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Chemical composition and seasonal variation of the volatile oils from *Siparuna guianensis* Aubl. leaves collected from Monte do Carmo, Tocantins

Composição química e variação sazonal dos óleos voláteis das folhas de *Siparuna guianensis* Aubl. coletadas em Monte do Carmo, Tocantins

Composición química y variación estacional de los aceites volátiles de *Siparuna guianensis* Aubl. hojas recolectadas de Monte do Carmo, Tocantins

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Abstract
Siparuna guianensis Aubl., known as “negramina”, “capitu”, is a small tree used for headaches, colds, fevers, as a healing agent, insect, and tick repellents. This study aimed to evaluate the chemical composition and seasonal variability of essential oils from the leaves of *S. guianensis*. Botanical material was collected in Monte do Carmo, Tocantins, Brazil. The powder from the leaves was submitted to hydrodistillation in a Clevenger apparatus, and the identification of the compounds was performed by GC-MS. In volatile oils, 21.32% to 55.44% of sesquiterpenes, 19.95 to 49.73% of oxygenated sesquiterpenes, 0.48 to 1.55% of oxygenated monoterpenes, 0 to 5.67% of monoterpene hydrocarbons were identified, 0 to 48.2% of other compounds. The major compounds were γ-muurolene (13.99 to 35.97%), Curzerene (7.22-19.15%), Curzerenone (7.3-18.13%), 2-undecanone (3.99- 10.63%). The presence of two clusters was verified: cluster I, discriminated by the compounds Curzerenone, β-selinene, δ-elemene, corresponding to the months with the lowest index, and cluster II, discriminated by the β-burbonene, corresponding to the months with the highest index rainfall index. Comparing the present study with data from the literature, it is concluded that *S. guianensis* presents great chemical variability, which can be explained by genetic factors, seasonality, light, temperature, which can alter the production of metabolites. As *S. guianensis* has broad therapeutic potential as an antimicrobial and promising larvicidal activity, there is a need for agronomic studies to obtain

specimens that require more interesting chemical components for the pharmaceutical industry. This study is the first carried out with oils from leaves collected in Monte do Carmo, Tocantins, Brazil.
Keywords: Siparunaceae; Essential oils; Insecticide; Negramin.

Resumo
Siparuna guianensis Aubl., conhecida como “negramina, capitu”, é uma arvoreta utilizada para dor de cabeça, resfriado, febres, como cicatrizante, repelentes de insetos e carrapatos. Objetivou-se avaliar a composição química e variabilidade sazonal dos óleos essenciais das folhas de *S. guianensis*. O material botânco foi coletado em Monte do Carmo, Tocantins, Brasil. O pó das folhas foi submetido à hidrodestilação em aparelho de Clevenger e a identificação dos compostos voláteis obtidos foi realizada por GC-MS. Foram identificados nos óleos voláteis 21,32% a 55,44% de sesquiterpenos, 19,95 a 49,73% de sesquiterpenos oxigenados, 0,48 a 1,55% de monoterpenos oxigenados, 0 a 5,67% de hidrocarbonetos monoterpenos, 0 a 48,2% de outros compostos. Os compostos majoritários foram γ-muurolene (13,99 a 35,97%), Curzerene (7,22-19,15%), Curzerenone (7,3-18,13%), 2-undecanone (3,99- 10,63%). Verificou-se a presença de dois clusters: cluster I, discriminado pelos compostos Curzerenone, β-selineno, δ-elemene, corresponde aos meses com menor índice pluviométrico, e o cluster II, discriminado composto pelo β -burbonene, corresponde aos meses com maior índice pluviométrico. Comparando o presente estudo com dados da literatura, conclui-se que a *S. guianensis* apresenta grande variabilidade química o que pode ser explicada por fatores genéticos, sazonalidade, luz, temperatura, que podem alterar a produção de metabólitos. Como a *S. guianensis* tem amplo potencial terapêutico como antimicrobiano e atividade larvicida promissora, há a necessidade de estudos agronômicos de forma a se obter espécimes que produzem compostos químicos interessantes em maior quantidade para indústria farmacêutica. Esse estudo é o primeiro realizado com os óleos das folhas coletadas em Monte do Carmo, Tocantins, Brasil.
Palavras-chave: Siparunaceae; Óleos essenciais; Inseticida; Negramina.

Resumen
Siparuna guianensis Aubl., Conocido como “negramina, capitu”, es un pequeño árbol utilizado para dolores de cabeza, resfriados, fiebres, como agente curativo, repelente de insectos y garrapatas. El objetivo de este estudio fue evaluar la composición química y la variabilidad estacional de los aceites esenciales de las hojas de *S. guianensis*. El material botánico fue recolectado Monte do Carmo, Tocantins, Brasil. El polvo de las hojas se sometió a hidrodestilación en un aparato Clevenger y la identificación de los compuestos volátiles obtenidos se realizó mediante GC-MS. En los aceites volátiles, se identificaron 21,32% a 55,44% de sesquiterpenos, 19,95 a 49,73% de sesquiterpenos oxigenados, 0,48 a 1,55% de monoterpenos oxigenados, 0 a 5,67% de hidrocarburos monoterpenos, 0 a 48,2% de otros compuestos. Los compuestos principales fueron γ-muuroleno (13,99 a 35,97%), curzereno (7,22 a 19,15%), curzerenona (7,3 a 18,13%), 2-undecanona (3,99 a 10,63%). Se verificó la presencia de dos conglomerados: el conglomerado I, discriminado por los compuestos Curzerenona, β-selineno, δ-elemeno, corresponde a los meses el índice de precipitación más bajo, y el conglomerado II, discriminado por el β-burboneno, corresponde a los meses con con mayor precipitación pluvial. Comparando el presente estudio con los datos de la literatura, se concluye que *S. guianensis* presenta una gran variabilidad química que puede ser explicada por factores genéticos, estacionalidad, luz, temperatura, que pueden alterar la producción de metabolitos. Dado que *S. guianensis* tiene un amplio potencial terapéutico como antimicrobiano y una actividad larvicida prometedora, es necesario realizar estudios agronómicos para obtener muestras que produzcan compuestos químicos interesantes en mayores cantidades para la industria farmacéutica. Este estudio es el primero que se lleva a cabo con aceites de hojas recolectadas en Monte do Carmo, Tocantins, Brasil.
Palabras clave: Siparunaceae; Aceites esenciales; Insecticida; Negramina.

1. Introduction

Siparuna guianensis Aubl. belongs to the Siparunaceae family and is popularly known as “negramina”, “limão bravo”, capitu”. It is a small tree up to 7 m in height, slightly roughened bark, gray in color, simple leaves with a smooth margin, opposite and glabrous, small greenish flowers, fleshy fruit, indehiscent, multiple, drupoid type, seed up to 4 mm in length. Fruit maturation occurs in the rainy season from January to March. The predominant habitat is forest, occurring in Mata de Galeria, Mata Seca and Cerradão. Present in the Cerrado, Amazon, Caatinga, Atlantic Forest and Pantanal domains (Kuhlmann, 2012).

S. guianensis leaves are hypostomatic, covered by a thin layer of cuticle, stellate non-glandular trichomes (Bierase & Sajo, 2009), capitate, and pelted glandular trichomes on both sides, and non-glandular trichomes (tectors), uniseriate, multicellular, unbranched, predominant in the abaxial epidermis. The secretory ducts, which are formed by elongated cavities resulting from the junction of several secretory, epithelial cells, being lined with adjacent cells (Portella et al., 2014).

Epidermal cells on the adaxial surface show cells with straight anticlinal walls, in front view, and on the abaxial surface sinuous anticlinal walls. In the central vein, it presents a main vascular system in the form of an arch with a collateral bundle in an adaxial position. The petiole is covered by a thin cuticle showing the main vascular system in the form of a continuous arch with two collateral bundles in an adaxial position. It is verified that idioblasts contain phenolic compounds in the central vein, petiole, and intervein (Bierase & Sajo, 2009).

The leaves, in the form of decoction and infusion, are indicated for headaches (blood coming out of the nose), colds (De La Cruz, 2008), as a healing agent (Kuhlmann, 2012), against pain and fever (slightly warm baths, as a relaxant and to relieve rheumatic, muscle and body pain) (decoction of 12-15 leaves/liter of water), to prevent lice in chicken nests, as insect repellents (Carvalho & Vilarinho, 2017) and ticks (Kuhlmann, 2012), for digestive problems, snake bite (leaves), nasal congestion, cold, abortive (fruit), as an emetic (root) and for herpes (shells) (Taylor, et al., 2006)

Chemical studies performed by Simas, et al. (2001) identified in fruit receptacles collected in Caratinga, Minas Gerais, Brazil, the alkaloids liriodenine, nantenin, N-methyl-laurotetanine, norglaucine, asimilobin, asimilobin, anonaine, nornantenine, and in fruit liriodenine, palmitic acid, acid linoleic acid, oleic acid, and stearic acid. Guimarães, et al. (2005) isolated from leaves collected in Crato, Ceará, Brazil the compounds quercetin, and two quercetin derivatives: quercetin, 3–OβD–glucopyranosyl(6-1)–rhamnoside, 2, and 7–OβD–glucopyranosyl(6-1)-rhamnoside, kaempferol, and the monoglycoside of kaempferol (rhamnoside). Facundo, et al. (2012) isolated from *S. guianensis* leaves collected in Mirante da Serra, Rondônia, Brazil the compounds kaempferolmethylether, kumatakenina (kaempferol-3,7-dimethyl ether), and kaempferol-3,7,3'-trimethylether. Marti et al. (2013) isolated from the leaves of *S. guianensis* collected in French Guiana the compounds (+) - bulbocapnine, (+) - N-methylindcarpine, (+) - actinodafinine, liriodenine and (+) - 11-methoxynornoelistin. Conegundes, et al. (2021) identified *S. guianensis* leaves collected from Matias Barbosa, the compounds kaempferol-pentosyl-pentoside-rhamnoside, kaempferol dirhamnoside, kaempferol-pentosyl-rhamnoside, rhamnosyl-caempferol and kaempferol dimethyl ether.

Taylor, et al. (2006) observed cytotoxic activity of the young leaf extract of *S. guianensis* for the SKBR3 breast cancer cell line. Andrade, et al. (2015) described antimicrobial activity of volatile oil from leaves against *Listeria monocytogenes* (MIC = 250 μg mL^{-1}), *Staphylococcus aureus* (MIC = 125 μg mL^{-1}), *Aspergillus flavus* (MIC = 7.81 μg mL^{-1}), *Aspergillus carbonarius* (MIC = 125 μg mL^{-1}), *Aspergillus niger* (MIC = 31.25 μg mL^{-1}), *Penicillium commune* (MIC = 31.25 μg mL^{-1}). Melo, et al. (2017) verified activity against *Mycobacterium tuberculosis*, *Mycobacterium kansasii* (MIC = 500 μg mL^{-1}) and *Mycobacterium avium* (MIC = 250 μg mL^{-1}), Moura, et al. (2020) against *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes* (MIC = 0.86 μg mL^{-1}) and *Staphylococcus aureus* (MIC = 1.30 μg mL^{-1}) and Oliveira et al. (2020) against *Streptococcus mutans*, *Enterococcus faecalis*, *Escherichia coli* and *Candida albicans*. Carvalho, et al. (2019) observed anthelmintic activity of ethanol extract and aerial part fractions against eggs and larvae of *Strongyloides venezuelensis in vitro*. Conegundes, et al. (2021) verified anti-inflammatory and antinociceptive activities of the dichloromethane fraction of the leaves.

Scientific studies with *S. guianensis* verified that essential oils from stem, leaves, and fruits showed high toxicity against fourth-stage larvae of *Aedes aegypti* and *Culex quinquefasciatus*. The LC_{50} values obtained for the stem, leaves, and essential fruit oils were 1.76, 0.98, and 2.46 μg mL^{-1} against *A. aegypti* and 1.36, 0.89, and 2.45 μg mL^{-1} against *C. quinquefasciatus* (Aguiar et al., 2015). Ferreira, et al. (2017) verified repellent activity of volatile oil from leaves against fourth stage larvae of *Achroia grisella* Fabricius and *Galleria mellonella* Linnaeus with LC_{50} of 0.08 μg/cm^2 and LC_{50} of 0.38 μg/cm^2, and adult individuals of both the species at a concentration of 0.30 μg/cm^2. Lourenço, et al., (2018) described the high insecticidal activity of volatile leaf oil against *Spodoptera frugiperda* and *Anticarsia gemmatalis*, which was associated with necrosis and apoptotic effects in vitro with lepidopteran cell lines, reproductive deficits (eg, lay deterrent of eggs and decreased egg viability), larval development (eg, inhibition of feeding) and locomotion (eg, walking activities of individual and clustered larvae). Ferreira, et al. (2019) encapsulated the volatile oil extracted from the leaves of *S. guianensis* in chitosan nanoparticles cross-linked with glutaraldehyde and verified increased physical stability, protection from degradation, controlled release of the oil in the control of third-stage larvae. *Ae. Aegypti*, having better larvicidal activity than pure oil. Moura, et al. (2021) developed cassava-based starch microparticles containing the volatile oil *S. guianensis* and found an increase of up to 8 days in ongoing lethal activities (more than 50%) against third-stage larvae of the *Aedes aegypti* and *Culex quinquefasciatus* mosquitoes in comparison with pure volatile oil. According to Toledo, et al. (2019), the volatile oil from the leaves had insecticidal activity against green aphids *Myzus persicae* (Sulzer) with an LC_{95} ¼1.08 mg/cm^2. It significantly repelled the aphids at concentrations as low as 0.14 mg/cm^2.

There is considerable interest by the scientific community in researching the beneficial properties of *S. guianensis* essential oils for the development of products in the pharmaceutical area, mainly due to the growing number of studies on their antimicrobial, insecticide, and repellent activities. In general, the demand for natural products has increased the number of researches related to the elucidation of the therapeutic properties of plants, based on the study of their active constituents (Dias, et al., 2012).

This study evaluates the chemical composition and seasonal variability of volatile oil from *Siparuna guianensis* leaves collected in Monte do Carmo, Tocantins, Brazil.

2. Methodology

Leaves of *Siparuna guianensis* Aubl were collected until 8 am, every 20th of the month, from 10 different individuals, in the municipality of Monte do Carmo – Tocantins, Brazil (-10.71S, -48.406 W, at an elevation of 243.2 m above sea level) for 12 months (April 2019 to March 2020). The specimens of *Siparuna guianensis* were identified by José Realino de Paula. A voucher specimen was deposited at the Herbarium of the Federal University of Goiás, Brazil, Conservation Unit PRPPG, number UFG-72205. The leaves were dried in air circulation stove at approximately 38º C. Climatic data for the period were obtained from the National Institute of Meteorology (INMET, 2021).

2.1 Volatile oils

For the extraction of essential oil, about 40 to 50g of dried botanical material (leaves) was crushed with a commercial crusher (Skymsen, LS-08MB-N) immediately before extracting the essential oil, avoiding losses by volatilization and subjected to hydrodistillation in Clevenger type appliance for 3 hours. Then, the volatiles oils were desiccated with Na_2SO_4, placed in amber glass bottles, and kept at a temperature of -18°C for further analysis.

The volatile oils were analyzed using a Shimadzu GC-MS QP5050A fitted with a fused silica SBP-5 (30 m x 0.25 mm I.D.; 0.25 µm film thickness) capillary column (composed of 5% phenylmethylpolysiloxane) and temperature- programmed as follow: 60-240º C at 3º C/min, then to 280º C at 10º C/min, ending with 10 min at 280º C. The carrier gas was He at a flow rate of 1 ml/min, and the split mode had a ratio of 1:20. The injection port was set at 225 oC. Significant quadrupole MS operating parameters: interface temperature 240º C; electron impact ionization at 70 eV with scan mass range of 40–350 m/z at a sampling rate of 1 scan/s. Constituents were identified by computer search using digital libraries of mass spectral data (NIST, 1998) and by comparison of their retention indices and authentic mass spectra (Adams, 2007), relative to C8–C32 n-alkane series in a temperature-programmed run (Van Den Dool & Kratz, 1963).

Principal Component Analysis (PCA) was applied to examine the interrelationships between the chemical constituents of the volatile oils from leaves collected in different months using the software Statistica (Stat Soft, 2004). A hierarchical cluster analysis (HCA) was used to study the similarity of samples based on the distribution of the constituents, and hierarchical clustering was performed according to the method of minimum variance Ward (Ward, 1963). To validate the cluster analysis was carried out using the canonic discriminant analysis (DCA).

3. Results

3.1 Volatile oils

During the collection period, the months of less rainfall were May (34.9 mm), July (0 mm), August (0 mm), September (31.5mm), with average temperatures ranging from 34.95º C to 20.26º C. The months with the highest rainfall were October (113.6 mm), November (54.8 mm), December (84.7 mm), January (326.1 mm), February (158.3 mm), March (168.0 mm), April (157.15 mm), with temperatures ranging from 34.85 °C to 23.45 °C (Table 1).

Table 1 - Climatic information of the period of collection of plant material of *Siparuna guianensis.*

Date	Rainfall Precipitation monthly (mm)	Relative humidity (%)	Daylight monthly (h)	Average temperature (ºC)	
				Maximum	Minimum
04/2019	157.1	78.36	185.8	33.36	23.92
05/2019	34.9	69.41	255.5	34.95	23.92
06/2019	0	56.09	286.9	35.70	21.26
07/2019	0	50.09	308.5	35.98	20.26
08/2019	0	42.42	304.8	37.45	21.30
09/2019	31.5	40.82	253.5	38.71	24.10
10/2019	113.6	67.70	153.9	34.85	23.85
11/2019	54.8	68.83	165.4	34.84	24.15
12/2019	84.7	68.58	196.6	34.75	24.10
01/2020	326.1	78.31	143.4	32.57	23.45
02/2020	158.3	70.49	134.7	32.71	23.73
03/2020	168	77.03	134	32.42	23.90

Source: INMET (2021). (Porto Nacional Station - OMM: 83064, Latitude: -10.71083333, Longitude: -48.40638888, Altitude: 243.28).

In the monthly analysis, 90.08% to 97.53% of the chemical compounds were identified in the volatile oils of *S. guianensis* leaves, with 21.32% to 55.44% of sesquiterpenes, 19.95 to 49.73% of oxygenated sesquiterpenes, 0.48 to 1.55% oxygenated monoterpenes, 0 to 5.67% hydrocarbon monoterpenes, 0 to 48.2% other compounds. The major compounds were γ-muurolene (13.99 to 35.97%), Curzerene (7.22-19.15%), Curzerenone (7.3-18.13%), 2-undecanone (3.99-10.63) (Table 2). The isopropyl tiglate compound was the majority in December, January, February, and March (18.3 to 24.9%) and was absent in the dry months. The compounds 2-undecanone, y-muurolene, Curzerene Curzerenone were sought in greater quantity in the dry period (Table 2).

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Table 2 - Percentage of the chemical constituents of the volatile oils from *Siparuna guianensis* leaves collected in Monte do Carmo - TO.

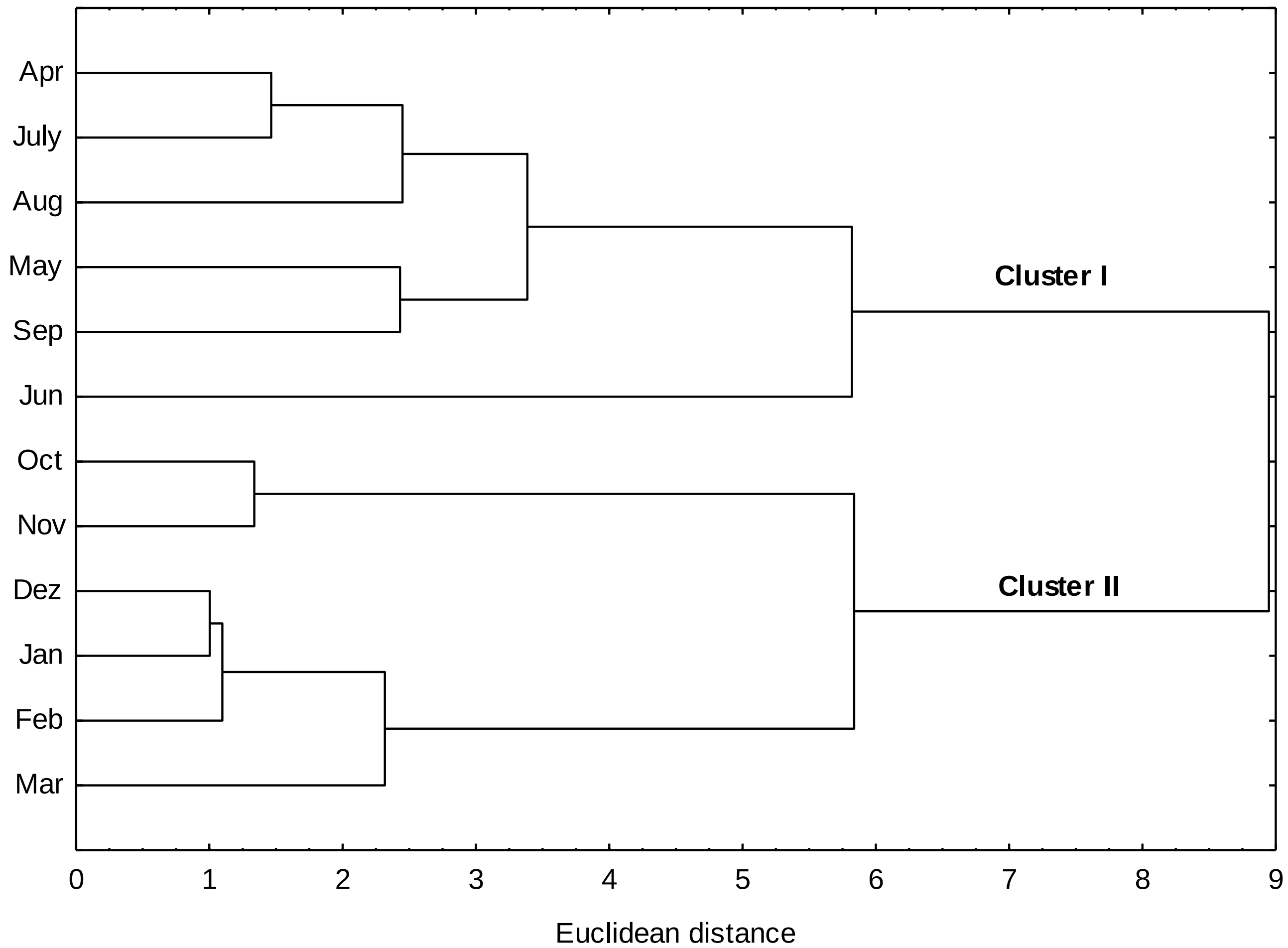
Compounds	KI	**2019**									**2020**		
		Apr	**May**	**Jun**	**July**	**Aug**	**Sep**	**Oct**	**Nov**	**Dez**	**Jan**	**Feb**	**Mar**
n-Octane	800	-	-	-	-	-	-	-	-	7.8	8.3	7.2	5.3
n-Nonane	900	-	-	-	-	-	-	-	-	0.4	0.4	0.3	0.2
Tricyclene	926	-	-	-	-	-	-	0.3	0.3	0.3	0.3	0.3	0.2
Allyl isovalerate	938	-	-	-	-	-	-	-	-	0.35	0.52	0.04	3.4
Isopropyl tiglate	976	-	-	-	-	-	-	-	-	22.8	24.9	21.9	18.3
Myrcene	990	5.5	5.02	-	0.2	0.5	0.3	-	0.3	-	-	-	-
β-phellandrene	1029	0.1	-	-	0.5	0.5	0.4	0.5	0.9	0.5	0.5	0.7	-
(Z)-β Ocimene	1037	-	0.1	-	-	-	-	-	-	-	-	-	-
Cyclohexyl propanoate	1134	-	-	-	0.4	0.5	-	1.3	1.4	0.6	0.96	1.1	0.6
n-Nonanol	1169	0.3	-	-	-	0.9	0.6	0.2	2.6	2.2	2.9	3.1	1.9
cis-chrysanthenyl acetate	1265	1.3	3.4	-	-	-	-	-	-	-	-	-	-
2-undecanone	1294	13.7	8.7	-	10.4	10.6	5.9	8.8	6.0	7.3	5.2	6.3	4.0
δ-elemene	1338	2.3	2.9	4.3	2.2	1.9	2.7	1.96	2.7	0.9	1.5	1.3	1.3
Decanoic acid	1366	0.2	-	-	-	-	-	-	-		1.95	1.98	0.54
β-burbonene	1388	0.61	0.9	0.89	1.16	1.32	1.66	2.95	3.05	1.76	1.76	1.06	0.87
1-Tetradecene	1389	0.2	-	-	-	0.37	-	1.72	0.79	0.84	3.07	2.96	1.02
β-Elemene	1390	0.81	1.27	1.18	1.19	1	1.06	1.07	1.21	0.53	0.84	0.93	0.7
Methyl eugenol	1403	1.13	0.64	1.55	1.38	1.4	1.2	1.09	1.53	0.48	0.85	0.87	0.87
β-Ylangene	1420	0.8	0.92	1.2	0.92	0.84	0.96	1.19	1.36	0.74	0.81	0.87	0.71
β-copaene	1432	0.23	0.27	0.46	-	-	-	0.4	0.48	0.92	-	-	-
α-trans-Bergamotene	1434	-	0.42	-	0.35	-	-	-	-	-	-	-	-
y-elemene	1436	2.75	3.4	1.9	3.13	2.35	2.3	2.35	2.44	0.92	1.57	1.84	2.19
Aromadendrene	1441	0.3	0.38	0.52	-	-	-	-	-	-	-	-	-
6,9-guaia diene	1444	0.3	0.47	0.63	-	-	0.46	0.55	0.64	0.32	-	-	-
y-muurolene	1479	15.8	23.0	35.97	16.1	20.7	24.3	22.1	27.2	10.6	14.2	14	19.2

β-selinene	1490	0.28	0.34	0.43	0.41	-	0.36	-	-	-	-	-	-
***γ*-amorphene**	1495	-	0.71	0.8	-	-	-	-	-	-	-	-	-
Curzerene	1499	16.6	18.7	18.3	19.2	18.7	20	14.7	16.8	7.2	8.7	8.9	12.1
trans-β-Guaiene	1502	0.52	0.49	0.9	0.45	0.44	0.58	-	0.61	-	-	-	-
δ-amorphene	1512	0.72	0.78	1.11	1.05	0.76	0.89	0.67	0.76	1.22	0.44	0.63	0.81
y-cadinene	1515	2.35	2.93	2.74	2.39	2.08	2.18	2.48	2.73	1.75	1.44	1.42	1.61
10-epi-cubebol	1535	0.43	0.58	0.6	-	-	-	-	-	-	-	-	-
Germacrene B	1561	2.33	4.33	2.42	3.56	3.72	3.25	3.73	3.65	1.67	2.15	2.39	3.14
Espathulenol	1578	0.46	0.88	0.62	2.03	1.35	1.1	1.59	1.28	4.04	0.92	1.49	1.1
Guaiol	1600	-	0.81	-	-	-	-	-	-	-	-	-	-
Curzerenone	1606	16	8.3	12.7	18.1	16.6	16.6	10.1	11.3	7.3	6.8	8.1	12.2
1,10-di-epi-Cubenol	1619	-	0.35	0.53	0.75	0.65	0.63	0.67	-	-		-	-
cis-Cadin 4-en-7-ol	1636	0.42	0.37	-	1.24	0.94	0.88	0.79	0.7	1.87	0.5	0.93	0.75
Cubenol	1638	0.62	0.33	0.62	0.6	-	-	-	-	-	-	-	-
α-muurolol	1646	2.54	1.56	2.69	3.08	2.23	2.49	1.75	2.2	3.19	1.31	1.62	1.74
α-eudesmol	1653	0.87	0.66	1.29	1.46	1.15	1.21	0.88	1	1.37	-	-	-
α-(Z)-Santalol	1675	0.49	0.25	-	-	0.38	0.41	-	-	-	-	-	-
Germacrone	1693	3.57	2.88	0.83	3.29	3.66	1.73	2.94	2.07	1.08	1.65	1.8	2.74
Hexacosane	2600	-	-	-	-	-	-	5.09	-	-	-	1.75	-
Octacosane	2800	-	-	-	-	-	-	4.2	-	-	-	1.59	-
Monoterpene hydrocarbons		5.67	5.13	0	0.66	0.98	0.74	0.81	1.41	0.78	0.85	0.95	0.21
Oxygenated Monoterpenes		1.13	0.64	1.55	1.38	1.4	1.2	1.09	1.53	0.48	0.85	0.87	0.87
Sesquiterpene hydrocarbons		30.1	43.5	55.4	32.9	35.1	40.7	39.5	46.8	21.3	24.7	24.4	30.5
Oxygenated sesquiterpenes		41.9	35.6	38.2	49.7	45.7	45.0	33.4	35.3	26.1	20.0	22.8	30.7
Others		15.7	12.1	0	10.74	12.4	6.5	21.3	10.8	42.2	48.2	48.2	35.2
Total		94.5	97.0	95.2	95.4	95.6	94.2	96.1	95.9	90.9	94.5	97.3	97.5
Yield (%)		0.3	0.4	0.3	0.2	0.3	0.4	0.2	0.3	0.1	0.2	0.3	0.4

Source: Authors.

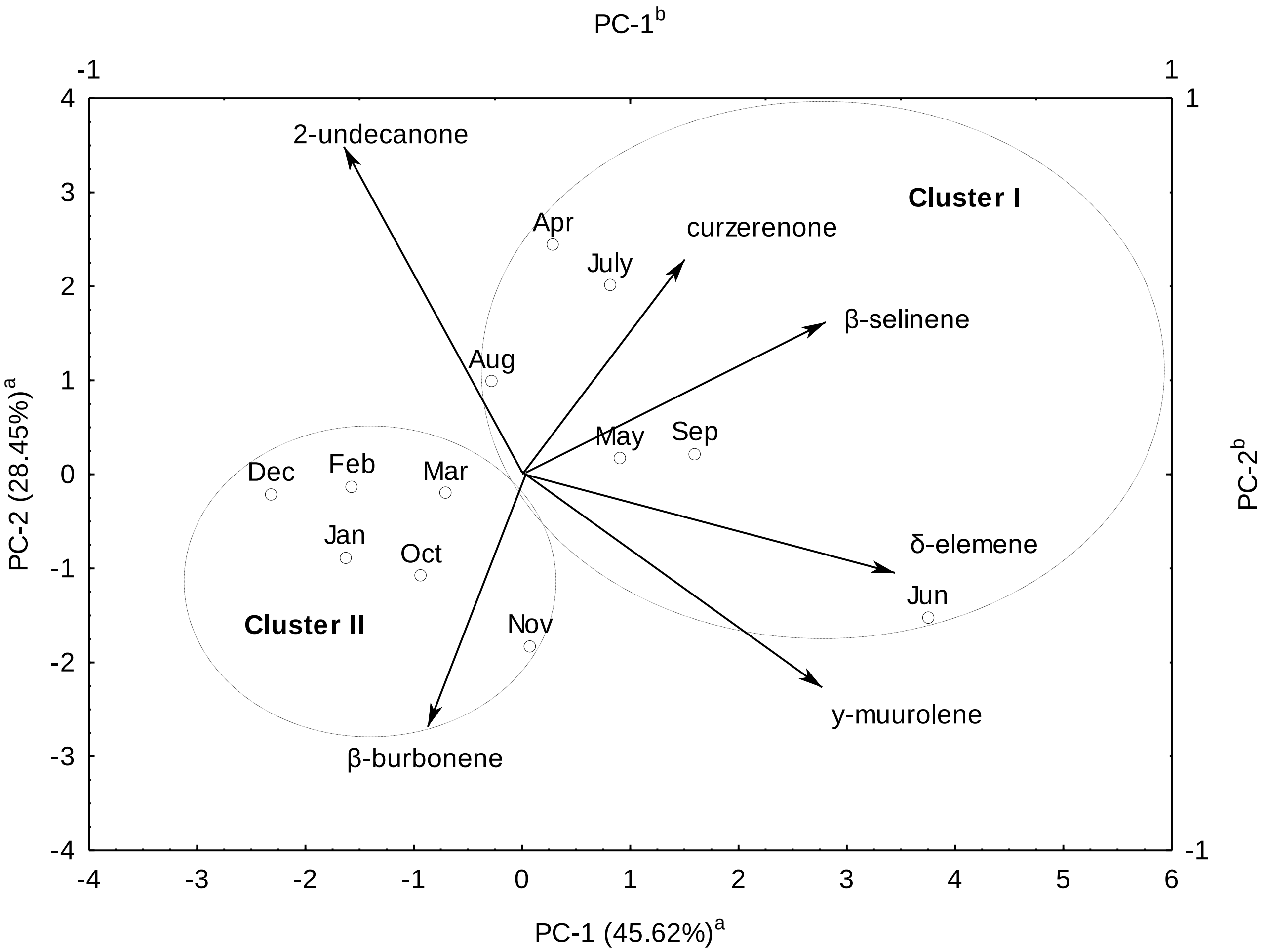
The results obtained in the PCA and the cluster analysis showed chemical variability between samples of volatile oils obtained from leaves of *S. guianensis* (Figure1). Figure 2 indicates that the relative position of the 2D axis originated at the PCA. This analysis suggests that cluster I, broken down by curzerenone, β-selinene, δ-elemene, corresponds to the months with the lowest rainfall (April, May, June, July, August, and September), and cluster II, broken down by β-burbonene corresponds to the months with the highest rainfall (October, November, January, February, and March) (Table 2).

Figure 1 - Dendrogram representing the chemical composition similarity relationships of *Siparuna guianensis* leaves volatiles oils according to Ward's variance minimization method.



Source: Authors.

Figure 2 - Scatterplot from PCA of leaves of *Siparuna guianensis*, samples collected from Monte do Carmo - TO belonging to the clusters I, and II. a. Axes refer to scores from the samples; b. Axes refer to scores from discriminant oil constituents represented as vectors from the origin.



Source: Authors.

Canonic discriminant analysis was performed to help predict the cluster analysis's grouping, and two predictive variables were employed: δ-elemene, and curzerenone; the two discriminant functions retain 91.7% % of well - classification in the original clusters by a cross-validation approach Table 3). Thus, the canonic discriminant analysis revealed that the classification proposed, and the variables employed are suitable to show that the findings of the HCA and the PCA were consistent. Besides, among the independent variables, δ-elemene and curzerenone presented a higher mean in the dry period (cluster I) when compared to cluster II.

Table 3 - Canonical discriminant analysis summary of leaves volatile oil from *Siparuna guianensis*.

	Canonical discriminant			
	Eingenvalues functions	Canonical R	Wilk's Lambda	*p*-level
F1	2.21	0.83	0.3108	0.0052
	Standardized Coefficients for Canonical Variables			
δ-elemene	-0.014			
curzerenone	-0.99			
Cumulative Proportion	1.0			
	Percent of total well-classification	**Cluster I**	**Cluster II**	
		p=0.5	p=0.5	
Cluster I	83.3%	5	1	
Cluster II	100%	0	6	
Total	91.7%	5	7	

Source: Authors.

4. Discussion

The major compounds found in the present study were γ-muurolene, curzerene, curzerenone, 2-undecanone. The isopropyl tiglate was the majority compound in December, January, February, and March (18.3 to 24.9%) and was absent in the dry months. The compounds 2-undecanone, y-muurolene, curzerene, and curzerenone were produced in greater quantity in the dry period. It was verified the presence of two clusters, one in the dry season and the other in the rainy season. Viana, et al. (2002) extracted essential oils from leaves, root bark, stem bark, stem wood, root wood, and fruits of *S. guianensis* collected in Amapá, northern Amazon Forest, Brazil, and identified monoterpenes, sesquiterpenes, sesquiterpene alcohols, and two aliphatic ketones, 2-undecanone and 2-tridecanone. α-Pinene, myrcene, y-cadinene, epi-α-cadinol were present in all samples. Still, epi-α -cadinol (11.9-39.9%) was the main component except for stem bark and oils of the fruits, where terpinolene (33.4%), and 2-undecanone (52.7%) were the majority, respectively. Zoghbi (1998) identified as the main constituents of the volatile oils from the leaves of *S. guianensis* collected in Moju, Pará, Brazil, the epi-α-bisabolol (25.1%) and spathulenol (15.7%), from the leaves collected in Rio Branco (AC) the spathulenol (22%), selin-in-4α-ol (19.4%), β-eudesmol (10%) and lemol (10%), and from those collected in Belém (PA), the germacrone (23.2%), germacrene D (10.9%), bicyclogermacrene (8.6%), and atractilone (31.4%). The main components of essential oils from the leaves of *S. guianensis* collected in Gurupi and Formoso do Araguaia, Tocantins, Brazil, were β-myrcene (79.71%) and 2-undecanone (14.58%), from the stem, were β -myrcene (26.91%), δ-elemene (20.92%), germacrene D (9.42%), α-limonene (7.91%) and bicyclogermacrene (7.79%) and from fruits, 2 -tridecanone (38.75%), 2-undecanone (26.5%) and β-myrcene (16.42%) (Aguiar, et al., 2015); of the leaves collected in Lavras, Minas Gerais, Brazil were β-myrcene (13.14%), germacrene-D (8.68%) and bicyclogermacrene (16.71%) (Andrade, et al., 2015); of the leaves collected in Gurupi, Tocantins, Brazil were β-myrcene (45.62%) and 2-undecanone (17.87%) (Ferreira et al., 2017). Melo, et al. (2017) verified in the volatile oil of fresh leaves of *S. guianensis* collected in Machado, Minas Gerais, the myrcene (1-16.0%), germacrene-D (2-10.0%), E,E-farnesol (3 - 18.0%) and siparunone (4-14.6 %); Ferreira et al. (2019) from leaves collected in Gurupi, Tocantins, Brazil, the β-myrcene (48.6%) and epicurzerenone (19.3%), Toledo et al. (2019) observed from leaves collected in Gurupi, Tocantins o β-myrcene (69.30%) and 2-undocanone (8.37%). Moura, et al. (2020) identified in leaves collected in Porto Nacional, Tocantins, Brazil, β-mircene (39.67%), germacrene D (14.34%) and epicurzerenone (18.16%); Oliveira, et al. (2020) from leaves collected in Belém, Pará o trans-βelemenone (11.78%), atractilone (18.65%), δ-elemene (5.38%), β-elemene (3.13%), β-yerani (4.14%) , γ-elemene (7.04%), germacrene D (7.61%), curzerene (7.1%), and germacrona (5.26%) and Morura, et al, (2021) from leaves collected in Gurupi, Tocantins o β-myrcene (39.16%)), epicurzerenone (16.02%) and β-copaene (9.33%).

Valentini, et al. (2010) evaluated the seasonal variation of volatile oil from *S. guianensis* leaves collected in Bosque Paulo Siqueira in Cuiabá MT, between November 2007 and October 2008, and found siparunone as major compounds (21.09 to 59.5%), spathulenol (2.8 to 17.21%), dihydrocarvyl acetate (5.4 to 23.89%), caryophyllene oxide (2.25 to 19.44 %), viridiflorol (0.56 to 26.47%), ledol (1.22 to 10.49%), limonene dioxide (1.74 to 10.81%) and 1,2-benzenedioic acid (0. 75 to 5.87%). As for the classes of compounds, they presented 1.74 to 10.81% of oxygenated monoterpenes, 1.35 to 5.23% of sesquiterpenes, 47.03 to 80.74 of oxygenated sesquiterpenes, and other non-terpenic compounds from 0.31 to 31.76%. In the essential oils from *S. guianensis* leaves collected in Cuiabá - MT, siparunone was the major component, with a monthly mean equal to 41.64 ± 12.68 (SD), and with the highest production in June 2008, season dry, with the lowest maximum and minimum temperatures. Ledol was identified in the samples of all months, and spathulenol was not identified only in August 2008, and they were identified in greater quantity in the dry-rainy transition season. These compounds identified in practically all monthly samples (siparunone, ledol and spathulenol) had a lower yield in the rainy months. Caryophyllene oxide, 1,2-benzenedioic acid, and dihydrocarvyl acetate had their highest production in the dry season (August 2008), where the highest average maximum temperature of the year was registered.

Comparing this study with data from the literature, it is concluded that *S. guianensis* has great chemical variability, which can be explained by genetic factors, seasonality, light, temperature, water availability, nutrition, herbivory, which can alter the production of metabolites (Gobbo-Neto & Lopes, 2007; Lima, et al., 2003).

5. Conclusion

As *S. guianensis* has broad therapeutic potential as an antimicrobial and promising larvicidal activity, there is a need for agronomic studies of soil, temperature, and light control to obtain specimens that produce interesting chemical compounds in greater quantities for the pharmaceutical industry. This study is the first carried out with oils from leaves collected in Monte do Carmo, Tocantins, Brazil. The results obtained here help to guide the best times for collecting leaves of the *S. guianensis* species and understanding the chemical variability profile of this species related to seasonality. We suggest more studies of the biological activities of purified major compounds in the search for active markers for products to be developed from *S. guianensis*.

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