbons; OS: oxygenated sesquiterpenoids; O: Other classes; Table 3.

The PCA represented 89.78% of the data variability (Figure 4). PC1 explained 40.37% of the data, presenting positive correlations with the classes of monoterpene hydrocarbons (MH: $\lambda = 2.56757$), oxygenated monoterpenoids (OM: $\lambda = 2.21248$), sesquiterpene hydrocarbons (SH: $\lambda = 0.18965$); negative correlations with oxygenated sesquiterpenoids (OS: $\lambda = -2.86488$), and others (O: $\lambda = -0.00149$). PC2 explained 27.93% and demonstrated positive correlations with oxygenated monoterpenoids (OM: $\lambda = 1.63089$), oxygenated sesquiterpenoids (OS: $\lambda = 0.93739$), and others (O: $\lambda = 1.41573$); negative correlations with monoterpene hydrocarbons (MH: $\lambda = -0.16124$) and sesquiterpene hydrocarbons

(SH: $\lambda = -2.67177$). PC3 described 21.48% and showed positive correlations with oxygenated monoterpenoids (OM: $\lambda = 0.38911$), sesquiterpene hydrocarbons (SH: $\lambda = 1.73078$), and others (O: $\lambda = 3.2476$); negative correlations with monoterpene hydrocarbons (MH: $\lambda = -1.46655$) and oxygenated sesquiterpenes (OS: $\lambda = -0.90097$).

Table 3. Compounds classes present in specimens used in the multivariate analysis.

	MH	OM	SH	OS	O	TI	Ref.
SG-1	0.4		27.9	68.5		96.7	*
SG-2			24.0	53.4	6.6	84.1	*
SG-3	0.2		12.4	74.1		86.7	*
SG-4			18.5	66.2	0.9	85.7	*
SG-5			4.1	94.1		98.2	*
SG-6	0.5		5.6	73.7		79.8	*
SG-7	10.6	0.3	20.1	50.7		81.7	[15]
SG-8	0.4	0.2	13.8	76.7		91.1	[15]
SG-9	4.8	1.0	35.1	58.2		99.1	[15]
SG-10	0.1		0.4	99.3		99.8	[16]
SG-11	1.6		74.7	8.4		84.7	[16]
SG-12	15.7	1.6	68.9			86.2	[16]
SG-13	59.3	3.0	2.9	1.3		93.5	[16]
SG-14	27.4		14.2	38.9		80.5	[17]
SG-15	8.4	83.1		5.7	2.3	99.5	[18]
SG-16	50.5	23.7	17.5	7.6		99.3	[19]
SG-17	17.9	1.7	38.0	21.6		79.3	[20]
SG-18	0.7	0.3	38.6	59.0		98.6	[21]
SG-19	39.7	9.8	25.5	24.1		99.0	[4]

* = see Table 2; MH = Monoterpene Hydrocarbons; OM = Oxygenated Monoterpenoids; SH = Sesquiterpene Hydrocarbons; OS = Oxygenated Sesquiterpenoids; O = Others; TI = Total Identified.

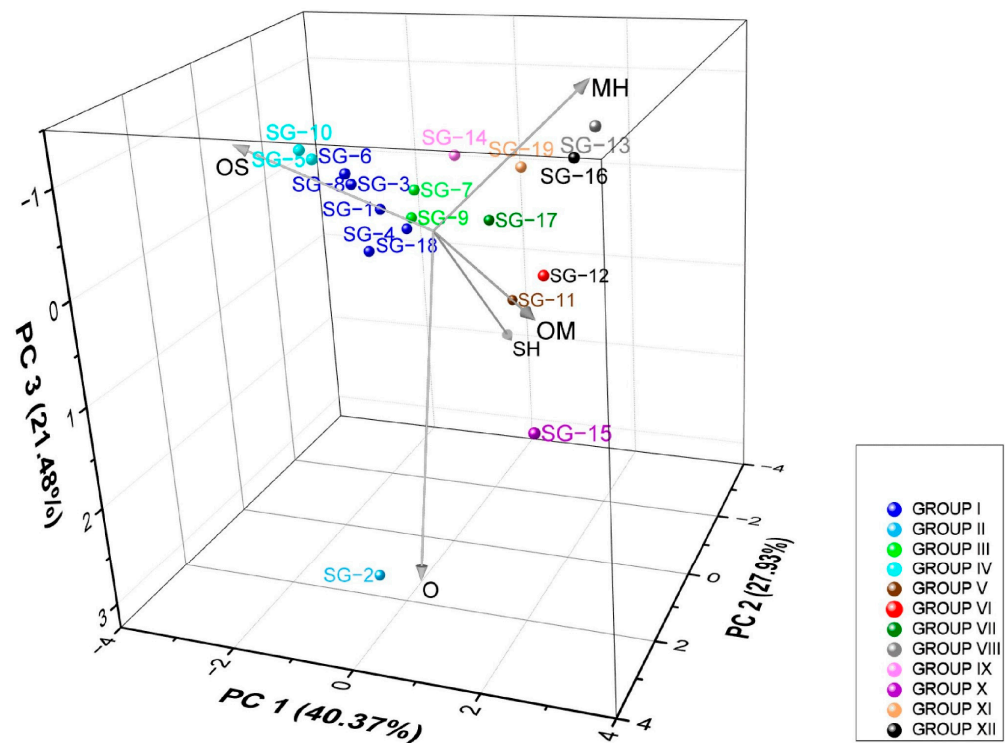


Figure 4. Principal components analysis (PCA) based on the compound classes of *Siparuna guianensis* essential oil samples.

The *S. guianensis* EO, extracted by hydrodistillation of leaves in this work (SG-1 to SG-6), and those reported in the literature (SG-7 to SG-18) were classified into 12 groups

according to PCA analysis. Group I (SG-1, SG-3, SG-4, SG-6, SG-8, and SG-18) was characterized by the predominance of oxygenated sesquiterpenoids (OS: 59.0–76.7%) followed by sesquiterpene hydrocarbons (SH: 5.6–38.6%) collected in Brazil from the state of Pará (SG-1, SG-3, SG-4, SG-6, SG-18) and Acre (SG-8). Group II (SG-2) was characterized by oxygenated sesquiterpenoids (OS: 53.4%), sesquiterpene hydrocarbons (SH: 24.0%), and others (O: 6.6%) of a specimen from Brazil (Pará state). Group III (SG-7 and SG-9) was characterized by oxygenated sesquiterpenoids (OS: 49.7%), sesquiterpene hydrocarbons (SH: 19.0%), and oxygenated monoterpenes (OM: 9.4%), both samples collected in Pará state. Group IV (SG-5 and SG-10, collected in Pará and Rondônia states) was rich in oxygenated sesquiterpenoids (OS: 94.1 and 99.3%), followed by sesquiterpene hydrocarbons (SH: 4.1 and 0.4%). Group V (SG-11) was characterized by sesquiterpene hydrocarbons (SH: 74.7%) followed by oxygenated sesquiterpenoids (OS: 8.4%) occurring in a specimen from Tocantins. Group VI (SG-12) was characterized by sesquiterpene hydrocarbons (SH: 68.9%), followed by monoterpene hydrocarbons (MH: 15.7%) in a sample from Rondônia state. Group VII (SG-17) was characterized by sesquiterpene hydrocarbons (SH: 38.0%), oxygenated sesquiterpenoids (OS: 21.6%) and monoterpene hydrocarbons (MH: 17.9%) collected in Minas Gerais state. Group VIII (SG-13) was characterized by hydrocarbon monoterpenes (MH: 59.3%) followed by oxygenated monoterpenoids (OM: 30.0%) from Rondônia. Group IX (SG-14) characterized by oxygenated sesquiterpenoids (OS: 38.9%), monoterpene hydrocarbons (MH: 27.4%) and sesquiterpene hydrocarbons (SH: 14.2%) collected in Amapá state. Group X (SG-15) was rich in oxygenated monoterpenoids (OM: 83.1%), and low amounts of monoterpene hydrocarbons (MH: 8.4%) and oxygenated sesquiterpenoids (OS: 5.7%) collected in São Paulo state. Group XI (SG-19) was characterized by monoterpene hydrocarbons (MH: 39.7%), sesquiterpene hydrocarbons (SH: 25.5%), and oxygenated sesquiterpenoids (OS: 24.1%) collected in Tocantins state. Group XII (SG-16) was characterized by monoterpene hydrocarbons (MH: 50.5%), oxygenated monoterpenoids (OM: 23.7%), and sesquiterpene hydrocarbons (SH: 17.5%) from Rondônia.

Sample SG-2 stands out in this study, due to the “other” class, as it presents a higher concentration compared to the other samples with the compound *n*-tetracosane, an unusual compound among samples due to this class, which is a determining factor for the formation of an isolated group (group II). The compound *n*-tetracosane found only in sample SG-2, is a 24-carbon linear hydrocarbon. According to previous reports it is a promising molecule with its potential uses as a biopesticide in the control of insects and larvae [22]. In addition to another potential explored in a preliminary study that points to pharmacological use, the bioactive shows significant cytotoxic action using MTT cancer cell testing (in vitro) [23].

The variability between the *S. guianensis* samples analyzed demonstrates variability in the chemical composition of the major constituents belonging to the classes of groups formed by the PCA (Appendix A).

It is notable that the 19 examples of EO oils based on their chemical composition were organized into 19 distinct chemical profiles according to the majority chemical composition (>5%) as follows: Profile I (SG-1) stood out for the presence of spathulenol (41.8%) and drimenol (6.4%). Profile II (SG-2) was composed of spathulenol (13.8%) and curzerenone (9.6%). Profile III (SG-3) had as main constituents elemol (30.3%), mustakone (16.52%), and spathulenol (9.8%). Profile IV (SG-4) revealed significant levels of spathulenol (33.2%), elemol (11.5%), and caryophyllene oxide (8.7%). Profile V (SG-5) exhibited a mixture of spathulenol (43.3%) and *epi*-longipinanol (36.1%). Profile VI (SG-6) was characterized by the presence of curzerenone (23.9%), drimenol (15.7%), and spathulenol (11.5%). Profile VII (SG-7) was dominated by *epi*- α -bisabolol (25.1%), spathulenol (15.7%), and α -pinene (6.3%). Profile VIII (SG-8) presented spathulenol (22.0%), selin-11-en-4 α -ol (19.4%), elemol (10.0%), and β -eudesmol (10.0%). Profile IX (SG-9) had as its main constituents atractylone (31.4%) and germacrone (23.2%). Profile X (SG-10) revealed the predominance of *E*-nerolidol, with 99.3%. Profile XI (SG-11) described γ -cadinene in a proportion of 47.8%, and γ -elemene in 12.6%. Profile XII (SG-12) was characterized by the presence of valencene (27.5%), *E*-caryophyllene (21.6%), and zingiberene (13.0%). Profile XIII (SG-13) presented α -pinene in

27.6%, 1,8-cineole in 22.6%, and *p*-cymene in 9.8%. Profile XIV(SG-14) exhibited a mixture between α -muurolol at 33.2% and terpinolene at 17.2%. Profile XV(SG-15) stood out for the significant presence of decanoic acid in 46.6%, and 2-undecanone in 31.7%. Profile XVI (SG-16) revealed the abundance of β -myrcene at 45.6%, and 2-undecanone at 17.8%. Profile XVII (SG-17) had as its main constituents β -myrcene in 13.1%, and germacrene D in 8.7%. Profile XVIII (SG-18) was characterized by the presence of atracylone in 18.6%, *trans*- β -elemenone in 11.8%, germacrene D in 7.6%, curzerene in 7.1%, γ -elemene in 7.0% and Profile XIX (SG-19) indicated the presence of the constituents β -myrcene (39.7%), *epi*-curzerenone (18.2%) and germacrene D (14.3%).

As observed, the 19 *S. guianensis* specimens were extracted using the same extraction method (hydrodistillation) and the same plant part (leaves). The EO chemical variability may be related to other issues, such as the age, size, stage of development of the plant at the time of collection [5]. The understanding of the chemical composition of the plant is paramount as recent research has shown promising pharmacological results on *in vivo* antitumor activity in the treatment of Ehrlich tumor attributed to the action of bioactive components from the plant under analysis [3].

To find the most widespread topics about *S. guianensis* EOs and identify their analyzed bioactive potentials, we investigated the co-occurrence of similar terms in titles and abstracts of 198 keywords found in the Scopus and PubMed databases from 1990 to 2024. Figure 5 represents this research and its associations. The size of the node indicates the extent of searches for the term. In other words, the larger the node, the more frequently the term was searched. The search terms are grouped according to their similarity. Thus, there was the grouping of 13 clusters (13 colors in Figure 5); the most prominent cluster (red) includes terms related to *in vivo* tests, such as “animal”, and “feeding behavior”, among others, followed by the yellow, orange, green, and blue clusters suggest bioactive tests with pharmacological uses and biological activities “antioxidants”, “anti-inflammatories”, “anti-cancer”. The purple and pink cluster proposes the use of “oils, volatile”, “plant extracts” and analysis of chemical composition, encompassed by green with “natural products”, “phytotherapy”, cyan cluster adds biopesticides “biological control” uses.

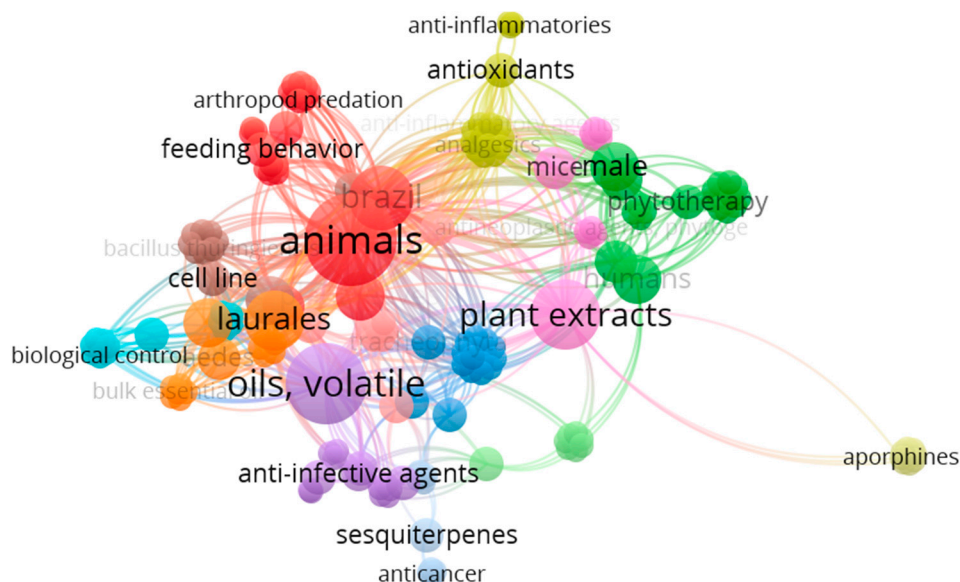


Figure 5. Network map of the most searched keywords and related to the theme, from 1990 to 2024.

The bibliometric analysis highlights the significant bioactive potential of *S. guianensis*, especially regarding its antitumor properties. The presence of a remarkable intraspecific chemical variability in this species is a relevant aspect to be considered. Such chemical diversity can have a significant impact on the effectiveness and consistency of the antitu-

mor effects associated with the plant. Therefore, conducting new studies is necessary to deepen the understanding of the impact of this variability in the chemical composition and its effect on the therapeutic use of the plant. This investigation aims to ensure more reliable and consistent results, thus expanding the possibilities of therapeutic application of *S. guianensis*.

3.3. Antioxidant Capacity

The evaluation of the antioxidant capacity of the six samples was carried out using two different methods. All six specimens were subjected to the DPPH free radical capture method and lipid peroxidation inhibition assay in the system composed of β -carotene and linoleic acid.

3.3.1. DPPH Anti-Radical Evaluation

The DPPH assay consists of free radicals. The mechanism of action aims to identify the oil's ability to inhibit the reactivity of 1,1-diphenyl-2-picrylhydrazyl. Through the donation of a hydrogen radical, when a compound can donate an atom of hydrogen, the DPPH radical is reduced simultaneously and the violet color is lost, then the free radical formed tends to undergo successive reactions to create a stable product. While DPPH can accept a hydrogen atom or an electron to form a stable, diamagnetic molecule, the oxidation of DPPH is difficult and irreversible [24].

The results of inhibitions (Table A2, Figure 6) do not demonstrate prominent inhibitions. The SG-2, SG-4, and SG-5 were similar in the Tukey test ($p > 0.05$), but the SG-3 showed a significant difference from the others.

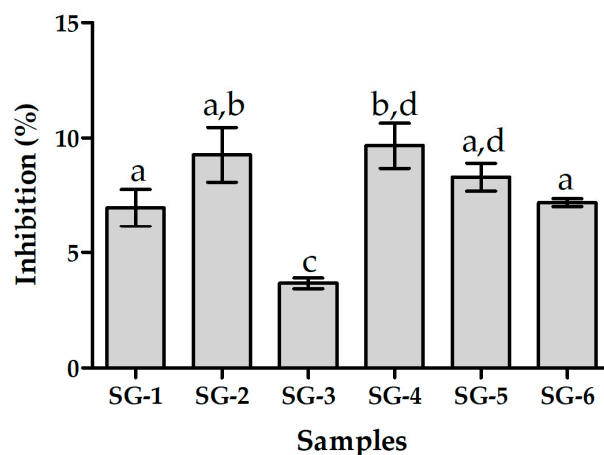


Figure 6. Comparison of inhibitions of *Siparuna guianensis* essential oils against DPPH. Values with the same letters in the bars do not differ statistically in the Tukey test ($p > 0.05$).

The antioxidant effect is proportional to the disappearance of radicals in the test samples. This reaction is stoichiometric in relation to the number of hydrogen radicals absorbed [25]. In this way, the total antioxidant capacity is expressed in Trolox equivalent capacity (TEAC, mg TE/g). The SG-4 value (108.1 ± 11.5) is about eight times lower than the Trolox, and the specimens have a low inhibition index.

The specimens' chemical composition is predominantly composed of oxygenated sesquiterpenoids. Since sesquiterpenes usually have low antioxidant action, *S. guianensis* EO compounds are not capable of donating hydrogen atoms to reduce the DPPH radical, which causes low antioxidant activity measured in the test [26]. Moreover, there is a lack of preliminary in vitro antioxidant studies in the literature regarding the *S. guianensis* anti-DPPH activity, leaving a gap for comparison.

3.3.2. Lipid Peroxidation Activity

The co-oxidation method of the β -carotene/linoleic acid system consists of analyzing the EO's ability to prevent and protect the oxidation of β -carotene against free radicals resulting from the peroxidation reaction of linoleic acid in contact with saturated oxygen water. Therefore, simulating an in vitro reactive oxygen species (ROS) attack process against important biomolecules to cellular biochemistry and the protective capacity to give bioactive compounds in experimentation [27].

Regarding the *S. guianensis* lipid peroxidation inhibitions (Figure 7 and Table A3), insignificant inhibitions were found in the EO samples, with no emphasis between them, as they are considered statistically similar in the Tukey test ($p > 0.05$). Thus, the percentage found to be less than 40% inhibition signals that antioxidant capacity is low [28].

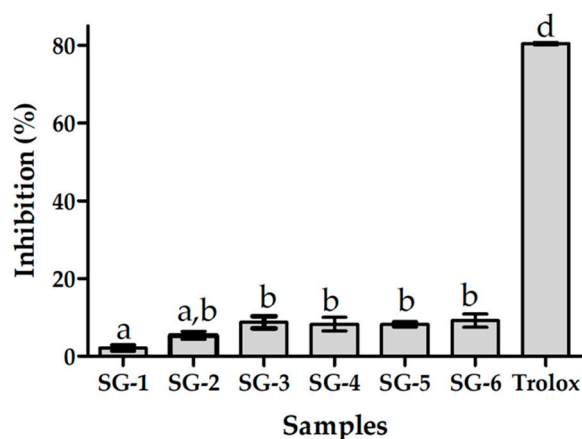


Figure 7. Comparison of *Siparuna guianensis* inhibition in the lipid peroxidation test. Values with the same letters in the bars do not differ statistically in the Tukey test ($p > 0.05$).

In previous studies that used the β -carotene/linoleic acid method to evaluate antioxidant capacity, a value of 15.5% was observed, approximately six times lower than the Trolox standard (90.9%) [3]. According to Andrade et al. [20], this indicates a moderate antioxidant capacity. However, this result differs from the values found in the present study (2.1–9.1%).

4. Conclusions

The effect of intraspecific chemical variability on *Siparuna guianensis* essential oil was significant with the formation of 19 chemical profiles; six profiles were reported for the first time. This variability can be related to different collection locations, seasonality and genetic variability.

There was no significant variability in relation to yield or antioxidant capacity through DPPH and β -carotene/linoleic acid. Moreover, due to the high pharmacological potential of the plant as an antitumor agent, it is necessary to consider the intraspecific variability in the chemical composition of *S. guianensis*, which suggests future studies focusing on seasonality, and comparisons between different plant tissues.

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Data Availability Statement: The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Table A1. *Siparuna guianensis* essential oil composition.

Sample Code	Occurrence	Plant Part/ Extraction Type	Primary Components (>5%)	Major Classes	Oil Yield (%)	Ref.
SG-1	Outeiro, Pará, Brazil	Leaves (HD)	Spathulenol (41.77%), drimenol (6.44%), curzerenone (9.60%), Elemol (30.30%), mustakone (16.52%), spathulenol (9.81%)	SO: 68.4%, SH: 27.9%	0.89%	*
SG-2	Outeiro, Pará, Brazil	Leaves (HD)	Spathulenol (13.85%), curzerenone (9.60%), Elemol (30.30%), mustakone (16.52%), spathulenol (9.81%)	SO: 53.4%, SH: 24.0%, O: 6.6%	1.13%	*
SG-3	Outeiro, Pará, Brazil	Leaves (HD)	Spathulenol (33.25%), elemol (11.47%), caryophyllene oxide (8.69%)	SO: 74.1%, SH: 12.4%	0.89%	*
SG-4	Outeiro, Pará, Brazil	Leaves (HD)	Spathulenol (43.31%), <i>epi</i> -longipinanol (36.08%)	SO: 66.2%, SH: 18.5%	0.81%	*
SG-5	Outeiro, Pará, Brazil	Leaves (HD)	Curzerenone (23.92%), drimenol (15.72%), spathulenol (11.52%)	SO: 94.1%	0.91%	*
SG-6	Salva terra, Pará, Brazil	Leaves (HD)	<i>epi</i> - α -Bisabolol (25.10%), spathulenol (15.70%), α -pinene (6.30%)	SO: 76.7%, SH: 5.6%	1.21%	*
SG-7	Moju, Pará, Brazil	Leaves (HD)	Spathulenol (22.00%), selin-11-en-4 α -ol (19.40%), elemol (10.00%), β -eudesmol (10.00%)	SO: 58.2%, SH: 35.1%, MH: 10.6%	0.20%	[15]
SG-8	Rio Branco, Acre, Brazil	Leaves (HD)	Atractylone (31.40%), germacrone (23.20%)	SO: 76.7%, SH: 13.8%	0.1%	[15]
SG-9	Belém, Pará, Brazil	Leaves (HD)	(E)-Nerolidol (99.30%)	SO: 58.2%, SH: 35.1%, MH: 4.8%	0.3%	[15]
SG-10	Porto Velho, Rondônia, Brazil	Leaves (SD)	γ -Cadinene (47.80%), γ -elemene (12.60%)	SO: 99.3%	0.5%	[16]
SG-11	Porto Velho, Rondônia, Brazil	Leaves (SD)	Valencene (27.50%), <i>E</i> -caryophyllene (21.60%), zingiberene (13.00%)	SH: 74.7%, SO: 8.4%	0.5%	[16]
SG-12	Porto Velho, Rondônia, Brazil	Leaves (SD)	α -Pinene (27.60%), 1,8-cineole (22.60%), β -cymene (9.80%)	SH: 68.9%, MH: 15.7%	0.5%	[16]
SG-13	Porto Velho, Rondônia, Brazil	Leaves (SD)	α -Muurolol (33.20%), terpinolene (17.20%)	MH: 59.3%, MO: 30.0%	0.5%	[16]
SG-14	Macapá, Amapá, Brazil	Leaves (SD)	Decanoic acid (46.60%), 2-undecanone (31.70%)	SO: 38.9%, MH: 27.4%, SH: 14.2%	1.50%	[17]
SG-15	Mogi-Guaçu, São Paulo, Brazil	Leaves (SD)	β -Myrcene (45.62%), 2-undecanone (17.83%)	MO: 83.1%, SO: 5.7%	0.49%	[18]
SG-16	Porto Nacional, Tocantins, Brazil	Leaves (HD)	β -Myrcene (13.14%), germacrene D (8.68%), spathulenol (4.16%), τ -muurolol (4.14%), α -bisabolol (3.53%), Atractylone (18.65%), <i>trans</i> - β -elemenone (11.78%), germacrene D (7.61%), curzerene (7.10%), γ -elemene (7.04%)	MH: 50.5%, MO: 23.7%, SH: 17.5%	ND	[19]
SG-17	Lavras, Minas Gerais, Brazil	Leaves (HD)	β -Myrcene (13.14%), germacrene D (8.68%), spathulenol (4.16%), τ -muurolol (4.14%), α -bisabolol (3.53%), Atractylone (18.65%), <i>trans</i> - β -elemenone (11.78%), germacrene D (7.61%), curzerene (7.10%), γ -elemene (7.04%)	SH: 38.0%, SO: 21.6%, MH: 17.9%	ND	[20]
SG-18	Belém, Pará, Brazil	Leaves (HD)	β -Myrcene (39.67%), <i>epi</i> -curzerenone (18.16%), germacrene D (14.34%)	SO: 59.0%, SH: 38.6%	1.42%	[21]
SG-19	Porto Nacional, Tocantins, Brazil	Leaves (HD)	β -Myrcene (39.67%), <i>epi</i> -curzerenone (18.16%), germacrene D (14.34%)	MH: 39.7%, SH: 25.5%	ND	[4]

SG = *Siparuna guianensis* samples; HD = Hydrodistillation; SD = Steam distillation; NI = Not described; MH = Monoterpenes Hydrocarbons; MO = Oxygenated Monoterpenes; SH = Sesquiterpene Hydrocarbon; SO = Oxygenated Sesquiterpenes; O = Others; * = see Table 2.

Appendix B

Table A2. Antioxidant activity of DDH inhibition essential oils.

Sample	Inhibition (%) *	TEAC
SG1	6.9 ^a	77.8 ± 9.0
SG2	9.3 ^{b,d}	103.9 ± 13.
SG3	3.7 ^c	41.3 ± 3.1
SG4	9.6 ^d	108.1 ± 11.5
SG5	8.3 ^{a,b,d}	93.0 ± 6.9
SG6	7.2 ^{a,b}	80.9 ± 2.1

* Mean ± Standard deviation. Values with the same letters in the column do not differ statistically in the Tukey test ($p > 0.05$).

Appendix C

Table A3. Antioxidant activity of essential oils in β -carotene assay.

Sample	Inhibition (%) *
SG1	2.1 ± 0.8 ^a
SG2	5.4 ± 1 ^{a,b}
SG3	8.7 ± 1.6 ^b
SG4	8.3 ± 1.7 ^b
SG5	8.2 ± 0.6 ^b
SG6	9.1 ± 1.7 ^b
Trolox	80.5 ± 0.3 ^d

* Mean ± Standard deviation. Values with the same letters in the column do not differ statistically in the Tukey test ($p > 0.05$).

Appendix D

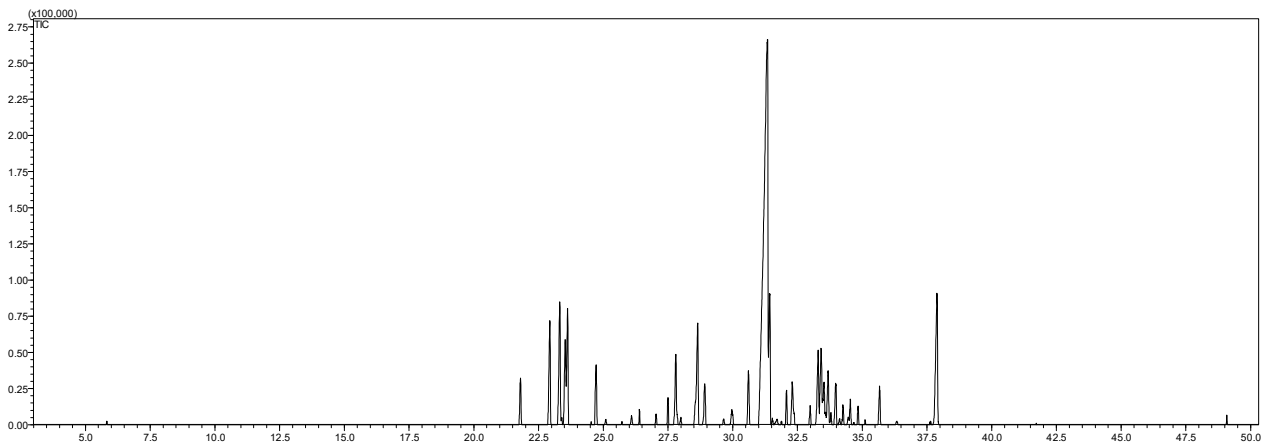


Figure A1. Ion-chromatogram from essential oil extracted from sample SG-1.

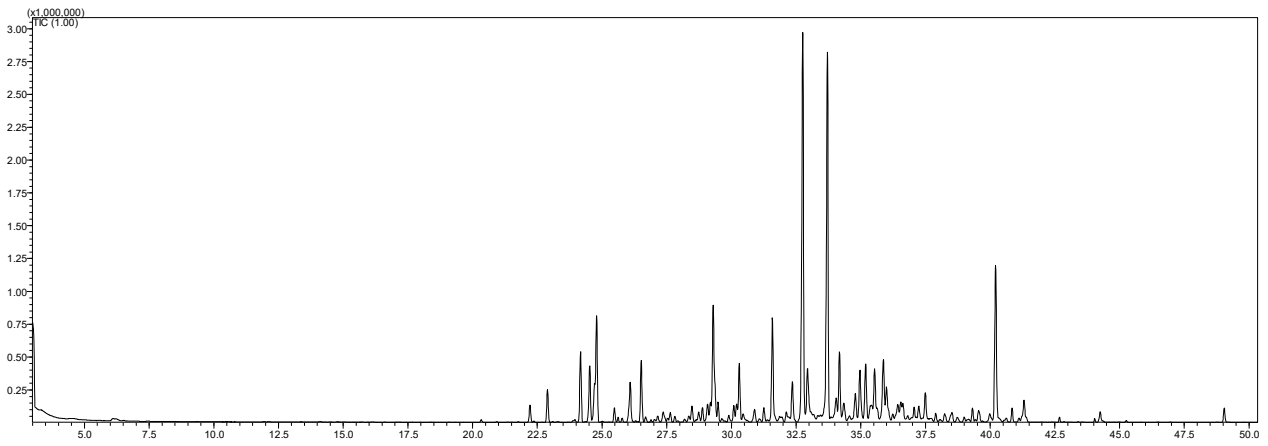


Figure A2. Ion-chromatogram from essential oil extracted from sample SG-2.

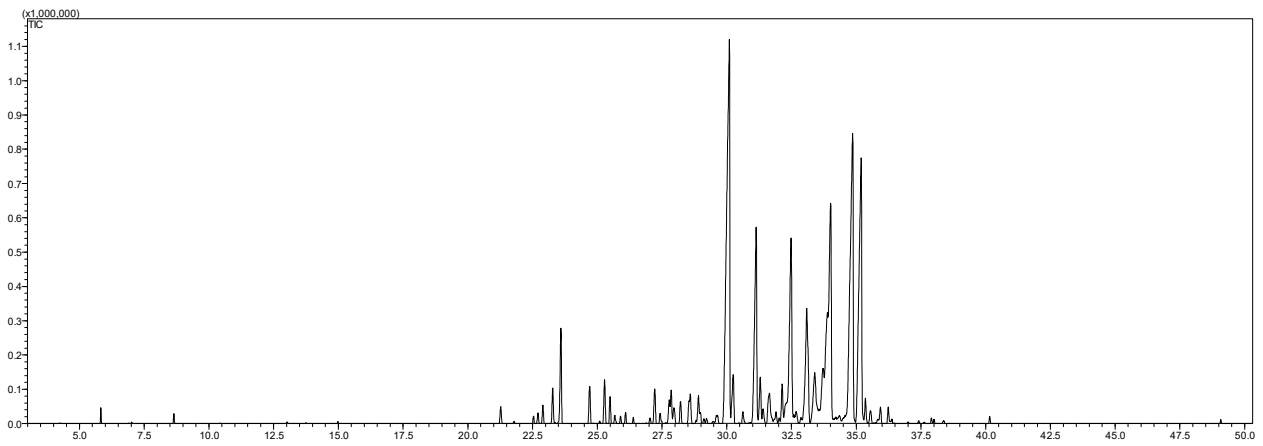


Figure A3. Ion-chromatogram from essential oil extracted from sample SG-3.

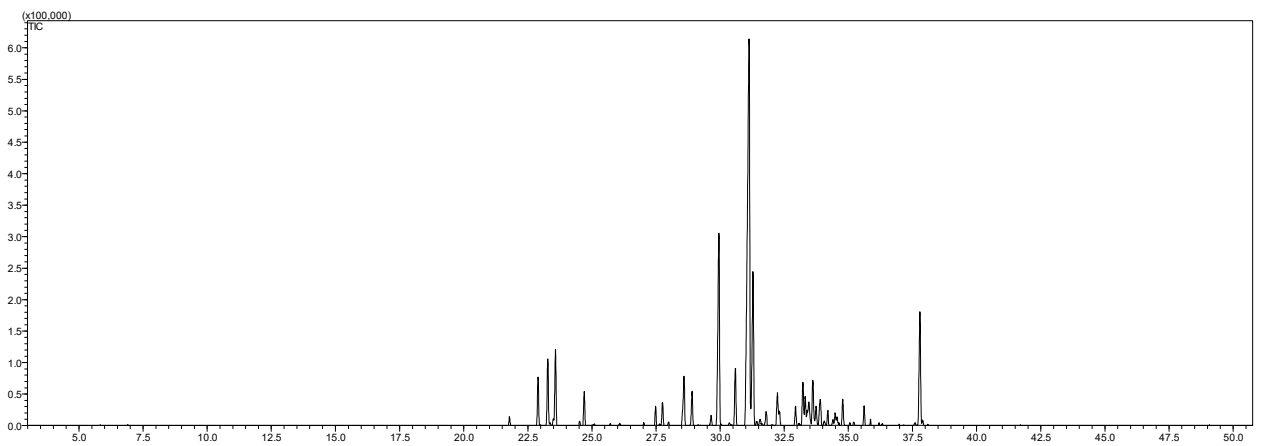


Figure A4. Ion-chromatogram from essential oil extracted from sample SG-4.

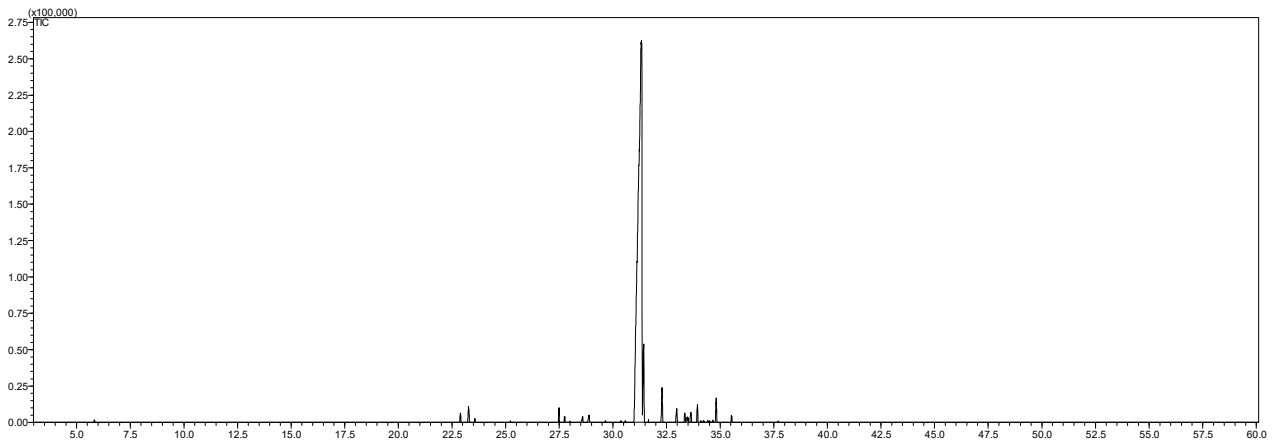


Figure A5. Ion-chromatogram from essential oil extracted from sample SG-5.

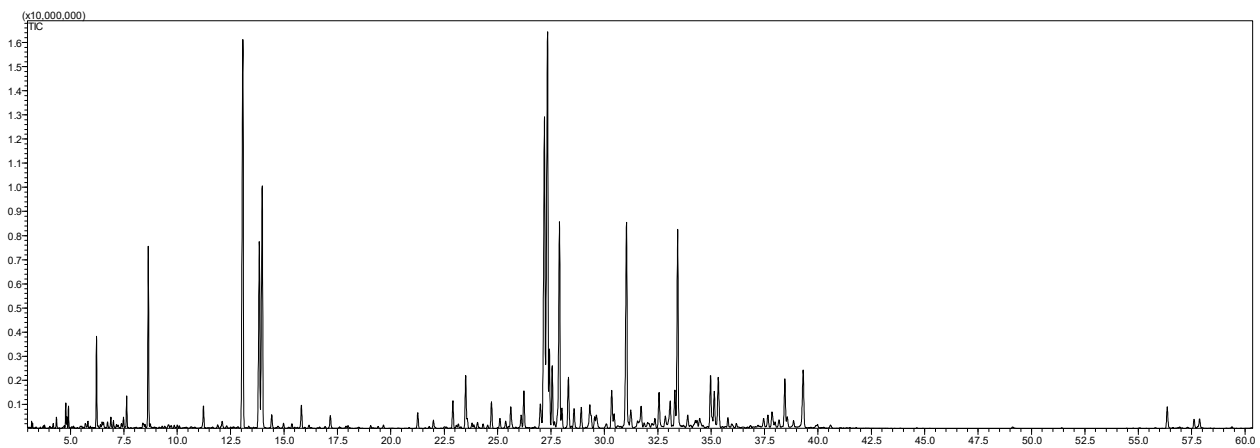


Figure A6. Ion-chromatogram from essential oil extracted from sample SG-6.

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