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Original Article

Chemical composition and evaluation of antimicrobial and antioxidant activities of essential oils from *Lippia hermannioides* and *Matayba marginata*, two plants on ironstone outcrops: an endangered Brazilian ecosystem

Composição química e avaliação das atividades antimicrobiana e antioxidante dos óleos essenciais de *Lippia hermannioides* e *Matayba marginata*, duas plantas em afloramentos de rochas ferruginosas: um ecossistema brasileiro ameaçado de extinção

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Abstract

Canga is the Brazilian term used to describe a weathered superficial ferruginous deposit. These geoenvironments shelter hundreds of rare species. Essential oils of *Lippia hermannioides* and *Matayba marginata*, species collected in the ferruginous rupestrian field on remaining *canga* at Federal University of Ouro Preto-UFOP, were isolated from fresh leaves by hydrodistillation in a Clevenger-type apparatus and characterized by GC-MS analyses. A total of 32 compounds were identified in the oil of *L. hermannioides*, while 15 were identified in the oil of *M. marginata*, representing 98.9% and 97.7% respectively, of the total volatile content. In both essential oils sesquiterpene hydrocarbons were the main class of constituents (53.4% for *L. hermannioides* and 92.1% for *M. marginata*). Elemicin (35.1%) and β-caryophyllene (21.3%) were found to be the major constituents in the essential oil of *L. hermannioides*, whereas germacrene A (48.8%) and β-elemene (29.5%) were encountered in the essential oil of *M. marginata*. Both essential oils showed bactericidal activity against Gram-positive bacteria, with the essential oil of *L. hermannioides* being more efficient. The oils did not present antioxidant activity and by $ORAC_{FL}$ assay. The oil of *L. hermannioides* showed weak DPPH radical-scavenging activity by thin-layer chromatography (TLC) autographic assay. The data presented in this study showed, for the first time in literature, the chemical composition of essential oil from *M. marginata* and their bactericidal and antioxidant properties. These findings contribute to the knowledge of the chemical composition and biological activities of *Lippia* and *Matayba* genera.

Keywords: ironstone outcrops, *canga*, essential oil, antimicrobial activity, antioxidant activity.

Resumo

Canga é o termo brasileiro utilizado para descrever um depósito ferruginoso superficial intemperizado. Estes geoambientes abrigam centenas de espécies raras. Os óleos essenciais de *Lippia hermannioides* e *Matayba marginata*, espécies coletadas no campo rupestre ferruginoso sobre *canga* remanescente na Universidade Federal de Ouro Preto-UFOP, foram isolados das folhas frescas por hidrodestilação em aparelho tipo Clevenger e caracterizados por análises de CG-EM. Um total de 32 compostos foi identificado no óleo de *L. hermannioides*, enquanto 15 foram identificados no óleo de *M. marginata*, representando 98,9% e 97,7%, respetivamente, do conteúdo volátil total. Em ambos os óleos essenciais, os sesquiterpenos hidrocarbonetos foram a principal classe de constituintes (53,4% para *L. hermannioides* e 92,1% para *M. marginata*). Elemicina (35,1%) e β-cariofileno (21,3%) são os principais constituintes do óleo essencial de *L. hermannioides*, enquanto germacreno A (48,8%) e β-elemeno (29,5%) foram encontrados no óleo essencial de *M. marginata*. Ambos os óleos essenciais apresentaram atividade bactericida contra bactérias Gram-positivas, sendo o óleo essencial de *L. hermannioides* mais eficiente. Os óleos não apresentaram atividade antioxidante e pelo ensaio $ORAC_{FL}$. O óleo de *L. hermannioides* apresentou uma fraca atividade de eliminação do radical DPPH por ensaio autográfico por cromatografia em camada delgada (CCD). Os dados apresentados neste estudo mostraram, pela primeira vez na literatura, a composição química do óleo essencial de *M. marginata* e suas propriedades bactericidas e antioxidantes. Estes resultados contribuem para o conhecimento da composição química e das atividades biológicas dos gêneros *Lippia* e *Matayba*.

Palavras-chave: afloramentos de rochas ferruginosas, *canga*, óleo essencial, atividade antimicrobiana, atividade antioxidante.

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1. Introduction

The Iron Quadrangle, in Brazil (state of Minas Gerais), covers approximately 7200 km^2 with the superficial iron crusts distributed on the tops of mountains, and is considered an area of extreme biological importance due the presence of endemic and endangered species, existence of unique habitats, and high anthropic pressure (Skirycz et al., 2014; Viana-Silva and Jacobi, 2012). An outstanding habitat typical of the Iron Quadrangle is that of ironstone outcrops, also known as "*canga*", distributed on mountaintop areas associated with major deposits of iron ore (Viana-Silva and Jacobi, 2012). However, the total area covered by *canga* is relatively small, approximately 100 km^2, and dispersed on mountain inselbergs (Skirycz et al., 2014). The outcrops are characterized by high UV exposure, high daily temperature variation, frequent winds, low water retention, and impermeable soils. For these reasons, the *canga* vegetation is underdeveloped and presents adaptations for survival in stressful, rupestral environments (Viana-Silva and Jacobi, 2012). Due to their very restricted area, difficult access, and because they are associated with high-quality iron-ore deposits, the plant communities over *canga* are among the most threatened and least studied in the ecosystems of southeast Brazil (Jacobi and Do Carmo, 2008). Hundreds of angiosperm species have been reported so far across remote *canga* inselbergs and different microhabitats. A floristic inventory, carried out in the ferruginous rupestrian field on remaining *canga* at Federal University of Ouro Preto-UFOP Campus, found 137 species distributed in 36 families (Scalon et al., 2012). Many of these species that were collected in this research (Scalon et al., 2012) are endemic and remain unexplored as to the study of their essential oils, emphasizing the importance of research aimed at greater knowledge from the chemical and biological point of view. Among these endemic species are the *Lippia hermannioides* and *Matayba marginata*.

The plant species of the genus *Lippia* Linn. belonging to the family Verbenaceae, comprising 200 species herbs, shrubs, and small trees and have a great geographic distribution are easily found in Brazil, Mexico, Central America, Africa, Paraguay and Argentina (Soares and Tavares-Dias, 2013; Viccini et al., 2005; Fabri et al., 2011). In Brazil, the genus *Lippia* is represented by nearly 120 species conspicuous for their appearance during their short blooming period and their generally strong and pleasant fragrance (Fabri et al., 2011). Most of them are endemic and concentrated at Espinhaço Range, Minas Gerais and Goiás state, where the occurrence of rocks is common and many *Lippia* species grow on them (Viccini et al., 2005). Since long time, several species are used traditionally in popular medicine in the treatment of gastrointestinal disorders, rheumatism, hypertension, stomach cramps, nausea, coughs, colds, allergic rhinitis, throat and mouth infections, skin and scalp disorders (Scio et al., 2012). Besides these known properties there are few published studies about *Lippia* and even less about the essential oil composition of the genus. *Lippia hermannioides* Cham. is a shrub that grows between 1.5 and 2.0 meters. The plant is native and endemic to Brazil and its geographic distribution includes the states of Bahia, Distrito Federal, Goiás and Minas Gerais (Salimena and Cardoso, 2024).

The genus *Matayba* (family Sapindaceae) contains around 50 species of shrubs or trees, distributed from Mexico to northern Argentina. In Brazil there are 30 species, 17 of them endemic (Coelho et al., 2017). The species of the genus more studied chemically are *M. arborescens* (coumarin cleomiscosin A), *M. elaeagnoides* (lupeol, α-amyrin, β-amyrin, β-sitosterol, scopoletin, umbelliferone, 3-β-O-D-glycopyranosyl-sitosterol and betulin), and *M. guianensis* (ether diglycosides: matayosides A–F, cupanioside and stigmasterol) (Arisawa et al., 1984; Souza et al., 2007; Mesquita et al., 2005, Assis et al., 2014). Pharmacological properties of the species of the genus include antinociceptive, antifungal and antiplasmodial (Souza et al., 2007; Mesquita et al., 2005; Assis et al., 2014). The composition of essential oils from the genus *Matayba* has not been extensively studied. *Matayba marginata* Radlk. is a tree or shrubs of 0.5 to 10 m in high, is native and endemic to Brazil, and its geographic distribution involves the states of Minas Gerais, Rio de Janeiro, São Paulo and Paraná (Coelho, 2024). The species is mostly found at elevations above 1000 meters (Coelho et al., 2017).

In this work we report on the chemical composition of the essential oils from fresh leaves of *L. hermannioides* and *M. marginata*, collected in the ferruginous rupestrian field on *canga*. The study included also an evaluation of the essential oils against a panel of microorganism strains and evaluation of the antioxidant capacity of the oils by TLC autographic assay for DPPH radical-scavenging and $ORAC_{FL}$ methods. The chemical composition and antinociceptive and anti-inflammatory potential of the essential oil from dry leaves of *L. hermannioides* have already been described in only one other study, however, this is the first report of the antimicrobial and antioxidant activities of the essential oil from fresh leaves of *L. hermannioides*. There are no reports in the literature concerning the chemical composition of essential oil from *M. marginata* and their antimicrobial and antioxidant properties.

2. Materials and Methods

2.1. Plant material

The two Brazilian species were collected in the ferruginous rupestrian field on remaining *canga* at Federal University of Ouro Preto-UFOP Campus, in the city of Ouro Preto, State of Minas Gerais, Brazil. Leaves of *L. hermannioides* (Figure 1A) and *M. marginata* (Figure 1B) were collected on 22 March 2018, and identified by comparison with voucher specimens present in the herbarium, previously identified. The choice of plants was based on two criteria: endemism and/or the fact that the plant had little or no chemical or biological study. A voucher of each species *L. hermannioides* (MCTB Messias: 2809) and *M. marginata* (MCTB Messias: 2794), was deposited in the herbarium José Badini, of the Federal University of Ouro Preto-UFOP. The registries in SisGen (Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado – National System of Management of Genetic Heritage and Associated

Figure 1. Plants on ironstone outcrops. (A) *L. hermannioides* and (B) *M. marginata*.

Traditional Knowledge) were performed (A0BAB20 for *L. hermannioides* and A1E7163 for *M. marginata*) according to the Brazilian legislation on access to biodiversity (Federal Law No. 13,123/2015).

2.2. Essential oils extraction

Approximately 100 g of fresh leaves of each species were subjected to hydro-distillation for 4 h, using a modified Clevenger type apparatus. The calculated essential oil yield was expressed in % (w/w) based on the fresh plant material.

2.3. Essential oils analysis

The chemical analysis of the essential oils obtained was carried out using a Shimadzu QP-2010 gas chromatograph interfaced to a mass spectrometer (GC-MS). The following conditions were used: ZB-5MS column Phenomenex Zebron (30 m × 0.25 mm × 0.25 µm); helium (99.999%) carrier gas at a constant flow of 1.1 mL/min; 1 µL injection volume; injector split ratio of 1:40; injector temperature 240 °C; electron impact mode at 70 eV; ion-source temperature 280 °C. The oven temperature was programmed from 100 °C (isothermal for 5 min), with an increase of 10 °C/min to 250 °C (isothermal for 5 min), and 10 °C/min to 280 °C (isothermal for 15 min).

2.4. Identification of constituents of essential oils

The identities of the essential oils constituents were carried out by comparison of their linear relative retention indices (RRI) calculated to a homologous series of *n*-alkanes (Van den Dool and Kratz, 1963), confirmed by comparison with published RRI values (Adams, 2007), and via fragmentation patterns in the mass spectra with those from the software database (Wiley 7 library and Nist 08 library).

2.5. Antimicrobial activity

The antimicrobial properties of the essential oils were examined using the broth microdilution method (96-well microtiter plates), as previously described (Salvador et al., 2002). Minimal inhibitory concentration (MIC) tests were performed according to the Clinical Laboratory Standard Institute (CLSI) protocol M07-A10 (CLSI, 2015), and the minimal inhibitory concentration (MIC) was calculated as the lowest concentration showing complete inhibition of microbial growth. Dimethyl sulfoxide (DMSO) was used as a solubilizing agent (final concentration 2.5%) while for the controls of sterility the solubilizing agent was 5% DMSO in MHB (Muller Hinton Broth). For evaluation of the antimicrobial activity of essential oils, strains of microorganisms were used as the multiple drug-resistant targets. The microorganisms used were resistant to methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus faecalis* (VREF), β-lactamase *Escherichia coli* (BLEC), and multiple drug-resistant *Candida albicans* (MDRCA). Inhibition tests (chloramphenicol 64 and 320 µg/mL for MRSA and VREF respectively) were performed for samples and controls together. Eight dilutions, starting from 1.0 mg/mL were assayed, and the MIC was determined after adding 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) and the optical density at 625 nm compared with the controls. In addition, the MICs of commercial antibiotics such as methicillin, vancomycin, and

chloramphenicol for MRSA and meropenem, vancomycin and chloramphenicol for VREF were performed. All the assays were performed in triplicate.

2.6. Antioxidant activity by $ORAC_{FL}$ kinetic assay

The antioxidant capacity of the essential oils was assessed through the Oxygen Radical Absorbance Capacity (ORAC) assay (Alencar et al., 2016). This assay is based upon the inhibition of the peroxyl radical-induced oxidation initiated by the decomposition of the peroxyl radical (2,2'-azobis(2-amidino-propane) dihydrochloride (AAPH, Aldrich, Milwaukee, WI), using fluorescein (Aldrich, Milwaukee, WI) as the fluorescent probe. The assay was carried out on a Synergy 2 (Biotek, Winooski, VT) multidetection microplate reader system. The temperature of the incubator was set at 37 °C and the procedure performed according to the method described by Huang et al. (2002). The data are expressed as µmol of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, Aldrich, Milwaukee, WI) equivalents (TE) per gram of oil on a dry basis (µmol of TE/g). The analyses were performed in triplicate. In this assay is considered active samples with antioxidant capacity ≥ 800.0 µmol de TE/g (Alencar et al., 2016). In these tests, quercetin, isoquercetin, caffeic acid, and chlorogenic acid were used as positive controls. The analyses were performed in triplicate.

2.7. Antioxidant activity by TLC autographic assay for DPPH radical scavenging

In this assay, 1:250, 1:100 and 1:50 (v/v), dilution of the essential oil in dichloromethane were made and then 10 µL of each dilution were applied on TLC plates (silica gel 60 GF254, Fluka, AG, Switzerland). The plates were sprayed with a 0.2% 2,2-diphenyl-1-picrylhydrazyl (DPPH, Sigma-Aldrich, St Louis, MO) solution, in methanol, and left at room temperature. Plates were observed 30 min after spraying. Active samples were observed as yellow spots against a purple background. Relative radical-scavenging activity was assigned as "strong" (samples that produced an intense bright yellow zone), "medium" (samples that produced a clear yellow spot), "weak" (samples that produce only a weakly visible yellow spot), or "not active" (samples that produced no sign of any yellow spot) (Alencar et al., 2016; Huang et al., 2002; Cavin et al., 1998). Quercetin, isoquercetin, caffeic acid, and chlorogenic acid were used as positive controls. The analyses were performed in triplicate.

3. Results

3.1. Essential oils extraction

The yields of essential oils from fresh leaves of *L. hermannioides* and *M.* marginata were 0.64 and 0.30% (w/w), respectively.

3.2. Identification of constituents of essential oils

The compositions of the essential oils, obtained by hydrodistillation, are listed (Table 1). The total number of chemical constituents identified in essential oil was 32 for *L. hermannioides*, representing 98.9% of the total oil content (Table 1). The essential oil is dominated by the presence of sesquiterpene hydrocarbons constituting 53.4%, followed by oxygenated sesquiterpenes (5.1%), monoterpene hydrocarbons (3.5%) and other compounds (36.8%). The major compounds were elemicin (35.1%), β-caryophyllene (21.3%), E-γ-bisabolene (12.0%), α-humulene (6,7%), and bicyclogermacrene (6.2%).

Fifteen compounds, constituting 97.7% of the leaf oil of *M. marginata*, were identified (Table 1). The essential oil had high amounts of sesquiterpene hydrocarbons (92.1%) and the most abundant components were germacrene A and β-elemene which constitute respectively 48.8% and 29.5% of the total oil composition.

3.3. Antimicrobial activity

Antimicrobial properties of the oils, evaluated by using the broth microdilution assay, are reported (Table 2). The oils were tested against two Gram-positive (*Staphylococcus aureus* ATCC 43300 and *Enterococcus faecalis* ATCC 51299) and one Gram-negative (*Escherichia coli* ATCC 25922) bacteria, and one yeast strain (*Candida albicans* ATCC 10231). The results show that essential oils of *L. hermannioides* and *M. marginata* inhibited both Gram-positive bacteria strains and did not show any activity to the other microorganisms tested in this series suggesting its selectivity. The essential oil of *L. hermannioides* was more efficient because it presented a better MIC value than the oil of *M. marginata* to the same bacteria (*S. aureus*). Susceptibility tests of multiple drug-resistant bacteria targets were performed against commercial antibiotics MIC. *S. aureus* ATCC 43300 was resistant to methicillin (MRSA), *E. faecalis* ATCC 51299 showed resistance to vancomycin and chloramphenicol (VREF), and *Escherichia coli* ATCC 25922 demonstrated resistance to the beta-lactam antibiotics (BLEC). The yeast strain, *Candida albicans* ATCC 10231, was resistant to multiple drug (MDRCA). The MIC for MRSA, VREF and BLEC against commercial antibiotics are shown (Table 2).

3.4. Antioxidant activity

The antioxidant activity was evaluated by two methods, by $ORAC_{FL}$ kinetic assay and by TLC autographic assay for DPPH radical scavenging. The results are described (Table 3).

3.5. Antioxidant activity by ORACFL kinetic assay

The in the $ORAC_{FL}$ assay, the results were 180 and 200 µmol TE/g for *L. hermannioides* and *M. marginata* essential oils, respectively. Both oils did not present antioxidant activity by $ORAC_{FL}$, since in this assay is considered active samples with antioxidant capacity ≥ 800.0 µmol TE/g.

3.6. Antioxidant activity by TLC autographic assay for DPPH radical scavenging

The samples that presented antioxidant activity produced yellowish spots and were classified according to the intensity of the yellow spots produced. The oil of *L. hermannioides* showed weak DPPH radical-scavenging

Table 1. Chemical composition of the essential oils from *L. hermannioides* and *M. marginata*.

RRI[a]	RRI[b]	Constituent	Yield %	
			LH	MM
930	924	α-Thujene	0.07	
939	932	α-Pinene	1.56	
954	948	Camphene	0.02	
975	971	Sabinene	0.40	
979	977	β-Pinene	1.26	
985	981	6-Methyl-5-Hepten-2-one	0.03	
990	988	Myrcene	0.06	
1011	1009	δ-3-Carene	0.06	
1029	1027	Limonene	0.10	
1058	1055	Isopentyl butanoate	0.11	
1232	1229	3*Z* Hexenyl 3-methyl butanoate	0.11	
1376	1372	α-Copaene		0.07
1388	1380	β-Bourbonene	0.47	
1390	1386	β-Elemene	0.08	**29.45**
1403	1397	Methyl eugenol	1.50	
1419	1416	β-Caryophyllene	**21.33**	2.96
1434	1430	α-*trans*-bergamotene	0.13	0.19
1454	1451	α-Humulene	**6.72**	4.34
1481	1476	Germacrene D	4.90	3.59
1490	1484	β-Selinene		0.96
1498	1491	α-Selinene		1.54
1500	1491	Bicyclogermacrene	**6.22**	
1505	1505	β-Bisabolene	0.53	
1509	1504	Germacrene A		**48.75**
1512	1514	δ-Amorphene		0.23
1515	1507	*Z*-γ-Bisabolene	0.72	
1515	1510	Cubebol		0.20
1523	1514	δ-Cadinene	0.14	
1531	1524	E-γ-Bisabolene	**12.01**	
1557	1547	Elemicin	**35.05**	
1575	1571	Germacrene-D-4-ol		0.13
1578	1571	Spathulenol	1.45	
1583	1576	Caryophyllene oxide	2.36	
1608	1603	Humulene epoxide II	0.45	
1637	1633	Gossonorol	0.40	
1640	1631	Caryophylla-4(12),8(13)-dien-5α-ol	0.13	
1642	1638	Epi-α-Muurolol		0.11
1654	1649	α-Cadinol	0.17	
1659	1651	Selin-11-en-4-α-ol		4.97
1660	1657	Neo-Intermedeol		0.19
1684	1681	Epi-α-Bisabolol	0.14	
		Monoterpenes		
		Hydrocarbons	3.53	
		Oxygenated		
		Monoterpenes total	**3.53**	
		Sesquiterpenes		
		Hydrocarbons	53.43	92.08
		Oxygenated	5.10	5.60
		Sesquiterpenes total	**58.53**	**97.68**
		Others	36.80	
		Total identified	**98.86**	**97.68**

RRI[a] = Relative Retention Indices. See Adams (2007); RRI[b] = Relative Retention Indices on ZB-5MS column (relative to *n*-alkanes). Essential oils: LH = *L. hermannioides*; MM = *M. marginata*. Bold text indicates the most representative components (>5%).

Table 2. Antimicrobial activity of the essential oils from *L. hermannioides* and *M. marginata.*

Microorganism	MIC[a] (μg/mL)		
	LH	MM	Controls[b]
Staphylococcus aureus ATCC 43300[1]	500	1000	20
Enterococcus faecalis ATCC 51299[2]	1000	1000	160
Escherichia coli ATCC 25922[3]	N	N	32
Candida albicans ATCC 10231[4]	N	N	0.5

Essential oils: LH = *L. hermannioides*; MM = *M. marginata*. [a]MIC: minimum inhibitory concentration in μg/mL. [b]Positive controls: chloramphenicol for bacterial strains 1 and 2 and amoxicillin for 3; voriconazole for yeast strain 4; N: no inhibition of microbial development. [1]MRSA: Methicillin-resistant *Staphylococcus aureus*; [2]VREF: Vancomycin-resistant *Enterococcus faecalis*; [3]BLEC: β-lactamase *Escherichia coli*; [4]MDRCA: Multiple drug-resistant *Candida albicans*.

Table 3. Antioxidant capacity of the essential oils from *L. hermannioides* and *M. marginata.*

Essential Oil / Positive Control	ORAC assay[a]	TLC-DPPH assay[c]
	(μmol of TE g^{-1})[b]	(5,0 mg/mL)
L. hermannioides	180 (20)	+
M. marginata	200 (25)	–
Quercetin[d]	5,60 (1,50)	+++ (1 mg/mL)
Isoquercetin[d]	5,15 (1,70)	+++ (1 mg/mL)
Caffeic acid[d]	2,85 (0,95)	+++ (1 mg/mL)
Chlorogenic acid[d]	2,65 (1,00)	+++ (1 mg/mL)

[a]Mean (%RSD, relative standard deviation) of triplicate assays; [b]ORAC data expressed as μmol of Trolox equivalents per g of essential oil (μmol of TE g^{-1}); [c]TLC-based 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenger antioxidant assay. Strong inhibition of DPPH radical reduction (+++), medium inhibition of DPPH radical reduction (++), low inhibition of DPPH (+) radical reduction, not active (-); [d]Positive controls: ORAC data expressed as relative Trolox equivalent, mean (%RSD, relative standard deviation) of triplicate assays.

activity by TLC autographic assay because it showed a weak yellow stain (Table 3).

4. Discussion

The essential oil from the leaves of *L. hermannioides* was found in only one study, and its content was 1.23% (v/w) from dry biomass (Oliveira eta al., 2024). Previous study carried out with another species of the genus *Lippia*, with *L. alba*, also from fresh plant material (leaves), demonstrated that the essential oil yield ranged from 0.33 to 0.67% (Barros et al., 2009). Qualitative and quantitative variations regarding the period of harvesting have been performed and the results were correlated with meteorological data, being 0.67% in January (summer), 0.60% in April (autumn), 0.33% in July (winter) and 0.40% in October (spring). Fresh leaves of *L. hermannioides* in the present study produced 0.64% yield of essential oil, and they were collected on the second day of autumn, showing a yield close to that of the study by Barros et al. (2009).

There is no data on the volatile chemical composition of the species *M. marginata* in the literature, which difficult the discussion of the results. An earlier investigation was conducted with *Matayba guianensis*, another species of the genus *Matayba*, using fresh leaves collected in March 2020, showed an essential oil yield of 0.21% (Jesus et al., 2020). Fresh leaves of *M. marginata* in the present study produced 0.30% yield of essential oil and were also collected in March, the yield being close to that of the study with *M. guianensis*.

L. hermannioides and *M. marginata* essential oils presented sesquiterpene hydBarocarbons as major constituents, however the oil of *M. marginata* is exclusive of sesquiterpenes (hydrocarbons and oxygenated). There is a variation in the content and chemical composition of the essential oils of the two different species in relation to the major constituents. Only five compounds were common to these two species: β-elemene, β-caryophyllene, α-trans-bergamotene, α-humulene and germacrene D. The second major compound present in *L. hermannioides* essential oil, β-caryophyllene (21.3%), is also the second major compound present in the essential oil of another species of the same genus, *L. schaueriana* (Souza et al., 2018). The detected constituents were not identical to those found in the only study on the composition of *L. hermannioides* essential oil previously reported by Oliveira et al. (2024), which showed just 13 of the 32 compounds identified in the present study. The main volatile constituents that have been found frequently in the essential oils of *Lippia* spp are geranial (= citral, or 3,7-dimethyl-2,6-octadienal or lemonal), carvone, carvacrol (= cymophenol), thymol, neral, 1,8-cineole, limonene, p-cymene, linalool, α-pinene

and β-caryophyllene (Silva et al., 2009). Only one study on the components of the essential oil of the *Matayba* genus was found for comparison. In this research carried out with *M. guianensis*, the essential oil from the leaves is composed of hydrocarbon sesquiterpenes (Jesus et al., 2020). The main constituents found were bicyclogermacrene (31.3%), germacrene D (28.4%), β-caryophyllene (15.5%) and germacrene B (5.2%), in contrast to our investigation, whose most abundant compounds were germacrene A (48.8%) and β-elemene (29.5%).

In recent years, the issue of microbial resistance, which affects how different diseases are treated, has come to light. According to Almeida et al. (2018), both Gram-positive and Gram-negative bacteria, including *Staphylococcus* and *Escherichia coli*, as well as opportunistic fungi like *Candida albicans*, have contributed to the fast spread of this resistance. In this way, susceptibility tests of multiple drug-resistant microorganism's targets were performed (Table 2).

The antibacterial activity of essential oil of *L. hermannioides* against *S. aureus* and *E. faecalis* may be a result of the presence of α-pinene (1.6%), β-pinene (1.3%), limonene (0.1%), spathulenol (1.5%) and caryophyllene oxide (2.4%), compounds that are known to possess antibacterial activity (Magiatis et al., 1999; Sökmen et al., 2003; Bougatsos et al., 2004). Although present in low concentrations, these compounds might have had a significant effect on the antibacterial activity of the oil. Some studies have concluded that the minor components are critical to the activity and may have a synergistic effect or potentiating influence (Sivasothy et al., 2011; Burt, 2004). In contrast, some researchers reported that there is a relationship between the chemical structures of the most abundant in the tested essential oil and the antimicrobial activity. The antimicrobial effect of β-caryophyllene was tested against some human pathogens, and the results showed that the β-caryophyllene demonstrated selective antibacterial activity against *S. aureus* (Dahham et al., 2015). The essential oil of wild *Daucus carota* L. was reported as antimicrobial against the human enteropathogen *Campylobacter jejuni* and the molecules that were responsible for the antibacterial activity were identified as (*E*)-methylisoeugenol and elemicin (Rossi et al., 2007). In this way, the antibacterial activity of the essential oil of *L. hermannioides* also which could be attributed due to the high percentage of β-caryophyllene (21.3%) and elemicin (35.1%).

Other species of the genus *Lippia* spp. have had their chemical makeup and pertinent antimicrobial properties thoroughly investigated, and some studies have already demonstrated antifungal and antibacterial activity against various microorganisms of agricultural, medical, and veterinary importance (Lemos et al., 1990; Pascual et al., 2001; Kunle et al., 2003; Albuquerque et al., 2006). Carvacrol and thymol, however, are the substances that numerous authors have claimed to be effective in this situation (Souza et al., 2018), both absent in the essential oil of *L. hermannioides* (Table 1).

Similarly, the antibacterial activity of the oil of *M. marginata,* against *S. aureus* and *E. faecalis*, can be attributed to the component β-elemene (29.5%), that exert action against *Mycobacterium tuberculosis* (Sieniawska et al., 2018). This compound is a rearrangement product of germacrene A (Adio, 2009), which in *M. marginata* essential oil has a high concentration (48.8%). In the study carried out by Jesus et al. (2020) essential oil obtained from the fresh leaves of *Matayba guianensis* was extracted and evaluated for their antibacterial activity against a panel of four standard and three clinical multiple drug-resistant bacterial strains. The tested oil showed moderate to good activity against at least four bacterial strains, including *Salmonella* Typhi and oxacillin–resistant *Staphylococcus* and strong inhibition of clinical *Staphylococcus* strains, which cause bovine mastitis and are related to milk-borne diseases. Twenty-two compounds were identified in the essential oil from the leaves of *M. guianensis*, which was shown to be composed almost exclusively of hydrocarbon sesquiterpenes (94.9%), such as bicyclogermacrene (31.3%), germacrene D (28.4%), β-caryophyllene (15.5%) and germacrene B (5.2%). Both germacrene D (3.6%) and β-caryophyllene (2.9%) are presents in the essential oil of *M. marginata* in small amounts (Table 1).

The antioxidant potential of different essential oils can be measured using numerous *in vitro* assays. A single method is not recommended for the evaluation due to the complex compositions of the oils. Therefore, the antioxidant effects of essential oils must be evaluated by combining two or more distinct *in vitro* experiments to obtain relevant data (Samojlik et al., 2010). With respect to this, the antioxidant activity was evaluated by two methods.

By the $ORAC_{FL}$ assay, none of the essential oils extracted in this study showed antioxidant activity. In previous investigations, the antioxidant capabilities of essential oils from various plants were assessed by $ORAC_{FL}$ assay, and it was discovered that γ-muurolene, δ-cadinene, germacrene D, bicyclogermacrene, α-copaene and β-caryophyllene were the main components in these oils (Costa et al., 2013). Therefore, the non-observed antioxidant capacity is attributed to the absence of mixtures of these compounds in large amounts.

Several compounds in the essential oil from *L. hermannioides* possess antioxidant activity. Trying to correlate the weak observed activity with the chemical composition of the oil, the study of Ruberto and Baratta (2000) should be cited, which examined the antioxidant activity of 98 pure essential oils chemical components. β-Caryophyllene, that possess antioxidant activity, occurs in appreciable amounts (21.3%) in *L. hermannioides*. In another study conducted with *Lippia*, the *L. alba* essential oil, obtained by hydrodistillation, showed IC_{50} = 12.45 mg/mL in DPPH assay (Reyes-Solano et al., 2017). Most of the oil was composed of 15.6% eucalyptol. The antioxidant properties of *Lippia* essential oils have not been extensively studied.

The oil of *M. marginata* did not present antioxidant activity by TLC-DPPH radical-scavenging assay. No studies on the antioxidant activity on essential oils of *Matayba* species were found.

5. Conclusions

The essential oil composition of the leaves of *M. marginata* is reported for the first time. The essential

oil of the leaves of *L. hermannioides* was particularly rich in sesquiterpene hydrocarbons and displayed biological activities. Considering that only a small percentage of the estimated 250,000-500,000 extant plant species has been investigated as for biological activity or chemical screened, this study comes to contribute to the biological and chemical knowledge on some of Brazilian flora species over ferruginous rupestrian field on *canga*.

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