

Artigo

Antimicrobial Activity of Seasonal Essential Oils From *Banisteriopsis Malifolia* (Ness & Mart.) B. Gates

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Atividade Antimicrobiana de Óleos Essenciais Sazonais de *Banisteriopsis Malifolia* (Ness & Mart.) B. Gates

Resumo: O uso de plantas para fins medicinais é crescente e as folhas do gênero *Banisteriopsis* apresentam usos medicinais pela população brasileira. Apesar do número de espécies de Malpighiaceae, a constituição química dessa família não é muito conhecida, e o foco principal do estudo fitoquímico se dá com a *Banisteriopsis caapi*. Assim, as composições químicas sazonais dos óleos das folhas de *Banisteriopsis malifolia* (Ness & Mart.) B. Gates foram determinadas. Terpenos (56,0 %) e álcoois alifáticos (29,8 %) estão presentes em grandes quantidades no óleo essencial da estação chuvosa em comparação com a estação seca (20,93 % e 20,48 %, respectivamente). Por outro lado, o óleo essencial da estação seca apresentou como compostos principais alcanos de cadeia longa (18,43 %) e ácidos graxos (16,03 %), destacando-se o hexatriacontano (18,43 %) e o ácido palmítico (10,62 %). O fitol foi o principal terpenoide presente em ambos os óleos essenciais (52,70 % versus 15,80 %). Em geral, o óleo essencial da estação chuvosa apresentou os melhores resultados antibacterianos (valores de MIC de 100 a 400 $\mu\text{g mL}^{-1}$) para todas as bactérias testadas. Os efeitos sinérgicos dos compostos presentes no óleo da estação chuvosa podem ser responsáveis pelos melhores efeitos inibitórios observados. Ambos os óleos essenciais não apresentaram atividade anti-*Candida* promissora.


palavras-chaves: *Banisteriopsis malifolia* (Ness & Mart.) B. Gates; Malpighiaceae; óleos essenciais sazonais; fitol; atividade antimicrobiana.

Abstract

The use of plants for medicinal purposes is increasing nowadays and the leaves of the genus *Banisteriopsis* have medicinal uses by the Brazilian population. Despite the number of Malpighiaceae species, the chemical constitution of this family is not very well known, and the main focus of the phytochemical study is with *Banisteriopsis caapi*. Thus, the seasonal chemical compositions of the *Banisteriopsis malifolia* (Ness & Mart.) B. Gates leaves essential oils were determined and subjected to antimicrobial activity tests. Terpenoids compounds (56.0 %) and aliphatic alcohols (29.8 %) are present in large amounts in rainy season essential oil compared to the dry season (20.93 % and 20.48 %, respectively). On the other hand, the dry season essential oil exhibited long-chain alkanes (18.43 %) and fatty acids (16.03 %) as principal compound classes and hexatriacontane (18.43 %) and palmitic acid (10.62 %) as major compounds. Phytol was the principal terpenoid compound present in both essential oils (52.70 % stood out in the rainy essential oil versus 15.80 % in the dry essential oil). In general, the EO of the rainy season presented the best antibacterial results (MIC values from 100 to 400 $\mu\text{g mL}^{-1}$) for all the tested bacteria. The compounds present in the rainy season oil may exert synergetic actions for the observed best inhibitory effects. Both essential oils had no promising anti-*Candida* activity.

keywords: *Banisteriopsis malifolia* (Ness & Mart.) B. Gates; Malpighiaceae; seasonal essential oils; phytol; antimicrobial activity.

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Antimicrobial Activity of Seasonal Essential Oils From *Banisteriopsis Malifolia* (Ness & Mart.) B. Gates

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1. Introduction

The Malpighiaceae family has great relevance in the world. It is estimated that 77 genera and 1.300 species of plants are known.¹ The genus *Banisteriopsis* stands out in the family because it has 92 species in Bolivia, Colombia, Ecuador and Peru, of which 47 are distributed in Brazil, and 11 are endemic to the Cerrado.² In Brazil, this is one

of the most present in Cerrado biome, being 28 genera and 237 endemic species.¹

In the literature, this genus is well known for having a variety of alkaloid metabolites (*B. caapi*), flavonoids, tannins and terpenes that are capable of presenting important pharmacological activities.³ Among these properties, antibacterial, anticholinesterase, antinociceptive, antitumor, antifungal, analgesic, vasorelaxant, hypothermic and analgesic agents are described.³⁻⁹

Banisteriopsis malifolia is a species commonly known as a “flor-do-dia” or “rama-de-moço”, with bushy leaves, with alternating arrangement of hairy leaves and two flower morphotypes, light pink and dark pink type.¹⁰ This species did not have a chemical study, only morphological¹⁰ and phenological ones,¹¹ which encouraged us to study its chemical composition and biological activities. Regarding to other species of *Banisteriopsis*, the essential oil of *Banisteriopsis campestris*, as well as the extracts of *Banisteriopsis laevifolia* and *Banisteriopsis oxyclada* have already been studied and showed compounds that are potential antimicrobial agents against bacteria related to oral diseases and yeasts related to Candidiasis.^{4,5,9}

For the treatment of oral infections, mouthwashes containing chlorhexidine gluconate have been widely used to their high antimicrobial power. This drug has been considered a gold standard in dentistry for more than 40 years¹² and is still very effective in several fields of clinical application, such as intensive care, hand soaps and as an oral antiseptic.¹³ However, in addition to its adverse effects, there are reports on the tolerance of clinical isolates to chlorhexidine.^{13,14} Considering the dependence on chlorhexidine in many health fields, the possibility of microbial resistance, and the consolidation of natural products as a source of active compounds, so it is important to explore new natural products that can replace or be precursors of new antibiotics.^{15,16,17} Another situation that has aroused concern, are diseases caused by fungi. Particularly *Candida* type yeasts, have been associated with various

opportunistic infections in hospital environments and immunocompromised patients, and are also related to increased resistance to current antifungals.¹⁸ Thus, one of the main goals is to replace the dependency on a few drugs used for the treatment of fungal infections (such as Amphotericin B and Griseofulvin) by alternative sources of bioactive compounds against *Candida* species.³ Some of the plant products are effective against fungal infections too, including *Banisteriopsis* species.^{3,7,8}

In this context, the objective of this work was to identify the chemical composition of EOs' leaves of *B. malifolia* (Figure 1) with seasonal variation (dry and rainy) and evaluate the antibacterial activity (against aerobic and anaerobic oral bacteria) and antifungal activity.

2. Experimental

2.1. Plant material

The leaves of *B. malifolia* were collected in two different periods, July / 2017 in the dry season (DS) and February / 2018 in the rainy season (RS), inside the Legal Reserve Area (ARL) of the *Clube Caça e Pesca* Itororó de Uberlândia (CCPIU), located at 18 ° 59 'S and 48 ° 18' W, and altitude of 863 m with a total area of 127.1 hectare. To identify the species, the aerial parts were compared to those deposited by Mamede 1994, voucher specimen number HUFU00013446), in the Herbarium Uberlândia (HUFU).



Figure 1. Photographs of *Banisteriopsis malifolia* (Ness & Mart.) B. Gates found at Clube Caça e Pesca Itororó, in Uberlândia/MG: bush (A) and flower of dark pink morphotype (B) of the species

2.2. Extraction of essential oil by hydrodistillation

Fresh leaves of *B. malifolia* from dry and rainy periods were ground and placed in Clevenger apparatus, extracted for 4 h under reflux. Thereafter, the obtained EO was extracted with dichloromethane and the organic phase was separated and dried with anhydrous sodium sulfate, filtered and kept in a closed vial under refrigeration (-10°C). The percent yield was calculated in relation to the dry mass of the initial sample; this procedure was performed in triplicate.

2.3. Identification of essential oil

The EO was analyzed by gas chromatography coupled to mass spectrometry (GC-MS, Shimadzu/QP2010) using an OV-5 bonded capillary column ($30\text{ m} \times 0.25\text{ mm} \times 0.25\text{ }\mu\text{m}$ film thickness). The carrier gas was helium at a flow rate of 1.0 mL min^{-1} ; detector and injector temperatures were 220°C and 240°C , respectively. The injection volume was $1.0\text{ }\mu\text{L}$ and split ratio 1:20. The oven temperature was programmed from 60°C to 240°C at a ramping rate of $3^{\circ}\text{C min}^{-1}$. The electron impact energy was 70 eV and fragments from 40 to 650 m/z were collected. The identification of the chemical constituents was carried out by comparison of the mass spectral fragmentation patterns with virtual libraries (Wiley, Shim and Nist), and Arithmetic Indices (AIs) were calculated and compared with AIs of the NIST Standard Reference Data, and Adams book.¹⁹⁻²⁰ The AIs were calculated using the equation proposed by Van Den Dool and Kratz²¹, which is based on the retention times of linear alkane standards (Sigma-Aldrich).

2.4. Antimicrobial assay

2.4.1. Antibacterial activity

The antibacterial activity of essential oils from *B. malifolia* leaves was determined in triplicate using the microdilution broth method in 96-well microplates.^{22,23} To evaluate the cell viability, resazurin in aqueous solution (0.01% the w v^{-1}) was used as a developing agent. The minimum inhibitory concentration (MIC) was correlated to the lowest concentration of oil capable of inhibiting

the growth of the microorganisms.²⁴ The tested concentrations varied between 400 to $25\text{ }\mu\text{g mL}^{-1}$. The microorganisms used in the evaluation of the antibacterial activity and their respective references from the American Type Culture Collection (ATCC, RockvilleMD, USA) were *Streptococcus mitis* (ATCC 49456), *Streptococcus mutans* (ATCC25175), *Streptococcus sanguinis* (ATCC10556), *Agregatibacter actinomycetemcomitans* (ATCC 43717), *Actinomyces naeslundii* (ATCC 19039), *Porphyromonas gingivalis* (ATCC 33277) and *Fusobacterium nucleatum* (ATCC 25586). The details of the methodology, as well as the controls used to validate the results are described in the work by Rocha et al., 2018.⁹

2.4.3. Antifungal activity

The assays were performed using broth microdilution method using the standards recommended by Clinical and Laboratory Standards Institute according to the reference protocol M27-A3²⁵. The tested microorganisms were *Candida albicans* (ATCC 28366), *Candida tropicalis* (ATCC 13803) and *Candida glabrata* (ATCC 15126), from American Type Culture Collection (ATCC, Rockville MD, USA). The minimum inhibitory concentration determination was performed using 96-well microplates, the oils where final concentrations were in the range from 1.46 to $3,000\text{ }\mu\text{g mL}^{-1}$. The MIC values were obtained after revelation with an aqueous resazurin solution 0.02% w v^{-1} . The details of this methodology are described in Rocha et al., 2018.⁹

3. Results and Discussion

3.1. Oils yield (%)

The content of the essential oils was distributed equally among the studied seasons. The yield for dry season essential oil was $0.01\% \pm 0.001\text{ (w/w)}$ and $0.02\% \pm 0.010\text{ (w/w)}$ for rainy season essential oil. During the summer in Cerrado biome, the rainfall (rainy season) is much higher than in the other seasons. But the temperature and humidity change dramatically during the spring (dry season). Despite this fact, the results are similar already demonstrated within the family.^{9,26}

3.2. Chemical composition of essential oil from leaves of *B. malifolia*

In total, 13 compounds were identified from the GC-MS chromatograms (Figure 2 and 3) of both seasonal essential oils, accounting for 87.7-91.2 % of the volatile constituents (Table 1).

Meanwhile, the dry season essential oil differs in composition from the oil collected in the rainy season. Table 2 presents the distribution of chemical classes of the volatile constituents present in the OE's.

The essential oil the in the dry season (DS) presented a greater number of compounds than in the rainy season (RS). In the DS essential oil 13 compounds were identified, being the main compounds phytol (**7**) (15.80 %), (Z)-hex-3-enol (**1**) (17.99 %), hexahydrofarnesyl acetone (**6**) (9.39 %), palmitic acid (**5**) (10.62 %) and hexatriacontane (**12**) (18.43 %). In the RS essential oil 6 compounds were identified, with phytol (**7**) (52.70 %) and (Z)-hex-3-enol (**1**) (26.22 %) compounds resulting 78.92 % of the total oil. The structures of these compounds are shown in Figure 4.

The contents of fatty acids and long-chain alkanes in the dry essential oil represent 34.46 % of the volatiles compounds and are absent in the rainy essential oil. Concerning terpenes, the contents of oxygenated monoterpenes and diterpenes in the essential oils were highest during the rainy season (58.8 %) and lower during the dry season (28.23 %). Linalool (**4**), hexahydrofarnesyl acetone (**6**), (*E, E*)-7,11,15-trimethyl-3-methylene-hexadeca-1,6,10,14-tetraene (**7**) and phytol (**9**) were the compounds of these classes found. Only one triterpene (squalene) was found in low concentration in the essential oil from the dry season.

The chemical composition of the essential oils of *Banisteriopsis* species has been little studied until today. The literature presents studies only for *B. laevifolia*, *B. oxyclada* and *B. campestris* (Table 3). The EO chemical composition of the *B. campestris* EO consisted predominantly of fatty acids, long-chain alkanes and oxygenated sesquiterpenes, with hexadecanoic acid, triacontane and (*E*)-nerolidol, as the main constituents of these classes.⁹ It is well know that in different seasonal periods the plant synthesizes different compounds,

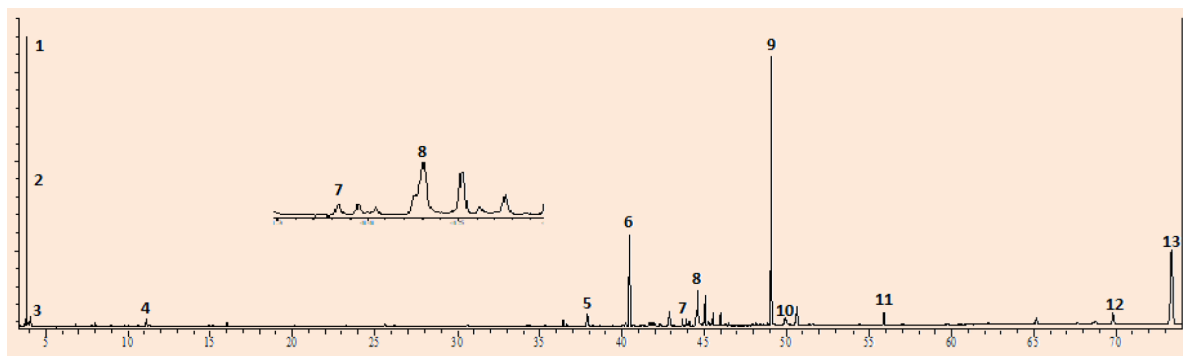


Figure 2. GC-MS chromatogram of dry season essential oil

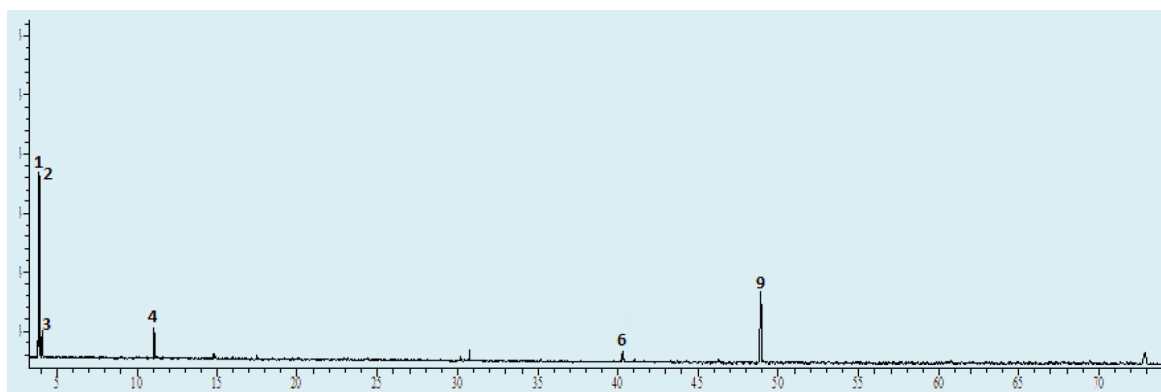


Figure 3. GC-MS chromatogram of rainy season essential oil

Table 1. Seasonal chemical composition of EO from leaves of *B. malifolia*

N°	Compound	Retention time (s)	AI calculated	AI reference	Composition, %	
					DS	RS
1	(E)-hex-2-en-1-al	3.792	845	846 a	1.48	2.55
2	(Z)-Hex-3-en-1-ol	3.879	851	850 a	17.99	26.22
3	Hexan-1-ol	4.087	866	863 a	2.49	3.64
4	Linalool	11.120	1097	1095 a	1.22	3.31
5	Myristic acid	37.959	1776	1775 b	2.80	-
6	Hexahydrofarnesyl acetone	40.474	1849	1850 b	9.39	2.79
7	(E,E)-7,11,15-Trimethyl-3-methylene-hexadeca-1,6,10,14-tetraene	43.878	1922	1922 b	1.82	-
8	Palmitic acid	44.570	1973	1971 b	10.62	-
9	Phytol	49.064	2119	2114 b	15.80	52.70
10	Linoleic acid	49.819	2144	2159 b	2.61	-
11	5-Methyl-5- (4,8,12-trimethyltridecyl) dihydro-2 (3H)-furanone	55.875	2357	2364 b	1.01	-
12	Squalene	69.818	2907	2833 b	2.09	-
13	Hexatriacontane	73.403	2987	3000 b	18.43	-
Total identified (%)					87.75	91.21

Dry season (DS); Rainy season (RS); AI = arithmetic index; a: Adams mass spectral-retention index library.²⁰ b: NIST: Standard Reference Data.¹⁹

Table 2. Chemical classes of the volatile constituents present in the essential oils of *B. malifolia*.

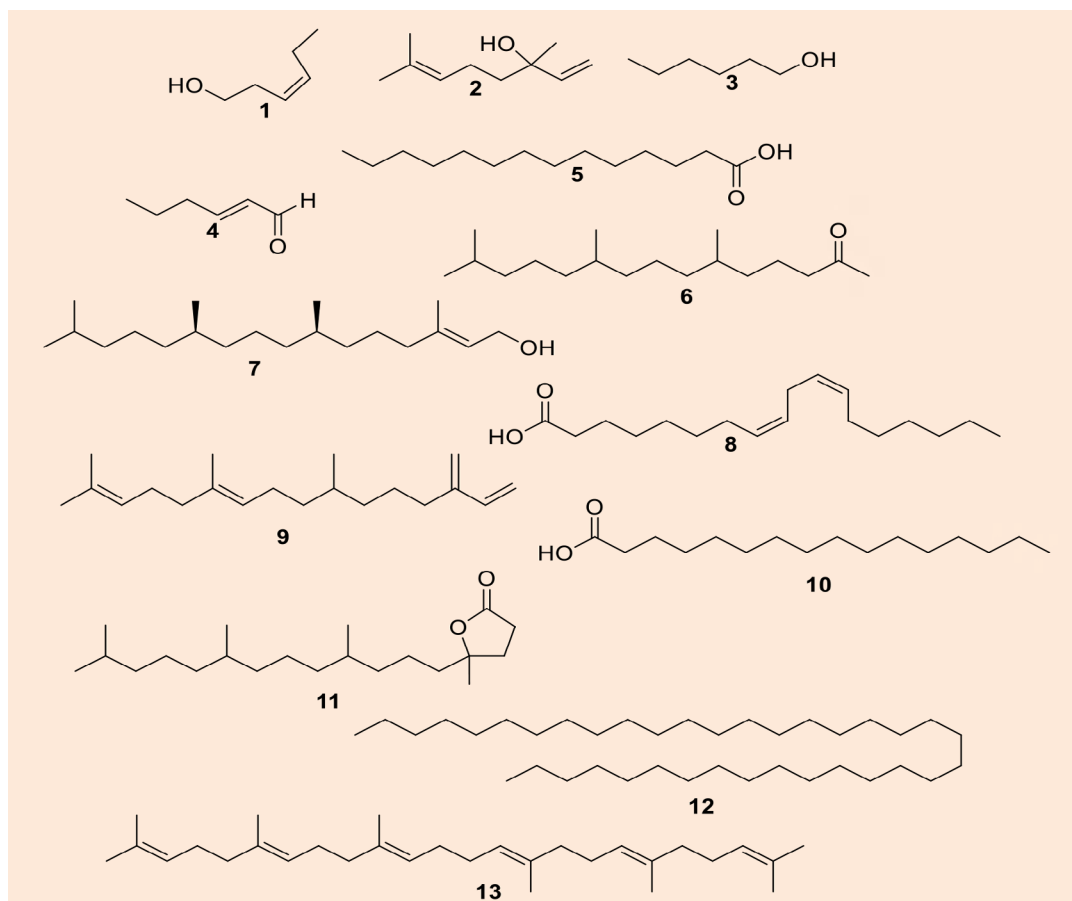
Functional groups	DS (%)	RS (%)
Fatty acids	16.03	-
Long-chain Alkanes	18.43	-
Aliphatic alcohols	20.48	29.86
Aldehydes	1.48	2.55
Ketones	1.01	-
Diterpenes	27.01	55.49
Oxygenated monoterpenes	1.22	3.31
Triterpenes	2.09	-

according to the environmental conditions.²⁷ The data presented here demonstrate this fact (Table 1). When comparing the chemical constituents present in the EOs of *Banisteriopsis* species there were significant differences in the composition.^{4,5} Anyway, for *B. oxyclada*⁵ and *B. campestris*⁹ the seasonality was not evaluated.

The EO chemical compositions are related to abiotic factors in the plant. So, they vary qualitatively and quantitatively among the same species and among different parts.²⁷ The dry season essential oil of *B. malifolia* presented approximately 21 % of terpenoids compounds (Table 2), with phytol (7) being an abundant

compound. In the rainy essential oil phytol was the most abundant compound (52.70 %). For comparison, the EO of leaves of *B. laevifolia* exhibited also phytol as the major compound in the dry period (9.85 %) and in the rainy season (14.9 %).²⁶ Phytol is present as the major compound also in the dry season essential oil of *B. oxyclada*,⁵ indicating a characteristic of the species in function of the seasonal variation.

Phytol is one of the major constituents of essential oils derived from plants, and much research has been done to prove that the antimicrobial and cytotoxic activity observed for these essential oils is related to phytol content.



1: (Z)-hex-3-enol; **2:** linalool; **3:** hexan-1-ol; **4:** (E)-hex-2-en-1-al; **5:** palmitic acid; **6:** hexahydrofarnesyl acetone; **7:** phytol; **8:** linoleic acid; **9:** (E,E)-7,11,15-Trimethyl-3-methylene-hexadeca-1,6,10,14-tetraene; **10:** myristic acid; **11:** 5-Methyl-5-(4,8,12-trimethyltridecyl)dihydro-2(3H)-furanone; **12:** hexatriacontane; **13:** squalene.

Figure 4. Chemical structures of the volatile constituents present in the leaves essential oils from *B. malifolia*.

Table 3. Major compounds identified in the essential oils of some species of *Banisteriopsis*

Compound	Species (season)	Part of the vegetable
	<i>B. malifolia</i> (dry and rainy)	
(Z)-hex-3-en-1-ol (1)	<i>B. laevifolia</i> (dry and rainy) ^{ref. 4}	Leaves
	<i>B. oxyclada</i> (dry season) ^{ref. 5}	
Hexahydrofarnesyl acetone (6)	<i>B. malifolia</i> (dry);	Leaves
Palmitic acid (5)	<i>B. malifolia</i> (dry);	Leaves
	<i>B. malifolia</i> (dry and rainy)	
Phytol (7)	<i>B. laevifolia</i> (rainy and dry) ^{ref. 4}	Leaves
	<i>B. oxyclada</i> (dry) ^{ref. 5}	
Untriacontane	<i>B. laevifolia</i> (dry and rainy) ^{ref. 4}	Leaves
	<i>B. laevifolia</i> (rainy) ^{ref. 4}	
(Z)-hex-2-en-1-ol	<i>B. oxyclada</i> (dry) ^{ref. 5}	Leaves
(E)-hex-2-en-1-al	<i>B. oxyclada</i> (dry) ^{ref. 5}	Leaves

This compound has various pharmacological properties, such as anxiolytic effects, metabolic modulators, cytotoxic, antioxidants, anti-quorum activity, antimicrobial, autophagic and apoptotic, antinociceptive, anti-inflammatory, immunomodulatory, antileishmanial and buccal type antibacterial effects.^{4,5,9,28}

The amount of aliphatic alcohols present in DS and RS were also significant (20.48 % and 29.86 %, respectively), evidencing that the rainy period also influenced the production of these compounds. The compound (Z)-hex-3-enol (**1**) (17.99 %; 26.22 %) was the most abundant for both periods (Table 2). This compound is popularly known as “leaf alcohol”, a colorless oil with freshly cut grass scent, pursuing a protective role against bacteria.²⁹

Fatty acids were found only during the dry season, with a total of (16.03 %). Among them, palmitic acid stands out (**5**), as the major acidic compound (10.62 %), linoleic acid (**8**) and myristic acid (**10**). These hydrophobic compounds have recognized antimicrobial activity.³⁰ Another relevant function for these long chain fatty acids is a protective barrier against water loss during the dry season in plants.^{31,27}

3.3. Antimicrobial assay

The results of the antibacterial activity against oral bacteria of the leaves essential oils of *B. malifolia* in different seasonal periods are presented in Table 4. The antibacterial activity is considered good for MICs values below 100 $\mu\text{g mL}^{-1}$ and moderate between 100 and 400 $\mu\text{g mL}^{-1}$.^{32,33}

In this study, the EO obtained in dry season (DS) presented moderate to weak activity for all the tested bacteria. The best results were observed against to the aerobic bacteria *S. mitis* and *A. actinomycetemcomitans*, and to the anaerobic *A. naeslundii* (MIC = 400 $\mu\text{g mL}^{-1}$). On the other hand, and in general, the EO of the rainy season (RS) presented the best antibacterial results, from moderate to good activity for all the bacteria tested. The typical characteristics of the rainy season (period from November to February), in the city of Uberlândia-MG (high temperatures, high precipitation index), favored the action of the rainy essential oil mainly against the bacteria *S. sanguinis* and *F. nucleatum*.

The seasonal variability of the antimicrobial activity of the essential oil may be related to the differences observed within the chemical composition and with the percentage of the major

Table 4. Minimum inhibitory concentration ($\mu\text{g mL}^{-1}$) of the *B. malifolia* essential oils in the dry and rainy seasons

Bacteria		MIC (Sample)		
		DS	RS	CHD
Gram-positive	<i>Streptococcus mutans</i> ATCC 25175	>400	400	0.922
	<i>Streptococcus mitis</i> ATCC 49456	400	200	3.688
	<i>Streptococcus sanguinis</i> ATCC 10556	>400	100	0.922
	<i>Aggregatibacter actinomycetemcomitans</i> ATCC 43717	400	400	0.460
	<i>Porphyromonas gingivalis</i> ATCC 33277	>400	200	3.68
	<i>Fusobacterium nucleatum</i> ATCC 25586	>400	100	1.84
Gram-negative	<i>Actinomyces naeslundii</i> ATCC 19039	400	400	1.84

Method validated with metronidazole: *Bacteroides fragilis* (MIC=0.36 $\mu\text{g mL}^{-1}$) and *Bacteroides thetaiotaomicron* (1.47 $\mu\text{g mL}^{-1}$). DS= dry season; RS = rainy season; CHD = Chlorhexidine dihydrochloride (positive control)

compounds identified in the oils. The diterpene phytol (**7**) (Figure 1) is present in a higher amount in RS (52.70 %) than in DS (15.80 %), whereas the compound (Z)-hex-3-enol (**1**) is present with 26.22 % in RS and 17.99 % in DS (Table 1). Generally, the biological properties of the essential oils are related to the chemical characteristics of the major compounds³⁴.

The dry season essential oil presented the higher amounts of fatty acids. It seems to be that the fatty acids did not influence the antibacterial activity to the tested gram-positive and gram-negative bacteria tested. On the other hand, the antibacterial activity observed to the rainy season essential oil may be related to the compounds of the classes of linear terpenes and alcohols. These classes of compounds have bacterial activity due to synergistic interactions with the compounds of high lipophilicity that, combined, may increase the activity^{35,36}.

In comparison, the flowers essential oils of *Banisteriopsis campestris* presented moderate and good activity against to oral bacteria (MIC values from 50 to 400 $\mu\text{g mL}^{-1}$)⁹, while leaf oils of *B. malifolia* showed moderate enhanced activity during the rainy season (MIC values from 100 to 400 $\mu\text{g mL}^{-1}$). These results were related to the composition of terpenoids compounds and fatty acids in their essential oils. The EO of leaves and barks of *Inga laurina* (Leguminosae) also showed relevant antibacterial activity against oral microorganisms (MIC values below 100 $\mu\text{g mL}^{-1}$), showing good relation to the seasonal variation and the terpenoids compounds³⁵. In short, it is proved that the concentration of the mixture of compounds in synergistic interaction is responsible for the antibacterial activity^{30,36}.

Studies show that many EOs have a higher antibacterial activity against Gram-positive aerobic bacteria, since Gram-negative anaerobic bacteria have a phospholipid bilayer in their cell wall, which prevents the penetration of macromolecules and hydrophobic compounds, increasing their resistance.^{37,38} So, it is important to highlight the relevance of the results found with the oil collected during the rainy season, since it inhibited the growth of Gram-negative bacteria in the same range as Gram-positive bacteria (100 to 400 $\mu\text{g mL}^{-1}$). *B. malifolia* oils have constituents with antibacterial potential because they have interfered against oral microorganisms involved with dental plaque, caries, periodontal and endodontic infections. The association of the active essential oils with mouthwashes can be an alternative to mouth rinse, minimizing the side effects, and even improve the antibacterial action of these products.³⁹

The *B. malifolia* EOs were also evaluated for anti-*Candida* activity, with concentrations between 1.46 and 3000 $\mu\text{g mL}^{-1}$. The MIC results of antifungal activity tests against *Candida* species are presented in Table 5. The criteria considered for evaluating the antifungal activity are those suggested by researchers Holetz *et al.*³², Rios and Recio³³ and Kuete⁴⁰.

DS and RS oils showed no promising activity against *Candida*-type yeasts, within the tested range of concentrations. MIC values were greater than 3000 $\mu\text{g mL}^{-1}$. In this case, the qualitative and quantitative variation in the composition of the oil promoted by the seasonality did not influence the inhibition of these yeasts tested. It is possible to suggest that the very weak antifungal activity is related to an insufficient concentration of bioactive

Table 5. Minimum inhibitory concentration ($\mu\text{g mL}^{-1}$) of yeasts of the *Candida* species of OE of leaves of *B. malifolia* in dry and rainy periods

Yeast	MIC ($\mu\text{g mL}^{-1}$)		
	DS	RS	Amphotericin B
<i>Candida albicans</i> ATCC 28366	>3000	>3000	0.25
<i>Candida glabrata</i> ATCC 15126	>3000	>3000	0.25
<i>Candida tropicalis</i> ATCC 13803	>3000	>3000	0.12

Positive control: Amphotericin B; The value of MIC > 3000 $\mu\text{g mL}^{-1}$ corresponded to the lack of antifungal activity. Amphotericin B-validated method: *Candida krusei* (MIC = 1.0 $\mu\text{g mL}^{-1}$) and *Candida parapsilosis* (0.25 $\mu\text{g mL}^{-1}$). DS= dry season; RS = rainy season

compounds as organic acids, oxygenated terpenes and phenolic compounds that traditionally contribute to the inhibition of these fungus species^{30,41}. Particularly, the phenolic compounds within the composition of EOs, have been listed as being the most important constituents to have considerable antifungal activity⁴². Some phenolics commonly found in EOs such as thymol, eugenol, carvacrol, have demonstrated relevant antifungal effect and their action mechanisms involve changes in the fungal membrane or cell wall causing cell death⁴³. The absence of anti-*Candida* effect found here, may also be related to the absence of this class in the oils of *B. malifolia*.

Studies of essential oils against fungi of *Candida* species with ($MIC \leq 1000 \mu g mL^{-1}$) are considered relevant³⁰. Until now, studies on antifungal activity using *Banisteriopsis* species show that only the essential oil from *B. laevifolia* leaves exhibited weak antifungal activity ($MIC = 1000 \mu g mL^{-1}$) against *Candida* spp.²⁶. However, another study with essential oil of flowers of *B. campestris*⁹ against the same *Candida* species showed very weak activity ($MIC > 3000 \mu g mL^{-1}$). Although the essential oils here studied presented chemical constituents that have an antifungal potential it was not possible to observe any effect on these microorganisms.

Yeasts were more resistant to the oils tested when compared to bacteria. In addition to some factors already mentioned that must be considered in antimicrobial activity such as oil composition, functional group of active constituents and their synergistic interactions,⁴⁴ microorganisms through their membranes and cell walls promote resistance to the external environment and the entry of potentially harmful molecules.⁴⁶ Particularly fungi, have a cell wall with peculiar characteristics, mainly due to the presence of chitin, a polysaccharide absent in human cells.^{43,45} Yeasts *Candida*-type have a complex internal cell wall containing layers formed by chitin, glucan and glycoproteins. Glucan and chitin interact by hydrogen bonds among the chains forming a fibrillar structure that provides support, rigidity and protection for the cell.^{45,46} Thus, the action of agents on the cell wall of the fungus is difficult. When the chemical structures of some drugs and potential candidates against candidiasis are observed,⁴⁵ there are several sites that give these molecules a more polar character. Thus, it is possible that the high hydrophobicity of *B. malifolia* oil was not a favorable condition to act

on the yeast cell wall or inactivate it by another action mechanism.

Although the results of *B. malifolia* oils against yeasts have not been satisfactory, due to the increased incidence of resistance to currently used antifungals, it is essential to evaluate alternative products for the development of new generations of therapeutic agents.⁴⁵

4. Conclusion

The seasonal essential oils from *B. malifolia* leaves presented significant differences in their chemical composition and remarkable antibacterial results, since the oil of the rainy season presented better antibacterial activity in comparison with the dry period. Additionally, no relevant anti-*Candida* activities were observed for the two essential oils tested. The seasonal study of these oils was important because it showed changes in chemical composition, quantity and interfered in the biological evaluation.

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