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Chemical Composition and Antimicrobial Activity of Essential Oil of Flowers from *Banisteriopsis campestris* (A. Juss.) Little

Abstract: The essential oil (EO) of the flowers from *Banisteriopsis campestris* was analysed by GC and GC-MS. Fatty acids were the most abundant class of compounds in the EO, followed by long-chain alkanes and oxygenated sesquiterpenes and monoterpenes. The main constituents were hexadecanoic acid (39.43 %), (*E*)-nerolidol (10.51 %), triacontane (9.08 %), heptacosane (5.49 %) and linalool (3.23 %). The antimicrobial activity of the EO was evaluated against aerobic and anaerobic oral bacteria and some yeasts by broth microdilution method. The EO inhibited the growth of all tested oral bacteria, showing strong activity against *S. sanguinis* with minimum inhibitory concentration (MIC) of 25 μg mL^{-1}. Considerable antibacterial activity was also observed for the anaerobes *Porphyromonas gingivalis* and *Actinomyces naeslundii* with MIC of 50 μg mL^{-1}. The EO of *B. campestris* flower showed moderate activity for *S. mutans*, *S. mitis*, *A. actinomycetemcomitans* and *Fusobacterium nucleatum* with MICs ranging between 200 and 400 μg mL^{-1}. The antifungal activity was evaluated against *C. albicans*, *C. tropicalis* and *C. glabrata*. The MIC results were above 3000 μg mL^{-1}, indicating inactivity against these yeasts. This study revealed that the OE of *B. campestris* flowers has promising antibacterial activity and this is the first report on its chemical composition and antimicrobial activity

Keywords: *Banisteriopsis campestris*, essential oil, CG-MS, antibacterial activity, antifungal activity.

Resumo

O óleo essencial (EO) das flores de *Banisteriopsis campestris* foi analisado por CG e GC-EM. Os ácidos graxos foram a classe de compostos mais abundantes no OE, seguidos por alcanos de cadeia longa, sesquiterpenos oxigenados e monoterpenos oxigenados. Os principais constituintes foram ácido palmítico (39,43 %), (*E*)-nerolidol (10,51 %), triacontano (9,08 %), heptacosano (5,49 %) e linalol (3,23 %). A atividade antimicrobiana do EO foi avaliada contra bactérias bucais aeróbias e anaeróbias e algumas espécies de *Candida* pelo método de microdiluição em caldo. O EO inibiu o crescimento de todas as bactérias bucais testadas, apresentando forte atividade contra a bactéria aeróbia *S. sanguinis* com concentração inibitória mínima (CIM) de 25 μg.mL^{-1}. Considerável atividade antibacteriana foi também observada para as bactérias anaeróbias *Porphyromonas gingivalis* e *Actinomyces naeslundii* com CIM de 50 μg.mL^{-1}. O EO da flor de *B. campestris* mostrou moderada atividade para *S. mutans*, *S. mitis*, *A. actinomycetemcomitans* e *Fusobacterium nucleatum* com concentrações inibitórias mínimas entre 200 e 400 μg.mL^{-1}. A atividade antifúngica foi avaliada contra *C. albicans*, *C. tropicalis* e *C. glabrata.* Os resultados de MIC estiveram acima de 3.000 μg.mL^{-1}, indicando inatividade contra estas leveduras. Este estudo revelou que o OE das flores de *B. campestris* tem promissora atividade antibacteriana e este é o primeiro relato sobre sua composição química e atividade antimicrobiana

Palavras-chave: *Banisteriopsis campestris*, óleo essencial, CG-EM, atividade antibacteriana, atividade antifúngica.

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Composição Química e Atividade Antimicrobiana do Óleo Essencial das Flores de *Banisteriopsis campestris* (A. Juss.) Little

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1. Introduction

Malpighiaceae is composed of 75 genera and approximately 1300 species of plants.[1] *Banisteropsis* is one of the most expressive genera of the family, with 92 species, 47 of which are distributed throughout Brazil and 11 are endemic species of the Cerrado.[2] *Banisteriopsis campestris* is a shrub that rarely exceeds 1.5 m in height with flowering occurring from December to February and fruiting in January.[3]

Many works involving the chemical composition and biological activity of *Banisteriopsis* species have already been

reported and show that they may be useful in the prevention of some human diseases.[4,5] Different classes of metabolites have been found in *Banisteriopsis* species such as alkaloids, flavonoids, tannins and terpenoides.[6,7,8] Antimicrobial, anticholinesterase, antinociceptive, antitumor, antifungal, analgesic, vasorelaxant, hypothermic and analgesic properties are also described in the literature.[6-12]

From this class of plants, it is possible to obtain essential oils with powerful biological applications. Despite the possible applications, few studies have been carried out with this genus of plants, which includes *Banisteriopsis campestris*. Only studies into the chemical composition and biological activity of the volatile EOs of leaves of *Banisteriopsis laevifolia* and *Banisteriopsis oxyclada* have been reported so far.[7,8] Among the antimicrobial activities studied for this genus are those related to oral diseases, such as caries and periodontitis.

Dental caries is a global health problem that affects human beings of different age groups.[13] Moreover, it has been suggested in recent years that oral bacteria associated to dental caries have some links with many systemic diseases such as pneumonia and cardiovascular disease.[14,15] Many synthesized antimicrobials, such as ampicillin, chlorhexidine, sanguinarine, metronidazole and phenolic and quaternary ammonium antiseptics, have been very effective in preventing tooth decay.[16,17] Chlorhexidine is one of the most widely used antimicrobial agents against oral bacteria. However, several studies have reported adverse effects, such as changes in tooth colour, oral and intestinal flora disorders and diarrhoea, associated with the use of these antimicrobials.[18] In addition, the resistance of pathogens to drugs is one of the largest problems in the treatment of microbial diseases.[19,20] In this context, natural antibacterial substances have attracted the attention of researchers.[21]

Therefore, the search for new natural products or prototypes with antibacterial activity becomes an important tool for the control and prevention of oral and systemic diseases in human health.[22]

In the literature, there are only morphological identifications data of *B. campestris* species. Therefore, the objective of this work was to evaluate the chemical composition of the EOs of *B. campestris* flowers and their antibacterial and antifungal activities

2. Experimental

2.1. Plant material

The samples of *Banisteriopsis campestris* (A. Juss) Little, Malpighiaceae, were collected in January, 2015, in a reserve from the Itororó Hunt and Fish Club, in Uberlândia (Minas Gerais), geographic coordinates 18º 58' 56.1" S 48º 17' 46.0" W. The plant was identified by a specialist researcher from the Botanical Institute of São Paulo, and a voucher specimen was deposited in Herbarium Uberlandensis under No. 72.462.

2.2. Extraction of essential oil by hydrodistillation

Fresh flowers of *B. campestris* were cut and extracted in a Clevenger apparatus for 4 h under reflux as described by Cunha and collaborators.[22] Then the EO obtained was extracted with dichloromethane and the organic phase was separated and dried with anhydrous sodium sulphate, filtered and kept in a closed flask under refrigeration (–10 °C). The percentage yield was calculated relative to the dried mass of the initial sample; this procedure was performed in triplicate.

2.3. Identification of essential oil

The EO was analysed by gas chromatography coupled to mass spectrometry (GC-MS, Shimadzu/QP2010) using an OV-5 bonded capillary column (30 m × 0.25 mm × 0.25 μm 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 μL and split ratio 1:20. The oven temperature was programmed from 60 °C to 240 °C at a ramping rate of 3 °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 with virtual libraries (Wiley and SHIM2205), and Arithmetic Indices (AIs) were calculated and compared with those in the literature. The AIs were calculated using the equation AI (X) = 100 PzC + 100 [(t (X) − t(Pz)) / (t(Pz+1) − t(Pz))], which is based on the retention times of linear alkane standards (Sigma-Aldrich).[23] Within the equation, t is the retention time in min, X is an unknown compound, C is the carbon number of the alkane Pz that runs before X, and Pz + 1 is the alkane that runs after X. The identification was based on a comparison of the mass spectra obtained and those of the virtual libraries, and the AI obtained was compared with AIs of the NIST Standard Reference Data, and Adams book.[24,25] The quantification of the compounds present in the EO was performed in gas chromatography equipment with flame ionisation detector (GC-FID, Shimadzu GC2014), using the same capillary column OV-5 under the same conditions as for GC-MS, but Nitrogen was used as carrier gas.

2.4. Antibacterial activity

The tested strains were obtained from the American Type Culture Collection (ATCC, RockvilleMD, USA). The following microorganisms were used in the evaluation of the antibacterial activity: *Streptococcus mitis* (ATCC 49456), *Streptococcus mutans* (ATCC 25175), *Streptococcus sanguinis* (ATCC10556), *Agregatibacter actinomycetemcomitans* (ATCC 43717), *Actinomyces naeslundii* (ATCC 19039), *Porphyromonas gingivalis* (ATCC 33277) and *Fusobacterium nucleatum* (ATCC 25586).

2.5. Broth microdilution method

The minimum inhibitory concentration (MIC) value is the lowest concentration of oil capable of inhibiting the growth of a microorganism. The antibacterial activity of *B. campestris* was determined in triplicate using the microdilution broth method in 96-well microplates.[26,27] The EO was dissolved in DMSO (Synth) at 8000 µg.mL^{-1}, followed by dilution in tryptic soy broth (Difco) for aerobic and Schaedler broth (Difco) supplemented with hemin (5 µg.mL^{-1}) and vitamin K1 (10.0 µg.mL^{-1}) for anaerobic; concentrations tested ranged from 400 to 25 µg.mL^{-1}. The final DMSO content was 4 % (v.v^{-1}), and this solution was used as a negative control. The inoculum was adjusted for each organism to yield a cell concentration of 5×10^5 colony forming units (CFUs) per mL. The microplates with the anaerobic microorganisms were incubated aerobically at 37 °C for 24 h. The anaerobic microorganisms were incubated for 48–72 h in an anaerobic chamber (Don Whitley Scientific Bradford, UK), in 5–10 % H_2, 0 % CO_2, 80–85 % N_2 atmosphere at 37 °C. Then, resazurin (Acros organics) (30 µL) in aqueous solution (0.01 % w v^{-1}) was added to the microplates, to indicate microorganism viability.[28] Chlorhexidine dihydrochloride was added as positive control, and the concentrations ranged from 0.0115 to 15.68 µg.mL^{-1}. Sterility tests were performed for the TSB and Schaedler broths, control culture (inoculum), positive control, oils and DMSO.

2.6. Antifungal activity

The microorganisms tested were *Candida albicans* (ATCC 28366), *C. tropicalis* (ATCC 13803) and *C. glabrata* (ATCC 15126), from American Type Culture Collection (ATCC, Rockville MD, USA). The assays were performed using broth microdilution method using the standards recommended by Clinical and Laboratory Standards Institute.[29]

The stock solutions were prepared by dissolving the EO in DMSO (Sigma-Aldrich, Co) in a concentration of 192,000 $\mu g.mL^{-1}$. Dilutions were made using the standard RPMI 1640 medium buffered to pH 7.2 with 0.165 $mol.L^{-1}$ of 3-*N*-morpholinepropanesulfonic acid (MOPS, *Acros Organics*, *Geel*, *Turnhout*, Bélgica) at 12,000 $\mu g\ mL^{-1}$. The inoculum was prepared using a spectrophotometric method (at 530 nm wavelength) and compared with a 0.5 McFarland scale to obtain the value of 6.0×10^6 $CFU.mL^{-1}$. Then, the dilutions recommended by CLSI were made with RPMI until the inoculums reached 1.2×10^3 $CFU.mL^{-1}$.

The MIC determination was performed using 96-well microplates, where dilutions with final concentrations were made in the range of 1.46 to 3,000.0 $\mu g.mL^{-1}$. Each well received 100.0 µL of the inoculum suspension and the final volume in each well was 200.0 µL. Amphotericin B was used as positive control, being diluted in broth at concentrations between 0.031 and 16.0 $\mu g.mL^{-1}$. The negative control (DMSO) was tested with concentrations ranging from 1 % to 10 % ($v.v^{-1}$) and did not influence yeast growth. The added inoculum medium was used as culture growth control. To validate the tests, the positive control amphotericin B were tested against reference strains of *Candida krusei* (ATCC 6258) and *C. parapsilosis* (ATCC 22019) in a range of MIC values between 0.25 and 2.0 $\mu g.mL^{-1}$. If the MIC value is within this range for these yeasts, the methodology and the results for the other tested yeasts are validated according to the reference protocol M27-A3.[29] After incubation, 30 µL of a 0.02 % aqueous resazurin (Sigma) solution was added to each well and the microplates incubated for further 30 min, for observation and descriptive analysis of the results. The MIC was calculated and correlated to the minimum concentration of sample that was able to inhibit the growth of yeasts. Resazurin is an oxyreduction probe that allows for immediate observation of microbial growth. The blue and red colours represent the absence and presence of microbial growth, respectively.[30]

2.7. Statistical analysis

All data on the biological tests were submitted to ANOVA treatment with a significance level of 5 %, using the Tukey method in GraphPad Prism 5.

3. Results and Discussion

In the EO of *B. campestris* flowers, 26 volatile compounds were identified by GC-MS, comprising 96.19 % of its composition. The yield of the EO of *B. campestris* flowers was 0.011 ± 0.002 %. The Table 1 shows the composition of the EO and Table 2 the chemical classes of the identified compounds.

Table 1. Chemical composition of EO from flowers of *B. campestris*

Compounds	Composition (%)	Theoretical (IA)	Calculated (IA)	Molecular formula	Identification method
(*Z*)-Hex-3-en-1-ol	0.55	850	862	$C_6H_{12}O$	a, b, c, d
Hexan-1-ol	2.01	867	878	$C_6H_{14}O$	a, b, c, d
Linalool	3.23	1095	1095	$C_{10}H_{18}O$	a, b, c, d
Phenylethyl Alcohol	0.54	1117	1114	$C_8H_{10}O$	a, c, d
(*E,Z*)-Nona-2,6-dien-1-ol	0.33	1159	1165	$C_9H_{16}O$	a, b, c, d
(*E*)-Non-2-en-1-ol	0.35	1163	1168	$C_9H_{18}O$	a, b, c, d
α-Terpineol	2.44	1195	1195	$C_{10}H_{18}O$	a, b, c, d
Nerol	0.40	1227	1230	$C_{10}H_{18}O$	a, b, c, d
Geraniol	1.86	1253	1256	$C_{10}H_{18}O$	a, b, c, d
Eugenol	1.00	1359	1361	$C_{10}H_{12}O_2$	a, b, c
(*Z*)-Caryophyllene	0.44	1408	1403	$C_{15}H_{24}$	a, c, d
N.I.	0.52	-	-	-	-
(*Z*)-Nerolidol	3.17	1531	1535	$C_{15}H_{26}O$	a, c, d
(*E*)-Nerolidol	10.51	1564	1570	$C_{15}H_{26}O$	a, c, d
α-Cadinol	0.42	1651	1658	$C_{15}H_{26}O$	a, b, d
Pentadecan-2-one	0.32	1697	1703	$C_{15}H_{30}O$	a, b, d
Myristic acid	2.2	1776	1781	$C_{14}H_{28}O_2$	a, b, d
Hexahydrofarnesyl acetone	2.95	1847	1848	$C_{18}H_{36}O$	a, b, d
Pentadecanoic acid	0.66	1887	1881	$C_{15}H_{30}O_2$	a, b, d
Hexadecanoic acid	39.43	1979	1980	$C_{16}H_{32}O_2$	a, b, c, d
(*E,E*)-Geranyl Linalool	0.97	2026	2033	$C_{20}H_{34}O$	a, c, d
Phytol	2.33	2119	2117	$C_{20}H_{40}O$	a, b, d
Linoleic acid	2.75	2155	2149	$C_{18}H_{32}O_2$	a, b, d
N.I.	1.60	-	-	-	-
Tricosane	1.52	2300	2279	$C_{23}H_{48}$	a, b, d
Pentacosane	1.24	2500	2458	$C_{25}H_{52}$	a, b, c
Heptacosane	5.49	2700	2654	$C_{27}H_{56}$	a, d
N.I.	1.60	-	-	-	-
Triacontane	9.08	3000	3000	$C_{30}H_{62}$	a, c
Compounds identified (%)	96.19				

AI = Arithmetic index on the OV-5 column (comparison with C8-C30 n-alkanes); n.i. = not identified. Identification method: a) Arithmetic index; b) Mass spectra library and Adams retention index; c) Comparison of the mass spectra with the Wiley library; d) comparison of arithmetic indices and mass spectra with NIST 2017

Table 2. Chemical classes of the identified compounds in EO

Functional group	EO of flowers (%)
Alcohols	3.78
Ketones	3.27
Long-chain alkanes	17.33
Oxygenated monoterpenes	8.93
Oxygenated sesquiterpenes	14.10
Non-oxygenated sesquiterpenes	0.44
Oxygenated diterpenes	3.30
Fatty acids	45.04
n.i.	3.72

The major components identified in the EO of the flowers of *B. campestris* were hexadecanoic acid (39.43 %), sesquiterpene (*E*)-nerolidol (10.51 %), long-chain alkanes triacontane (9.08 %) and heptacosane (5.49 %) and monoterpene linalool (3.23 %) (Figure 1). Fatty acids (45 %), long-chain alkanes (17.33 %), oxygenated sesquiterpenes (14.10 %) and oxygenated monoterpenes (8.93 %) were the predominant classes, comprising 85.36 % of the total. Monoterpenes (linalool, α-terpineol, nerol and geraniol), sesquiterpenes ((*Z*)-caryophyllene, (*Z*)-nerolidol, (*E*)-nerolidol and α-cadinol) and diterpenes ((*E,E*)-geranyl linalool and phytol) represented 26.77 % of the oil composition. Fatty acids and long-chain alkanes represented 62 % of the oil composition. The presence of fatty acids in the flower oil of *B. campestris* has been reported. In the study by Baronio and Del Claro, *B. campestris* oil was obtained by friction in microcapillary tubes, followed by immersion in ethyl acetate, evaporation of the solvent and reaction of the remainder with diazomethane.[31] In the characterisation of the final product by GC-MS, eight esters corresponding to their methylated fatty acids were observed. Methyl hexadecanoate, methyl-3-acetoxyoctadecanoate and 3,9-diacetoxydocosanoic acid methyl ester were the major constituents. Figure 1 shows the chromatogram obtained for the EO of the flowers of *B. campestris* and Figure 2 the structure of the major compounds.

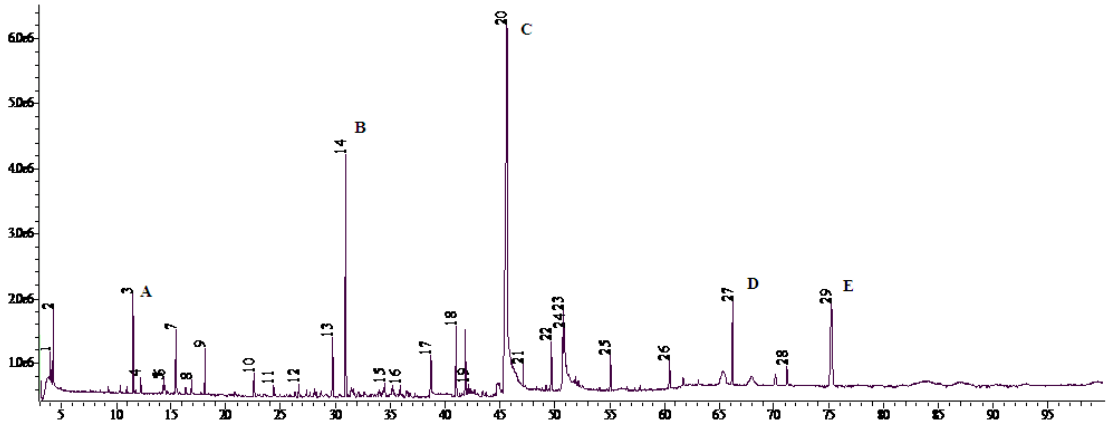

Figure 1. Chromatogram by GC-MS of the EO of flowers of *Banisteriopsis campestris* (A) Linalool; (B) (*E*)-nerolidol; (C) Hexadecanoic acid; (D) Heptacosane; (E) Triacontane

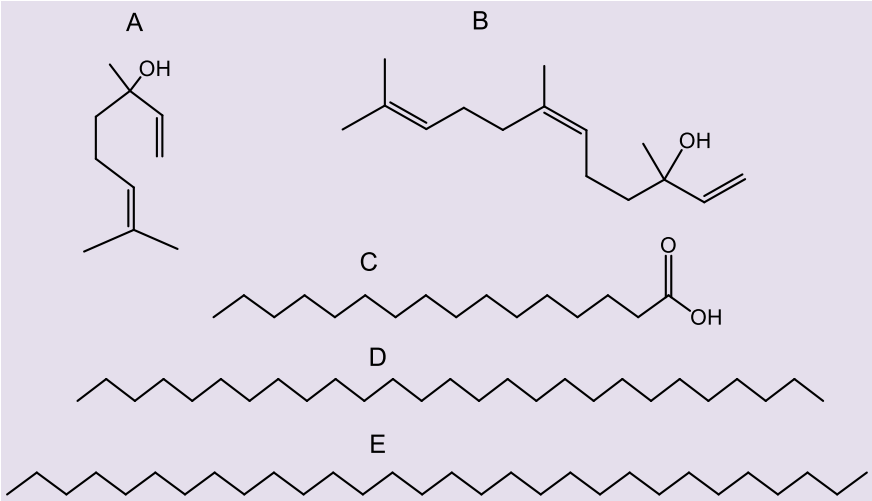

Figure 2. Major compounds identified in EO of *B. campestris* flowers

The chemical constitution and the biological potential of the EOs of *Banisteriopsis* species have been little investigated so far. Studies involving the chemical characterisation of EOs were only found for *B. laevifolia* and *B. oxyclada* (Table 3).[7,8] The major components found for the EO of flowers of *B. campestris* are not similar to those observed as predominant in the leaves of *B. laevifolia* and *B. oxyclada*. However, when comparing the chemical composition of the EOs of *B. campestris*, *B. oxyclada and B. laevifolia*, although with different contents, (*Z*)-hex-3-en-1-ol, linalool and phytol were identified in the three species. Hexan-1-ol, phenylethyl alcohol, (*E,Z*)-nona-2,6-dien-1-ol, geraniol, (*E*)-nerolidol, hexadecanoic acid, (*E,E*)-geranyl linalool, pentacosane and heptacosane were identified in *B. campestris* and at least one of the other species. The chemical constituents of EOs can vary qualitatively and quantitatively between species, between the same species and between different parts.[22,32,33] This may occur because many factors influence the composition of EOs, such as soil properties, seasonality, light intensity, climatic conditions, genetic factors and extraction techniques.[34,35]

Table 3. Essential oils of some species of *Banisteriopsis* and their major constituents

Species	Part of the vegetable	Major compounds	Reference
B. campestris	Flowers	Hexadecanoic acid (39.4 %) (*E*)-nerolidol (10.5 %) Triacontane (9.08 %)	This work
B. laevifolia (Rainy season)	Leaves	(Z)-hex-3-en-1-ol (17.0 %) Phytol (14.9 %) Untriacontane (15.3 %)	7
B. laevifolia (Dry season)	Leaves	(Z)-hex-3-en-1-ol (19.4 %) Phytol (9.8 %) Untriacontane (7.5 %)	7
B. oxyclada	Leaves	(*Z*)-hex-3-en-1-ol (15.26 %) (*Z*)-hex-2-en-1-ol (13.0 %) Phytol (10.06 %) (*E*)-hex-2-en-1-al (9.7 %)	8

The EO of flowers of *B. campestris* is rich in compounds with proven antibacterial and antifungal effects.[36–44] In particular, in relation to the majorities, hexadecanoic acid, (*E*)-nerolidol and triacontane (a long-chain alkane) have recognised antimicrobial activity.[40,45,46]

The evaluation of the antimicrobial activity of the EO of flowers of *B. campestris* was determined against aerobic and anaerobic oral bacteria and some yeasts. The antimicrobial activity of the EO of this species has not yet been described. The analysis was carried out using the broth microdilution method, aiming at the determination of the MIC. Tables 4 and 5 show MIC values for the antibacterial and antifungal activities, respectively.

Table 4. Antibacterial activity of EO of flowers of *B. campestris*

	Bacteria	Essential oil flowers MIC (μg.mL^{-1})	Chlorhexidine MIC (μg.mL^{-1})
Aerobic	*Streptococcus mutans*[a] (ATCC 25175)	400	0.92
	Streptococcus mitis[a] (ATCC 49456)	200	3.68
	Streptococcus sanguinis[a] (ATCC 10556)	25	0.92
	Aggregatibacter actinomycetemcomitans[a] (ATCC 43717)	200	0.46
Anaerobic	*Porphyromonas gingivalis*[b] ATCC 33277	50	3.6
	Fusobacterium nucleatum [b] ATCC 25586	200	1.8
	Actinomyces naeslundii [b] ATCC19039	50	1.8

[a] Gram-positive bacteria; [b] Gram-negative bacteria; MIC. Positive control: chlorhexidine dihydrochloride

Some authors support the relevant antibacterial effect described here for the EO of *B. campestris* flowers.[47,48] MIC values below 100 μg.mL^{-1}, between 100 and 500 μg.mL^{-1} and between 500 and 1,000 μg.mL^{-1} correspond to promising, moderate and weak activities, respectively, while MIC values above 1,000 μg.mL^{-1} indicate inactivity. Recently, new considerations on the antibacterial activity of EOs against cariogenic bacteria such as *S. mutans* and *S. sanguinis* have been proposed. For MIC values less than or equal to 100 μg.mL^{-1}, activity is considered to be very strong; 101–500 μg.mL^{-1}, strong; 501–1000 μg.mL^{-1}, moderate; 1,001–2,000 μg.mL^{-1}, poor; and above 2,001 μg.mL^{-1}, inactive.[43]

In this study, the EO of the flowers of *B. campestris* showed strong antibacterial activity against *P. gingivalis* and *A. naeslundii* with MIC values of 50 μg.mL^{-1}, and moderate activity for *F. nucleatum* with an MIC value of 200 μg.mL^{-1}. Regarding the aerobic bacteria, the EO of the flower showed a very strong activity against *S. sanguinis* (MIC of 25 μg.mL^{-1}), strong activity against *S. mutans* (MIC of 400 μg.mL^{-1}) and moderate effect against *S. mitis* and *A. Actinomycetemcomitans.* Although the EO of the flower of *B. campestris* was active against all microorganisms tested, we highlight here the results found for the anaerobic bacteria. Studies show that some 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.[49,50] It is also important to note that the bacteria evaluated in this study cause oral diseases such as caries, endodontic infections and periodontitis and systemic diseases such as infective endocarditis.

The inhibitory concentration results for the EO of *B. campestris* and other species are shown in Table 5.

In Table 5, the EO of the flower of *B. campestris* showed MIC values lower than other studies in the literature, and promising activity against three oral bacteria with MIC values below 100 $\mu g.mL^{-1}$. The biological activity of EOs has often been related to the major compounds, but in some studies, the isolated main constituents showed less antimicrobial activity than the EO.[39,53] Thus, it becomes increasingly clear that biological activities of the EOs are involved with the synergism between the major components and the minority constituents. The antibacterial activity for this work is probably related to the synergism of several compounds present in the oil with recognised antimicrobial activity.

Saturated and unsaturated fatty acids, alkanes, alcohols and long-chain ketones accounted for 63.3 % of the oil composition. These compounds have been shown to have potential antifungal and antibacterial effects.[40,41,46,55,56] The EO of the leaves of *Inga laurina* collected in rainy season, rich in hexadecanoic acid and phytol, showed significant antibacterial effect against aerobic and anaerobic oral bacteria.[51] Although many classes of metabolites may be present in EOs, the antimicrobial activity is also attributed to the presence of terpenes.[32,35,43,44,51,55] The monoterpenes linalool and α-terpineol have already demonstrated an effect against cariogenic bacteria, including *S. mutans*, the main etiological agent of dental caries. The non-terpene compound eugenol is considered a very promising antibacterial agent against these microorganisms.[43] Some sesquiterpenes were found in flowers EO of *B. campestris*.

The EOs from the seeds of *Aframomum dalzielii, Aframomum letestuianum* and *Aframomum pruinosum*, rich in (E)-nerolidol and the isolate (E)-nerolidol showed a strong effect against *Micrococcus luteus* and *Escherichia coli*.[45] Some EOs rich in (E)-nerolidol and β-caryophyllene were considered promising against cariogenic bacteria and several other microorganisms.[32,43] Thus, the sesquiterpenes found here may exert some influence on the inhibition of the tested bacteria.

Recent studies have shown that diterpenes are very effective in inhibiting different microorganisms, including oral pathogens.[44,57] The diterpene, phytol, although not abundant in *B. campestris* flower oil, may be an important contributor to the activity, since its role in antibacterial activity against oral microorganisms has already been described.[44,51]

Table 5. MICs comparison of essential oils from other plant species against oral bacteria

Minimum Inhibitory Concentration (MIC), µg.mL^{-1}											
Bacteria	***Banisteriopsis Campestris*** [d]	***Inga laurina*** [a 51]	***Banisteriopsis laevifolia*** [a 7]	***Kielmeyera coriacea*** [a 52]	***Kielmeyera coriacea*** [b 52]	***Cassia bakeriana*** [a 22]	***Plectranthus neochilus*** [a 39]	***Eugenia Calycina*** [a 20]	***Artemisia absinthium*** [a 53]	***Eugenia klotzschiana*** [a 54]	***Eugenia klotzschiana*** [c 54]
S. mutans	400	50	>400	>400	100	62.5	3.9	>400	250	50	50
S. mitis	200	50	>400	>400	100	62.5	31.3	400	62.5	200	200
S. sanguinis	25	50	>400	>400	100	125	62.5	400	1000	400	400
A. actnomycetem.	200	-	>400	-	-	125	-	-	-	-	-
P. gingivalis	50	50	-	-	-	125	-	100	-	-	-
F. nucleatum	200	200	>400	-	-	1000	-	-	-	-	-
A. naeslundii	50	400	>400	>400	400	62.5	-	>400	-	-	-

Part of plant: [a] leaves; [b] outer bark; [c] flower. (-) not reported; (>) EO not active within the tested concentration range

The EO of the *B. campestris* flower showed very strong to moderate activity against all the aerobic and anaerobic bacteria tested and could be a natural alternative in the prevention of diseases caused by oral microorganisms. The EO was also evaluated against some yeasts and the results are shown in Table 6.

The EO showed no activity against the yeasts of *C. albicans*, *C. tropicalis* and *C. glabrata* within the range of the tested concentrations (1.46–3000 µg mL^{-1}). Although these results are negative, it was considered important for future research with the species. Even though the flower oil of *B. campestris* presented several constituents that are potential antifungal agents, it was not possible to observe any effect on these microorganisms.

Table 6. Antifungal activity of EO of *B. campestris* flowers

Yeasts	Essential oil flowers MIC (μg mL^{-1})	Amphotericin
Candida albicans	>3000	0.25
Candida tropicalis	>3000	0.25
Candida glabrata	>3000	0.12

Yeast control by protocol M27-A3 CLSI (2008): *Candida krusei* – MIC 1 μg mL^{-1} *Candida parapsilosis* – MIC 0.25 μg mL^{-1}

4. Conclusions

The chemical composition of the EO of the flowers of *B. campestris* 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. The EO of the flowers of *B. campestris* presented relevant activity against the aerobic and anaerobic oral bacteria tested and did not indicate any anti-*Candida* activity. These results contributed to the chemical and biological knowledge of the species *B. campestris* that is endemic to the Brazilian Cerrado.

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