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Research Article

In Vitro Antibacterial Activity and *in Silico* Analysis of the Bioactivity of Major Compounds Obtained from the Essential Oil of *Virola surinamensis* Warb (*Myristicaceae*)

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Essential oils are well known for their antimicrobial activity and they are used as an effective food preservative. *Virola* is one of the five genera of *Myristicaceae* and this genus is native to the American continent, especially in neotropical regions. The largest number of species of this genus is found in the Amazon region and the most important species include *Virola surinamensis* Warb. and *Virola sebifera* Aubl. In the present study, we describe the chemical composition of the essential oil of the *V. surinamensis* obtained at two different periods of the day in two seasons (rainy and dry), as well as their antimicrobial activity against pathogenic bacterial strains of *Pseudomonas aeruginosa, Escherichia coli,* and *Staphylococcus aureus*. In addition, we investigated, using *in silico* tools, the antimicrobial activity of the major chemical compounds present in the essential oil of *V. surinamensis*. The samples collected at different seasons and times showed a similar chemical profile, characterized by the major constituents α-pinene (>33%) and β-pinene (>13%). The essential oil of *V. surinamensis* showed an interesting antibacterial activity, exhibiting low inhibitory concentrations against the tested bacterial species. The computational investigation indicated that limonene, myrcene, and β-pinene could be related to the antibacterial activity against the tested pathogenic bacterial strains. Our results shed light on the possible constituents of essential oil that could be related to its activity against bacterial species and might be useful for further experimental tests that aim to discover new potential antibacterial agents for food preservation.

1. Introduction

Virola is one of the five genera of *Myristicaceae* and this genus is native to the American continent, especially in neotropical regions. The largest number of species is found in the Amazon and some of the most important species include *Virola surinamensis* Warb. and *Virola sebifera* Aubl. [1]. *V. surinamensis* is an Amazon species, which habits the floodplain and "*igapós*." In the Amazon region, it is popularly known by its Portuguese names *Ucuúba, Sucuúba, Súcuba*, and *Leite-de-mucuiba* [2, 3]. It is described by its medicinal properties in the treatment of infections and inflammations [4, 5].

The antibacterial activity of the genus *Virola* sp. has been investigated in some studies. Cordeiro et al. 2019 [6] obtained the oil extract from the seeds of *V. surinamensis* using Soxhlet and supercritical fluid extraction. They evaluated the antimicrobial activity against *Candida albicans,*

Staphylococcus aureus, and *Escherichia coli,* highlighting that the oil extract obtained by supercritical fluid extraction, which was characterized by high levels of myristic acid and lauric acid, was sensitive for *S. aureus*. In another study, Velasco et al. 2005 [7] showed that the aqueous extract of *V. sebifera* Aubl. had activity against the infection caused by the methicillin-resistant strain of *S. aureus*. The essential oil of *V. surinamensis* was reported by its antimalarial activity, which was associated with the sesquiterpene nerolidol [5, 8]; antitumoral activity in colon cancer cells, which was investigated using the essential oils from bark and leaves, both composed mostly by the sesquiterpenes aristolene, β-elemene, and byciclogermacrene [2]. To the best of our knowledge, there are no studies about the use of the essential oil of *V. surinamensis* as an antibacterial agent. Essential oils with antimicrobial properties may have applications in the food industry, acting as preservatives.

Food safety is fundamental in the food industry and is related to food preservation. There are a variety of synthetic substances that are used as food preservatives, such as nitrates, sorbates, and benzoates. However, natural products have a great appeal to the consumer due to the negative perception associated with synthetic preservatives. Therefore, the scientific community has been looking for substances from natural products that act as food preservatives [9, 10]. Since ancient times, aromatic plants with antimicrobial properties have been recognized as food preservatives and traditional medicines. Essential oils are composed of bioactive compounds which are described by their antioxidant and antimicrobial activities. Thus, those natural products are natural alternatives to replace or reduce the use of synthetic preservatives and they can be potentially used for food preservation, as well as for food packing [11–14]. Different studies have shown the feasibility of using essential oils in their free form or encapsulated form as food preservatives in bakery products [15, 16], vegetables [17, 18], ice cream [19], meat products [20, 21], and in fruit preservation [22–24].

In the present study, we describe the chemical composition of the essential oil of the *V. surinamensis* leaves collected at two different periods of the day (8 a.m. and 4 p.m.) in rainy and dry seasons, and their antimicrobial activity against the American Type Culture Collection (ATCC) bacterial strains of *Pseudomonas aeruginosa* ATCC 27253*, Escherichia coli* ATCC 25922, and *Staphylococcus aureus* ATCC 29213. In addition, we investigated using *in silico* tools the potential antibacterial activities of the major chemical constituents present in the essential oil of *V. surinamensis*. Our results could be useful for further experimental studies that aim to develop and discover new antibacterial agents for food conservation.

2. Material and Methods

2.1. Plant Materials. The leaves of *V. surinamensis* were collected in the municipality of Belém (Pará state, Brazil) in the rainy season (March) and dry season (September) at 8 a.m. and 4 p.m. After collection, the samples were dried in a forced convection oven at 35°C, grounded in a blade mill, and submitted to hydrodistillation. The botanical identification was performed by comparison with an authentic specimen of *V. surinamensis* (MG 180285) previously deposited in the Herbarium of the Museu Paraense Emílio Goeldi (Belém, Pará state, Brazil).

2.2. Essential Oil Extraction. Leaves of *V. surinamensis* obtained from rainy and dry seasons were subjected to hydrodistillation using a modified Clevenger glass extractor for 3 h. We utilized 160 g of the plant material for each experiment. The yield (%) of the essential oil was expressed as the percentage of essential oil concerning the dry matter of the leaves [25–27].

2.3. Chemical Analysis. The chemical composition was analyzed with gas-phase chromatography coupled to mass spectrometry (GC/MS) using a Shimadzu QP 2010 Plus System (Shimadzu Corporation, Japan), equipped with silica capillary column (DB-5MS, length of 30 m, inner diameter of 0.25 mm, and film thickness of 0.25 μm); carrier gas: helium, linear velocity: 36.5 cm·s^{-1}; type of injection: splitless (solution of 2 μL of oil in 500 μL of hexane); injector temperature: 250°C, temperature range: 60°C to 250°C, the gradient of 3°C·min^{-1}; electron impact mass spectrometry: 70 eV; ion source temperature and connection parts: 220°C. The quantification of each component was performed by peak-area normalization using a flame ionization detector (GC-FID, Shimadzu, QP 2010 system) under the same conditions as GC/MS, except for the carrier gas, which was hydrogen.

The components were identified based on the retention index (RI), which was calculated using the retention times of a homologous series of n-alkanes (C8–C40, Sigma-Aldrich, USA). The pattern of fragmentation observed in the spectra was compared with existing patterns of authentic samples in data system libraries and the literature [28].

2.4. Antibacterial Activity

2.4.1. Bacterial Strains. V. surinamensis essential oil was tested against two Gram-negative bacteria: *P. aeruginosa* ATCC 27253 and *E. coli* ATCC 25922 and one Gram-positive bacteria *S. aureus* ATCC 29213. These microorganisms were provided by the Microbiology Laboratory of the Institute Ophir Loyola, Belém, Pará, Brazil. We used ATCC bacterial strains from the Ophir Loyola Hospital that are used in the quality control of the microbiology laboratories, so they were not originated from clinical samples.

2.4.2. Agar Diffusion Assay. For the preparation of the bacterial inoculum, 3 to 4 colonies of each strain isolated in nutrient agar (Kasvi, Brazil) were added in 1 mL of 0.9% physiological solution and compared with 0.5 MacFarland standard to adjust the turbidity of bacterial suspension with approximate cell count density of 1.0×10^8 colony forming units·mL^{-1} (CFU·mL^{-1}).

The antibacterial activity of the essential oil was performed by the disc diffusion method in agar, according to the Clinical and Laboratory Standards Institute. Using a swab, the inoculum (1.0×10^8 CFU·mL^{-1}) was sowed on the surface of the Mueller–Hinton agar in three different directions. Sterile filter paper discs of 6 mm in diameter were impregnated with 10 μL of essential oil *of V. surinamensis*, placed on the surface of the inoculated Mueller–Hinton agar, and incubated for 18 hours at 37°C. The antimicrobial activity was evaluated for each microorganism by measuring the diameter of the inhibition zone (in millimeters ± standard deviation). The tests were performed in triplicate. Bacterial colonies that showed inhibition zones smaller than 8 mm were considered insensitive to the essential oil of *V. surinamensis*.

2.4.3. Minimum Inhibitory Concentration (MIC) Assay. The minimum inhibitory concentration (MIC) was determined by the microdilution method, using the Mueller–Hinton broth and sterile 96-well microplates. The *V. surinamensis* essential oil was solubilized in 2% Tween 80 and successive dilutions were performed to obtain different concentrations (350, 175, 87.5, 43.75, 21.87, 10.93, 5.46, 2.73, and 1.36 μg mL^{-1}). Then, each dilution was added to the wells in triplicate. After that, the suspension of microorganisms was added to each well. One of the wells received the 2% Tween solution, being the test blank, to verify if the solvent interfered with bacterial growth. For the positive control, the Mueller–Hinton broth and bacterial suspension were used; the negative control consisted of the culture medium and sterile saline solution. The plates were incubated at 36°C for 24 h and posteriorly an 0.5% (v/v) aqueous solution of 2,3,5-triphenyl tetrazolium chloride—TTC (Merck, Germany) was added to each well. Then, the plates were incubated at 36°C for 3 hours. The analyses were performed by colorimetric changes in each well. The red color indicates metabolic activity and bacterial growth, so it indicates that the analyzed bacterial strain is not sensitive to the essential oil. No color change indicates the absence of bacterial growth and demonstrates bacterial sensitivity to the specified concentration of the essential oil, indicating that the lowest concentration of the essential oil was able to inhibit bacterial growth.

2.5. Statistical Analyses. Principal component analysis (PCA) was used to investigate similarities in the chemical composition between the essential oil samples extracted at different times (8 a.m. and 4 p.m.) and different seasons (rainy and dry). The analysis was performed with the chemical constituents identified and quantified by CG/MS and CG-FID, respectively, according to. Hierarchical cluster analysis (HCA) was also performed using the Euclidean distance and complete connection [29, 30]. Statistical analyses were performed in the Software Statistica® 13.1 using a correlation of the matrix type (TIBCO Software Inc., USA).

2.6. Computational Prediction of the Antibacterial Activity. To identify potential bacterial species that are targets of the natural compounds of the *V. surinamensis* essential oil, we compared the structure of the major constituents of the essential oil with the structure of bioactive compounds deposited in ChEMBL [31]. The ChEMBL is a chemo-structural database that contains information regarding the bioactivity of compounds belonging to different chemical classes. Initially, the structures of the major compounds of the essential oil were obtained using the Marvin Sketch program [32], and the valency and stereochemical errors were corrected using the Structure Checker program [32]. Then, to perform the structural search, we used the TargetHunter server [33], applying a cutoff 2D similarity equal to 0.7 for each compound structure. The molecular targets were only considered in the final results if they had a value of an inhibitory activity expressed by Ki, IC_{50}, ED_{50}, MIC, and EC_{50}. Finally, a summary table was organized with information on each natural product from essential oils, their SMILES code, ChEMBL accession codes, matched compounds, predicted molecular targets, and references relating to bioactivity.

3. Results and Discussion

3.1. Yield and Chemical Composition of the Essential Oils. Our analyses demonstrated that the collection time did not influence the yield of essential oil; however, higher yields were achieved in the dry season (September), which correspond to the period of lower incidence of rainfall (Table 1). In previous studies, Anunciação et al. (2020) [2] reported yields equivalent to 1.21 ± 0.10% (bark) and 1.78 ± 0.17% (leaves) of the *V. surinamensis* essential oil collected in the Amazon region (Amazonas state, Brazil). Several factors can influence the yield of essential oils, such as seasonal and circadian variations [34, 35]. The Brazilian amazon climate is characterized only by dry and rainy seasons and this classification is directly related to the spatial and seasonal heterogeneity of rainfall in the Amazon region [36].

Differently from the results reported in the present study, Lopes et al. (1997) [37] reported that seasonal and circadian variations did not influence the yield of the *V. surinamensis* essential oil, which was approximately constant (0.5%); however, they noted a strong variation in the essential oil chemical composition. Information about the influence of seasonality and circadian rhythm on the yield of *V. surinamensis*, or its influence on species of the *Virola* genus remains scarce. However, several studies have investigated the possible correlations between essential oil yield and environmental factors along with seasonal and circadian variations. For example, Ribeiro et al. (2014) [38] evaluated the influence of the collection time and seasonality on the yield of *Lippia origanoides* Kunth (*Verbenaceae*) essential oil. As in the present study, for the seasonal study, they also found better yields during the dry period. Furthermore, they pointed out that these results are useful to program the plant collection according to time, month, and chemosystematics interest. In another study, Figueiredo et al. (2018) [39] studied the seasonal influence on the

Table 1: Chemical constituents and their percentages present in the essential oils of *Virola surinamensis* leave collected in the rainy season (March) and dry season (September) at 8 a.m. and 4 p.m.

N°	RI*	Compound	Chemical class	*Relative peak area (%)*			
				Rainy season (March)		*Dry season (September)*	
				8 a.m.	4 p.m.	8 a.m.	4 p.m.
1	936	α-Pinene	MH	34.69	36.16	33.82	38.27
2	979	β-Pinene	MH	17.28	19.27	13.58	19.63
3	991	Myrcene	MH	4.68	5.62	2.5	2.95
4	1016	α-Terpinene	MH		0.34	0.04	0.22
5	1024	p-Cymene	MH		0.05		
6	1029	Limonene	MH	6.31	7.41	3.56	6.08
7	1046	(E)-β-ocimene	MH			0.11	0.08
8	1057	γ-Terpinene	MH	0.36	0.51	0.09	0.32
9	1088	Terpinolene	MH	0.31	0.43	0.09	0.3
10	1099	Linalool	OM	0.74	0.7	0.26	0.75
11	1177	Terpinen-4-ol	OM	0.41	0.53	0.19	0.32
12	1191	α-Terpineol	OM	0.49	0.68	0.54	0.67
13	1340	δ -elemene	SH	1.49	1.17	2.02	1.43
14	1378	α-Copaene	SH	0.09	0.07		0.05
15	1396	β-Elemene	SH	1.86	1.55	2.57	1.72
16	1406	Methyl-eugenol	PH	0.21	0.18	0.01	0.24
17	1425	β-Maaliene	SH	8.64	6.6	15.21	8.42
18	1433	β-Copaene	SH			0.03	
19	1436	γ-Elemene	SH	0.17	0.13	0.22	0.15
20	1446	6,9-Guaiadiene	SH	0.26	0.22	0.24	0.4
21	1455	Spirolepechinene	SH	0.12	0.11	0.13	0.02
22	1457	α-Humulene	SH			0.03	0.05
23	1476	Thujopsadiene	SH	0.12	0.11		0.08
24	1480	γ-Gurjunene	SH	0.19	0.18	0.1	0.18
25	1485	α-Amorphene	SH	1.06	0.87	1.01	0.74
26	1488	Selina-4,11-diene	SH	0.58	0.5	0.38	0.51
27	1491	β-Selinene	SH	0.55		0.42	0.46
28	1496	δ-Selinene	SH	7.37	6.44	13.17	6.12
29	1498	Viridiflorene	SH	2.95	1.66	1.91	2.74
30	1509	α-Bulnesene	SH		0.62		
31	1511	δ-Amorphene	SH	0.57	0.52	0.4	0.52
32	1524	7-Epi-α-selinene	SH	0.92	0.81	1.1	0.82
33	1527	δ-Cadinene	SH	0.23	0.31	0.19	0.23
34	1529	Zonarene	SH				0.03
35	1552	Elemol	SH		0.02		
36	1562	Elemicin	PH	3.96	3.18	3.15	3.01
37	1567	Germacrene B	SH	0.08	0.37	0.4	0.79
38	1569	trans-Dauca-4 (11)-diene	SH	0.85	0.38	0.42	0
39	1588	Globulol	OS			0.06	0.06
40	1626	cis-Cadin-4-en-7-ol	OS	1.45	1.25	1.35	0.92
41	1633	α-Acorenol	OS	0.18	0.15		
42	1637	1-Epi-cubenol	OS	0.06	0.06		
43	1645	Cubenol	OS		0.07		
44	1651	α-Epi-Muurolol	OS				0.03
45	1657	α-Cadinol	OS	0.29	0.18	0.14	0.11
46	1659	Selin-11-en-4-α-ol	OS	0.1	0.1	0.12	0.12
47	1664	Intermedeol	OS			0.07	0.07
			MH	63.63	69.79	53.79	67.85
			MO	1.64	1.91	0.99	1.74
			SH	28.1	22.64	39.95	25.28
			PH	4.17	3.36	3.16	3.25
			OS	2.08	1.81	1.74	1.31
			Total	99.62	99.51	99.63	99.43
				8 am	4 pm	8 am	4 pm
			Yield (%)	0.8	0.8	1.2	1.2

Note. RI*: Retention index calculated using n-alkane standard solutions (C8–C40) in column DB5-MS; MH : Monoterpene hydrocarbons; MO : Oxygenated monoterpenes; SH : Sesquiterpene hydrocarbons; PH : Phenylpropanoid; OS : Oxygenated sesquiterpene.

essential oil yield of *Ocimum campechianum* (*Lamiaceae*, methyleugenol chemotype) and found that the climatic conditions during the seasonal variation affected the yield and this parameter showed a moderate correlation with rainfall. In another study, Ramos et al. (2021) [40] evaluated the influences of seasonal variation and circadian rhythm on the yield of *Piper gaudichaudianum* Kunth (*Piperaceae*) essential oil. During the seasonal study, the yields obtained were in the range of 0.02 to 0.23%, and comparing the average yields of the dry and rainy seasons, there was no significant difference. In the circadian rhythm, the highest values were recorded at 6 a.m. (0.23%) in the rainy season and 12 p.m. (0.16%) in the dry season. The authors highlighted that plant species show different patterns of qualitative plasticity in essential oil yield, which can be influenced by the level of light intensity, temperature, and relative humidity [40]. Therefore, the influence of seasonal and circadian factors is particular for each species and it can vary according to several environmental factors.

Forty-seven chemical constituents were identified in the *V. surinamensis* essential oil (Table 1). More than 54% of the constituents belong to the class of monoterpene hydrocarbons, followed by sesquiterpene hydrocarbons (content above 23%).

The α-pinene (monoterpene hydrocarbon) was the major constituent in the samples collected at different seasons (rainy and dry) and hours (8 a.m. and 4 p.m.), with contents ranging from 33.82% (dry/8 a.m.) to 38.27% (dry/4 p.m.), followed by β-pinene with a variation from 13.58% (dry/8 a.m.) to 19.63% (dry/4 p.m.). The two major constituents of *V. surinamensis* essential oil, the α-pinene and β-pinene, have been reported by several biological activities, such as antibacterial [38, 41], antifungal [42, 43], antioxidant [44], and larvicidal [45, 46].

In our samples, limonene ranged from 3.56% (dry/8 a.m.) to 7.41% (rainy/4 p.m.). The chemical constituents β-maaliene and δ-selinene presented higher levels in the dry season at 8 a.m., with percentages equal to 15.21% and 13.17%, respectively. The main chemical constituents ($\geq$5.0%) identified in the essential oils of *V. surinamensis* during seasonal and circadian variation are shown in Figure 1.

There are still few studies reporting the chemical composition of the essential oil of *V. surinamensis* leaves. Lopes et al. (1997) [37] evaluated the influence of seasonality and circadian rhythm on the composition of the essential oil of *V. surinamensis* specimen collected in the metropolitan region of Belém (Amazon region, Pará state, Brazil) in February, June, and October, at 6 a.m., 12 a.m., 6 p.m., and 9 p.m. The authors did not identify the constituents β-maaliene and δ-selinene. According to their results, the chemical composition varied throughout the seasonal and circadian rhythms. Only limonene was among the major constituents of *V. surinamensis* essential oil, regardless of collection time and season. In February samples, in addition to limonene (11.87–19.85%), high levels of phenylpropanoid elemicin (17.65–26.51%) were also found. In June, limonene (22.92–26.67%) and elemicin (9.19–12.02%) remained among the major constituents, accompanied by α-pinene (12.25–14.44%). The October samples were rich in limonene (10.11–10.64%), caryophyllene (20.40–24.50%), and valencene (23.95–25.44%).

In another study, Anunciação et al. (2020) [2] evaluated the inhibition of essential oil samples obtained from bark and leaves of *V. surinamensis* against HCT116 carcinoma cells using *in vitro* and *in vivo* assays. They reported as major compounds the β-elemene (9.61 ± 1.02%), bicyclogermacrene (8.1 ± 2.0%), germacrene D (7.44 ± 1.80%), α-cubebene (5.69 ± 0.67%), and α-copaene (5.02 ± 0.77%); they indicated that some of these major compounds of *V. surinamensis* essential oil can be an alternative in the development of anticancer compounds to treat the colon cancer.

Multivariate analysis was used to investigate possible similarities in the chemical composition (Table 1) between the samples of *V. surinamensis* essential oils related to the seasonal variation and circadian rhythm. By analyzing the PCA data (Figure 2(a) and 2(b)), it was possible to explain 83.12% (PC1 + PC2) of the chemical variation in the samples. Two groups were formed (Figures 2(b) and 2(c)): Group 1 consisted of samples collected in the rainy season (8 a.m. and 4 p.m.) and dry season (4 p.m.) and Group 2 consisted only of the sample collected in the dry season at 8 a.m.

In qualitative terms, the samples collected at different seasons and times showed a similar chemical profile. Multivariate analyses showed that the variation in the collection time in the rainy season did not influence the chemical composition of essential oils. In addition, the samples collected during the rainy season provided essential oils with a chemical composition similar to the sample extracted from the *V. surinamensis* leaves at 4 p.m. in the dry season. Evaluating the chemical composition of the samples collected in the dry season, we observed that the variation in the collection time caused a quantitative change in the chemical profiles of the essential oil samples analyzed in this season (Figure 2(c)). *V. surinamensis* essential oil presented a chemical composition composed of forty-seven chemical constituents (Table 1). Possibly, the percentage variation of sesquiterpene hydrocarbons β-maaliene and δ-selinene were some of the factors that most influenced the formation of the groups, since the percentage of these chemical constituents was approximately twice higher in the essential oil of the sample collected at 8 a.m. in the dry season.

3.2. Antibacterial Activity

3.2.1. Disk-Diffusion Assay. Table 2 shows the inhibition analyses against the bacterial strains using the disk-diffusion and minimum inhibitory concentration (MIC) assays with the essential oil obtained from *V. surinamensis* leaves collected at 8 a.m and 4 p.m in March and September.

In the initial screening, the essential oil samples showed antibacterial activity against *E. coli* and *S. aureus* that are above the reference values (greater than 8 mm in diameter). However, the disk-diffusion tests performed with the *P. aeruginosa* (Gram-negative bacteria) strain showed inhibition zone values equal to or less than 8 mm. These results

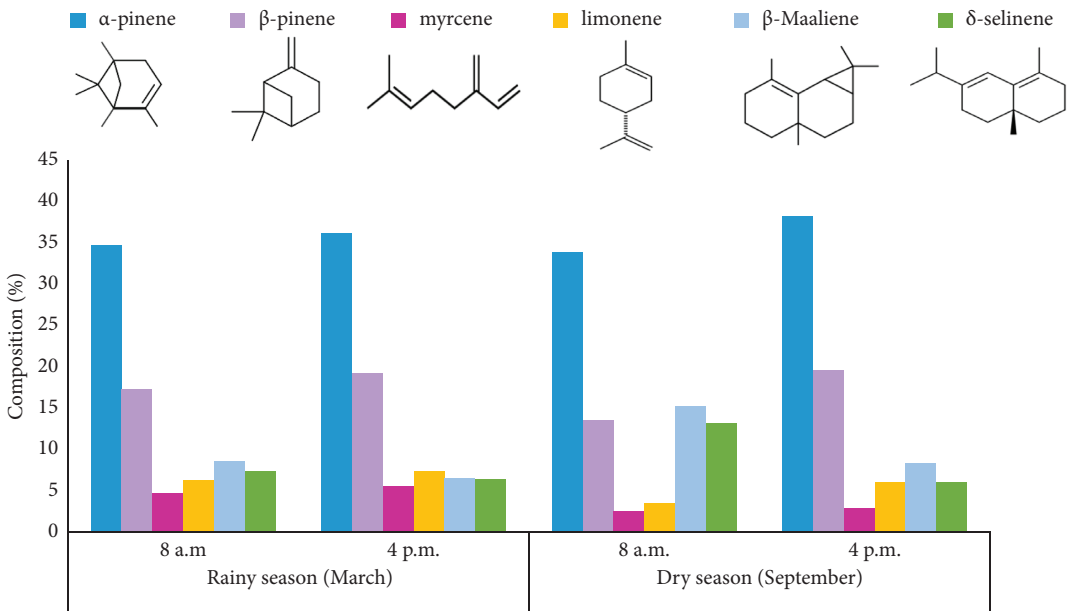


Figure 1: Main chemical constituents (≥5.0%) identified in the essential oils of *Virola surinamensis* leaves, collected in the rainy season (March) and dry season (September) at 8 a.m. and 4 p.m.

were obtained independently of the collection sample time and the seasonal period, thus indicating that essential oil showed to be inactive against this bacterial species.

To the best of our knowledge, the literature did not report similar results of the *V. surinamensis* essential oil against the bacterial species investigated in our study. Analyzing the antimicrobial activities against *E. coli*, we obtained inhibition zones of 12 mm (rainy/8 a.m.) and 14 mm (dry/8 a.m.). The samples obtained in the rainy season (4 p.m.) and dry season (4 p.m.) showed inhibition zones of diameters equal to 13 mm. Different from our results, previous studies did not identify the formation of inhibition zones when testing the *V. surinamensis* extract from stem bark [47] and oil from seed [6] against *E. coli*.

The *V. surinamensis* essential oil tested against the *S. aureus* (Gram-positive) strain showed inhibition zones equal to 12.4 mm for samples from the rainy season (8 am and 4 pm) and inhibition zones equal to 13.7 mm for the dry season (8 am and 4 pm). Interestingly, Costa et al. (2008) [47] reported inhibition zones greater than 8 mm when they evaluated the antibacterial activity of *V. surinamensis* extract from stem bark against *S. aureus*; however, Cordeiro et al. (2019) [6] verified that the oil extracted from the *V. surinamensis* seeds were inactive against *S. aureus*.

3.2.2. Minimum Inhibitory Concentration. The essential oil of *V. surinamensis* showed an interesting antibacterial activity, exhibiting low inhibitory concentrations against the tested bacterial species. The MIC values ranged from 2.73 $\mu g \cdot mL^{-1}$ to 87.5 $\mu g \cdot mL^{-1}$ for the essential oil samples tested (Table 2). It is worth mentioning that the blank sample (2% tween 80) did not interfere with bacterial growth.

The essential oil obtained from the rainy season showed MIC values equal to 10.93 $\mu g \cdot mL^{-1}$ (8 a.m./rainy) and 2.73 $\mu g \cdot mL^{-1}$ (4 p.m./rainy) against the *S. aureus* strain. Regarding the dry season, the essential oil samples obtained from this period showed MIC values equal to 87.5 $\mu g \cdot mL^{-1}$ (8 a.m./dry) and 21.87 $\mu g \cdot mL^{-1}$ (4 p.m./dry). Thus, we noticed that the circadian and seasonal effects did not show a clear relationship with the antibacterial activity of the essential oil samples against the *S. aureus* strain.

The essential oil samples obtained in the dry season showed MIC values equal to 21.87 $\mu g \cdot mL^{-1}$ (8 a.m. and 4 p.m.) against the *E. coli* strain. Regarding the rainy season, the essential oil samples showed MIC values equal to 87.5 $\mu g \cdot mL^{-1}$ (8 a.m. and 4 p.m.). Thus, we noted a significant difference in the antibacterial activity of the essential oil samples due to the seasonality against the *E.coli* strain. We noted that in the HCA plot (Figure 2(c)), majority of chemical constituents showed quantitative differences related to the collection period and it could be corroborated by the variation of the antibacterial activity of the essential oil samples. Despite the chemical profile showing quantitative variability as a function of the seasonal and circadian study, the MIC value for essential oil samples of *V. surinamensis* against the *P. aeruginosa* was equal to 87.5 $\mu g \cdot mL^{-1}$, independently of the season and time of collection. It is important to highlight that the *P. aeruginosa* strain is a Gram-negative bacteria that forms a biofilm that could impair the permeability of substances with antibacterial activity [48–52]. Some essential oils and their chemical constituents can destabilize the bacterial lipid layer, promoting cell membrane degeneration and bacterial death [53, 54].

The chemical composition of all collected samples of *V. surinamensis* essential oils was characterized by a high percentage of α-pinene (>33%) and β-pinene (>13%), which probably influenced their antibacterial activities. Studies have demonstrated that these two compounds in the isolated

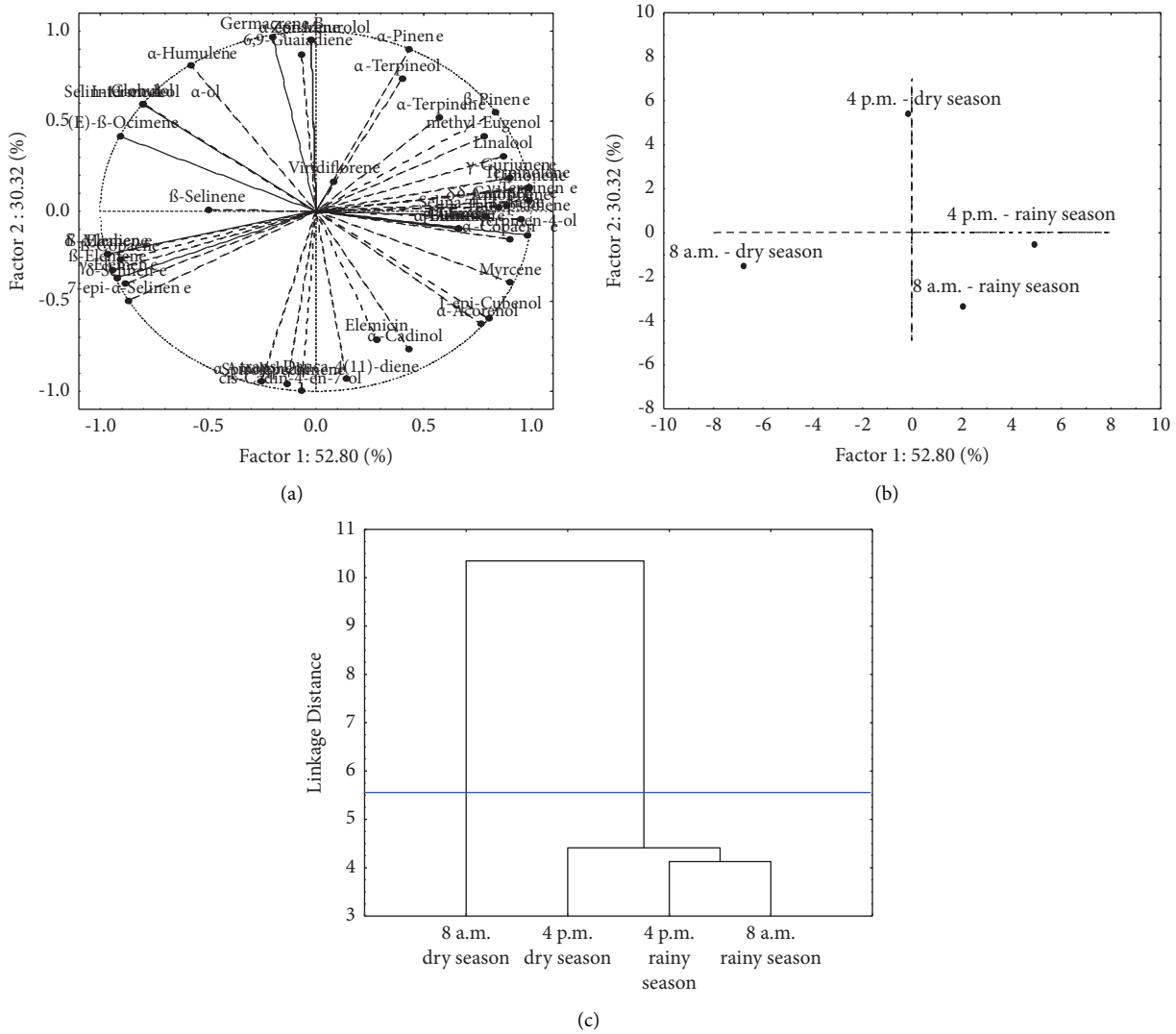


Figure 2: Multivariate analysis of the chemical composition of the essential oils obtained from *Virola surinamensis* leaves collected in the rainy season (March) and dry season (September) at 8 a.m. and 4 p.m. Panels (a) and (b) : Principal Component Analysis (PCA). Panel (c) : Hierarchical Cluster Analysis (HCA). Blue lines indicate the identified groupings.

Table 2: Inhibition zone (IZ) diameter and minimum inhibitory concentration (MIC) for the analysis of the essential oil of *Virola surinamensis* leaves against some bacteria strains.

Bacterial species	*Iz (mm)**				*MIC (μg/mL)**			
	Rainy season (March)		*Dry season (September)*		*Rainy season (March)*		*Dry season (September)*	
	8 a.m.	4 p.m.	8 a.m.	4 p.m.	8 a.m.	4 p.m.	8 a.m.	4 p.m.
Escherichia coli ATCC 25922	12.0 ± 0.0	13.0 ± 0.0	14.0 ± 0.0	13.0 ± 0.0	87.5 ± 0.0	87.5 ± 0.0	21,87 ± 0.0	21,87 ± 0.0
Pseudomonas aeruginosa ATCC 27253	7.0 ± 0.0	8.4 ± 0.8	7.0 ± 0.0	7.0 ± 0.0	87.50 ± 0.00	87.5 ± 0.0	87.5 ± 0.0	87.5 ± 0.0
Staphylococcus aureus ATCC 29213	12.4 ± 0.5	12.4 ± 0.5	13.7 ± 1,2	13.7 ± 1.5	10.93 ± 0.00	2.73 ± 0.0	87.50 ± 0.00	21.87 ± 0.00

*Values are expressed as mean ± standard deviation ($n = 3$).

form show antibacterial activity against different bacterial species [55–57]. Furthermore, essential oils with high content of α-pinene and β-pinene have been reported by antibacterial activity against strains of *P. aeruginosa* [58], *E. coli* [59], and *S. aureus* [60].

However, it is important to highlight that essential oils are composed of a high variety of substances, and thus, their chemical constituents act synergically in the organisms [61, 62]. The antibacterial activity could be attributed to different substances that compose the

essential oil and not only to the majority of components [63, 64].

3.2.3. Computational Identification of Molecular Targets. Different *in silico* methods have been used to identify the molecular targets and the bioactivity of chemical compounds, thus offering a low-cost and effective approach to explore their potential applications and elucidate their molecular mechanisms of action [65–69]. In the present study, we performed a similarity search using the TargetHunter server to identify the bioactivity of the major constituents present in the essential oil samples of *V. surinamensis*. TargetHunter uses a fingerprint-based algorithm named "*Targets Associated with its Most Similar Counterparts*" (TAMOSIC) to explore similar compounds in ChEMBL, a chemo-structural database that contains information on compound structures and their bioactivity [33]. The β-pinene exhibited a high structural similarity with (+)-aromadendrene (ChEMBL code: CHEMBL2269083, TargetHunter score: 0.71), which is the main chemical constituent of the volatile extract of *Callicarpa japonica* leaves which showed antibacterial activity against different bacterial species, such as *E. coli, S. aureus,* and *Bacillus cereus* [70].

Regarding myrcene, we identified a similarity with squalene that has demonstrated inhibition against *Mycobacterium tuberculosis* (TargetHunter score: 0.79) [71]. Similarly, limonene showed structural matches with two diterpenes compounds containing the dolabellane skeleton obtained from the extracts of the brown alga *Dilophus spiralis.* These diterpenes are related to inhibition of *S. aureus* strain exhibiting MIC $>128\,\mu g{\cdot}mL^{-1}$ (CHEMBL1689074, TargetHunter score: 0.87, and CHEMBL1689085, TargetHunter score 0.83) [72]. Additional information could be seen in Supplementary material 01.

Natural products have been widely investigated for the development of new bioinspired drugs [69, 73, 74] and computational methods have been used as complementary and useful predictive approaches to identify interesting molecular targets of small compounds, their biological activities, and their pharmacokinetic properties [67, 75–79]. However, it is important to highlight that our computational results are only indicative of the antibacterial activity of these major constituents of the essential oil; thus, posterior experimental studies must be performed to confirm the inhibitory activity of these compounds against these bacterial species.

Several essential oils obtained from aromatic plants, such as clove, rosemary, ginger, thyme, and basil, have been used in food matrices due to their antimicrobial activities [11, 12, 23]. It is important to emphasize that after proving the antimicrobial action of essential oil, further studies are needed to ensure that the essential oil can be an effective agent in food preservation. Our *in silico* analyzes could shed light on the main compounds that are involved with the antibacterial activity of essential oil, thus favoring the rational choice for their isolation or synthesis to be applied in food matrices.

4. Conclusions

The essential oil yield was not influenced by the circadian rhythm; however, the higher yields were achieved in the dry season (September). The monoterpene hydrocarbons α-pinene and β-pinene were the major compounds identified in the essential oil samples of *V. surinamensis,* regardless of seasonal or circadian variations. Concerning the chemical profile of the samples of essential oils, in qualitative terms, the chemical composition was very similar.

The essential oil samples of *V. surinam*ensis showed satisfactory antibacterial activity against *S. aureus* and *E. coli*, compared with *P. aeruginosa.* Our computational investigation indicated that some major chemical components from the essential oil, such as limonene, myrcene, and β-pinene could be related to the antibacterial activity against the tested pathogenic bacterial strains. The essential oil of *V. surinamensis* extracted from the leaves collected in the rainy season showed promising results against *S. aureus*, indicating that this essential oil could be a potential natural source of bioactive compounds with antibacterial activity and an alternative to control the growth of microorganisms in food matrices. However, additional experiments related to food safety must be carried out.

Data Availability

The data used to support the findings of this study are available upon request to the corresponding author.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Conceptualization was done by L. D. N; E. H. A. A, and F. A. M. C.. Investigation was done by L. Q. A; K. S. C.. L. D. N., and F. A. M. C. K. S. C., L. Q. A, and L. D. N. wrote the draft. C. S. S. J., K. S. C., F. A. M. C, and E. H. A. A reviewed the article. All authors have read and agreed to the published version of the manuscript.

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Supplementary Materials

Additional information about the TargetHunter analysis of the major compounds of *Virola surinamensis* essential oil is

listed in Supplementary material 01. (*Supplementary Materials*)

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