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## Glandular trichome density and essential oil composition in leaves and inflorescences of *Lippia origanoides* Kunth (Verbenaceae) in the Brazilian Cerrado

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### ABSTRACT

The essential oils from leaves and inflorescences of *Lippia origanoides* Kunth present aromatic and medicinal potential and have been used to treat several diseases, including melanoma. In Brazil, *L. origanoides* is commonly found in campo cerrado and cerrado *stricto sensu*, physiognomies featured mainly by the differential light conditions to which short and medium-sized plants are subjected. Our aim was to investigate the glandular trichome density and the yield and chemical composition of the essential oils in leaves and inflorescences of *L. origanoides* from campo cerrado and cerrado *stricto sensu*. For glandular density analysis, leaves and inflorescences were processed according to conventional techniques for scanning electron microscopy. The essential oils of leaves and inflorescences were obtained by hydrodistillation and identified with gas chromatography. Bracts and sepals showed the highest glandular density, followed by petals and leaves. The glandular density in the abaxial leaf surface was higher in individuals from the campo cerrado. In both populations the essential oil yield was higher in inflorescences than in leaves. The chemical composition of the essential oils varied among individuals from different areas and inside a same population. Our results demonstrated the chemical plasticity of *L. origanoides* suggesting the importance of monitoring its popular use.

**Key words:** campo cerrado, cerrado *stricto sensu*, external glands, terpenes.

### INTRODUCTION

Species of Verbenaceae are known for the presence of glandular trichomes that secrete essential oils. Many of these species are widely exploited by drug manufacturers and used in popular medicine (Judd et al. 2009, Pascual et al. 2001, Souza and Lorenzi

2008). *Lippia origanoides* Kunth, a native shrub of the Brazilian Cerrado, is widely distributed in South America from Guiana to northern Argentina, and also occurs in Central America (O'Leary et al. 2012). The essential oil extracted from leaves of *L. origanoides* shows antimicrobial, antiviral, acaricidal, antioxidant, antibacterial and anti-inflammatory activities (Cavalcanti et al. 2010,

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Gomes et al. 2012, Oliveira et al. 2007, Rocha-Guzmán et al. 2007, Stashenko et al. 2010, 2013, Veras et al. 2013). Different chemotypes of *L. origanoides* were reported in literature and p-cimene, thymol and carvacrol are the major components most commonly found (Cavalcanti et al. 2010, Oliveira et al. 2007, Stashenko et al. 2010, 2013).

In the Brazilian Cerrado, *L. origanoides* is commonly found in areas of campo cerrado and cerrado *stricto sensu*. These Cerrado physiognomies are featured mainly by the differential light intensity that reaches the short and medium-sized plants. The cerrado *stricto sensu* is characterized by the occurrence of medium-sized trees, scattered shrubs and some grasses. In this area the individuals of *L. origanoides* are shaded by nearby trees in the early hours of the morning and in late afternoon. In the campo cerrado, grasses and small shrubs are predominant, with some sparse trees (Maroni et al. 2006), and individuals of *L. origanoides* are exposed to full sunlight throughout the day.

Studies indicate that both yield and chemical composition of the essential oils and the glandular trichome density, can be influenced by environmental factors, including herbivory, temperature, water availability, altitude, circadian cycle, seasonality, and light intensity, among others (Argyropoulou et al. 2007, Gianfagna et al. 1992, Gobbo-Neto and Lopes 2007, Gonzáles et al. 2008, Juliani Jr et al. 2002, Martínez-Natarén et al. 2011, Pérez-Estrada et al. 2000, Viljoen et al. 2005, Werker 2000). Whether the different organs in a same plant respond similarly to the environmental fluctuations concerning the essential oil composition is still unknown.

Since leaves and inflorescences of *L. origanoides* are exhaustively exploited by people living in Cerrado areas in Brazil for medicinal purposes, knowledge on the occurrence of variation in the essential oil composition in plants living in different environmental conditions can be important in order to avoid it being misused.

We investigated the density of the glandular trichomes and the yield and chemical composition of essential oils in leaves and inflorescences of *L. origanoides* from campo cerrado and cerrado *stricto sensu*.

## MATERIALS AND METHODS

### STUDY AREA AND PLANT MATERIAL

Two wild populations of *L. origanoides* were located in two different Cerrado physiognomies, cerrado *stricto sensu* (S 22°49'11.39" and W 48°44'51.18") and campo cerrado (S 22°53'29.82" and W 48°29'25.81"), both in midwestern state of São Paulo, Brazil. The climate of both areas is Cwa, according to Köppen.

Data on the temperature and relative humidity from November 2012 through March 2013 were obtained from meteorological stations in the cerrado *stricto sensu* and campo cerrado areas. The photosynthetic photon flux density (PPFD) was measured with a PAM fluorometer on successive days. The altitude data were recorded with a GPS.

Table I shows the data for temperature, relative humidity, PPFD and altitude in both areas.

**TABLE I**  
**Environmental data from areas of cerrado *sensu stricto* and campo cerrado in São Paulo State, Brazil.**

	cerrado <i>stricto sensu</i>	campo cerrado
Temperature (°C)	22.5	21.7
Air relative humidity (%)	82.0	79.5
PPFD ( $\mu\text{mol.m}^{-2}.\text{s}^{-1}$ )	1837	2045
Altitude (m)	719	833

Twelve adult plants of *L. origanoides* were sampled in each population. Leaves and inflorescences were collected during the summer, in February 2013. All individuals were in the same phenological stage.

Vouchers were deposited in the Herbarium Irina Delanova Gemtchújnicov (BOTU), Department of Botany, Universidade Estadual Paulista (UNESP), Botucatu, state of São Paulo.

## DENSITY OF GLANDULAR TRICHOMES

To evaluate the density of glandular trichomes, two fully expanded leaves were collected from each plant in both populations. To estimate the density of glandular trichomes in reproductive organs, two inflorescences were collected from each individual in the campo cerrado.

Samples of flowers and the middle part of leaf blades were fixed in glutaraldehyde (2.5% with 0.1 M phosphate buffer, pH 7.3, overnight at 4 °C), dehydrated in a graduated acetone series, critical-point dried, mounted on aluminum stubs, gold-coated (Robards 1978), and examined with a Fei Quanta scanning electron microscope.

The glandular density in leaves and flowers was calculated in 1 mm<sup>2</sup> using the Scandium software with an image-capture system coupled to the scanning electron microscope. We compared the glandular density between a) the adaxial and abaxial leaf surfaces in individuals from the same population; b) leaves from different populations; and c) leaves and floral parts (bracts, sepals, petals) from the same population. The data were submitted to ANOVA followed by Tukey test, at a 5% probability level.

## YIELD AND CHEMICAL COMPOSITION OF THE ESSENTIAL OILS

For the essential oil analyses, leaves and inflorescences were collected from four individuals growing in the cerrado *stricto sensu* (numbered 1 to 4) and from three individuals growing in the campo cerrado (numbered 1 to 3). Individual number 3 in the campo cerrado was located on an ant colony.

The samples were dried at 40 °C and were subjected to hydrodistillation in a Clevenger-type apparatus (Craveiro et al. 1981) for 2 h.

The qualitative analysis of the essential oil was performed on a gas chromatograph coupled to a mass spectrometer (CG-MS, Shimadzu, QP-5000), with an OV-5 fused silica capillary column (30 m x 0.25 mm x 0.25 µm, Ohio Valley Specialty Chemical, Inc.), operating at an MS ionization

voltage of 70 eV, with helium as the carrier gas (1.0 mL/min.). The following chromatography conditions were used: injector at 240 °C, detector at 230 °C, injection volume: 1 µL of solution (1 mg of essential oil/1 mL of ethyl acetate), split 1/20, and the temperature program: 60 °C – 240 °C, 3 °C/min. The compounds were identified by comparison of the acquired mass spectra with those stored in the GC/MS database of the system (NIST 62 lib.) and retention indices (Adams 2007). The retention indices (RI) were obtained from the injection of a mixture of *n*-alkanes (Sigma-Aldrich, C<sub>9</sub>-C<sub>24</sub>), employing the same temperature program conditions described above for GC/MS, applying the equation of Van den Dool and Kratz (1963).

The separation and quantification (area normalization method) of the substances were carried out by gas chromatography (GC-FID, Shimadzu, GC-2010/AOC-20i), equipped with a flame ionizer, using a DB-5 capillary column (J and W Scientific; 30 m x 0.25 mm x 0.25 µm), with helium as the carrier gas (1.0 mL/min), temperature injector at 240 °C, detector at 230 °C, split 1/20, injection volume 1 µL of solution (1 mg essential oil/1 mL ethyl acetate). The following chromatography conditions were used: 60 °C – 160 °C, 5 °C/min; 160 °C – 230 °C, 3 °C/min; 230 °C – 280 °C, 10 °C/min.

The yield of essential oil was analyzed using factorial analysis, and compared among the plant organs and populations by ANOVA, followed by Tukey test at a 5% probability level.

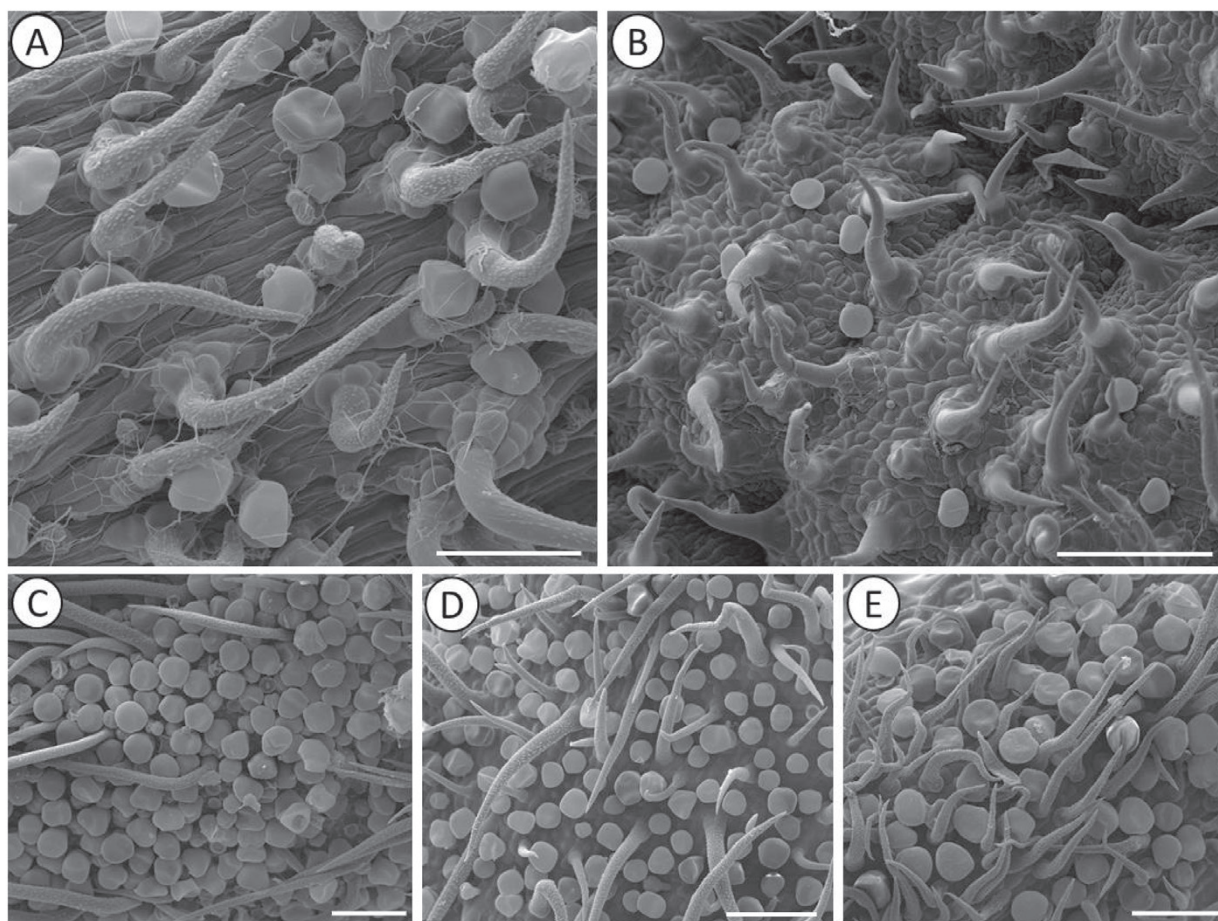
## RESULTS

## DISTRIBUTION AND DENSITY OF GLANDULAR TRICHOME

Glandular trichomes were observed in both leaf (Fig. 1A, B) and bract (Fig. 1C) surfaces, as well as the abaxial side of sepals (Fig. 1D) and petals (Fig. 1E) of *L. origanoides*. In the petals, these glands occurred exclusively in the distal region.

In the individuals from the campo cerrado, the glandular density was higher in the inflorescences than in the leaves ( $F_{(4,55)}=144.3919$ ;  $P<0.00001$ )





**Figure 1** - Scanning electron micrographs of leaves and floral parts of *Lippia origanoides* Kunth showing glandular and non-glandular trichomes. A. Abaxial leaf surface. B. Adaxial leaf surface. C. Abaxial bract surface. D. Abaxial sepal surface. E. Abaxial petal surface. Scale bars: A-E = 100 µm; B = 200 µm.

(Table II). Bracts and sepals were the organs with the highest glandular density (Table II). The density of glandular trichomes was three to four times higher on the abaxial surface of the leaf (Fig. 1A) than on the adaxial (Fig. 1B) for individuals from both the campo cerrado and the cerrado *stricto sensu* (Table II).

The abaxial leaf surface of individuals from the campo cerrado showed a higher density of glandular trichomes in comparison to individuals from the cerrado *stricto sensu* ( $F_{(1,22)}=15.6354$ ;  $P=0.0009$ ). Differences in the glandular density were not observed for the adaxial leaf surfaces of individuals from the different populations ( $F_{(1,22)}=1.1297$ ;  $P=0.2998$ ) (Table II).

#### YIELD AND CHEMICAL COMPOSITION OF THE ESSENTIAL OIL

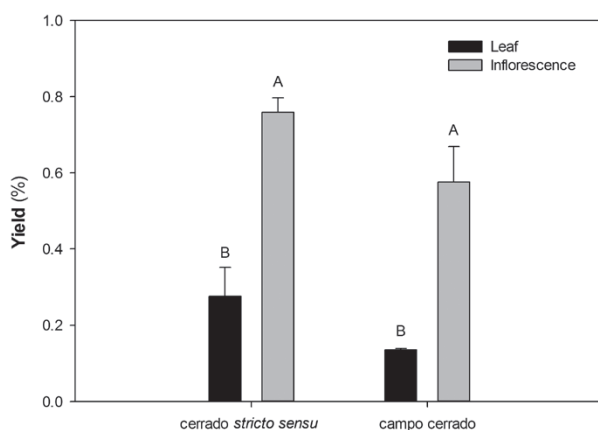
The yield of the essential oil of *L. origanoides* was higher in inflorescences than in the leaves (Fig. 2) in individuals from both cerrado physiognomies ( $F_{(3,8)}=22.7906$ ;  $P<0.0001$ ) (Fig. 2). No significant differences were observed in the oil yield between individuals from the cerrado *stricto sensu* and the campo cerrado, for both leaves ( $P=0.390$ ) and inflorescences ( $P=0.233$ ).

During the analysis of the essential oil yield, the samples from individual 3 (the one that was located on an ant colony in the field) from the campo cerrado were not used, due to the discrepant values obtained for its inflorescences (1.98) and leaves (3.97).

**TABLE II**  
**Glandular density (mm<sup>2</sup>) in leaf blades and inflorescences of *Lippia***  
***origanoides* Kunth from two physiognomies of the Brazilian Cerrado.**

Organ	Glandular density	
	campo cerrado	cerrado <i>stricto sensu</i>
Bract (abaxial surface)	624.00 A	-
Sepal (abaxial surface)	544.42 A	-
Petal (abaxial surface)	271.85 B	-
Leaf (abaxial surface)	97.62 Ca	69.52 Ab
Leaf (adaxial surface)	21.43 Da	19.16 Ba

Means followed by different capital letters indicate statistical differences in the glandular density among organs and different lowercase letters indicate statistical differences in the glandular density between populations (Tukey test  $p < 0.05$ ).



**Figure 2** - Yield of essential oil of leaves and inflorescences of *Lippia origanoides* Kunth from two populations in the Cerrado (Means followed by different letters indicate statistical differences in the essential oil yield between organs inside a same population and between different populations by Tukey test at 5% significance level).

We identified 49 substances in the essential oil extracted from leaves and inflorescences of *L. origanoides*. The amount of substances found in the inflorescences was higher than in the leaves, in both populations. The major compounds varied between the populations, among the individuals from the same population, and between the organs of the same individual (Table III).

In the cerrado *stricto sensu*, *trans*-caryophyllene was the major compound in the inflorescences of individual 1 (63.4%), 2 (34.2%) and 4 (39.3%), whereas  $\alpha$ -humulene was the major compound in

individual 3 (58.7%). Concerning the leaves, *trans*-caryophyllene (33.7%) and  $\delta$ -cadinene (10.3%) were the major compounds in individual 2; individuals 3 and 4 showed  $\alpha$ -humulene (66.3% and 40.0%, respectively) and *trans*-caryophyllene (9.5% and 24.9%, respectively) as their major compounds.

In the campo cerrado,  $\alpha$ -pinene (27.2%) and *trans*-caryophyllene (38.6%) were the major compounds in the inflorescences, and *trans*-caryophyllene (60.6%) in the leaves of individual 1.  $\beta$ -elemene was the major compound in the inflorescences (10.2%), and  $\alpha$ -humulene in the leaves (10.3%) of individual 2; *trans*-caryophyllene,  $\gamma$ -selinene and 7-*epi*- $\alpha$ -selinene were the majority compounds in both the inflorescences (11.2%, 14.9% and 10.5%) and the leaves (9.5%, 16.7% and 13.5%) in this individual. 1,8 cineole was the major compound in both the inflorescences (52.2%) and the leaves (58.2%) of individual 3; differently from the other individuals, oxygenated monoterpenes were predominant in the inflorescences (61.8%) and leaves (71.3%) of this individual (Table III).

## DISCUSSION

We have found a higher density of glandular trichomes on the abaxial leaf surface of *L. origanoides* plants growing in the campo cerrado; in this area the relative humidity was lower and the altitude and PPFD were higher. Little is known

**TABLE III**  
**Chemical composition of the essential oils (%) from inflorescences and leaves of *Lippia origanoides* Kunth from cerrado *stricto sensu* and campo cerrado in São Paulo state, Brazil.**

Compound	Inflorescences							Leaves							RI*	RI**
	cerrado <i>stricto sensu</i>				campo cerrado			cerrado <i>stricto sensu</i>			campo cerrado					
	1.0	2.0	3.0	4.0	1.0	2.0	3.0	2.0	3.0	4.0	1.0	2.0	3.0			
Hydrocarbon Monoterpenes																
$\alpha$ -thujenew	nd	nd	nd	nd	nd	nd	0.6	nd	nd	nd	nd	nd	0.8	927	930	
$\alpha$ -pinene	3.7	0.8	8.4	nd	27.2	nd	4.6	0.5	0.7	nd	2.4	nd	4.2	934	939	
camphene	1.2	3.0	1.3	0.4	2.4	0.2	0.2	1.5	0.1	0.1	0.2	nd	tr	948	954	
sabinene	nd	nd	nd	nd	nd	nd	5.8	nd	nd	nd	nd	0.7	4.2	975	975	
$\beta$ -pinene	nd	nd	nd	nd	1.3	nd	2.3	nd	nd	nd	nd	nd	2.3	977	979	
myrcene	nd	0.6	0.5	nd	0.7	tr	1.5	tr	tr	nd	nd	nd	1.5	991	990	
$\alpha$ -phellandrene	nd	nd	0.4	nd	tr	nd	0.3	nd	nd	nd	nd	nd	0.4	1006	1002	
$\alpha$ -terpinene	nd	0.6	tr	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.6	1017	1017	
p-cymene	1.0	1.9	0.3	tr	2.1	0.2	1.6	0.9	nd	nd	nd	nd	1.0	1024	1024	
limonene	0.4	1.9	3.0	nd	nd	nd	nd	nd	nd	nd	0.4	0.2	1.8	1029	1029	
Z- $\beta$ -ocymene	nd	nd	nd	nd	nd	nd	nd	1.4	nd	0.3	nd	nd	nd	1037	1037	
E- $\beta$ -ocymene	nd	7.9	1.8	nd	nd	1.7	tr	4.5	0.2	tr	nd	0.8	tr	1047	1050	
$\gamma$ -terpinene	tr	5.1	1.2	nd	nd	nd	1.9	0.3	nd	nd	nd	nd	1.3	1058	1059	
terpinolene	nd	0.2	tr	nd	tr	nd	0.3	tr	nd	nd	nd	nd	0.2	1089	1088	
Oxygenated Monoterpenes																
1-octen-3-ol	0.2	0.3	0.5	nd	nd	nd	nd	0.9	tr	0.4	nd	nd	nd	977	979	
1,8 cineole	0.4	8.7	0.4	0.3	0.2	0.3	52.2	4.3	1.3	nd	nd	nd	58.2	1031	1031	
linalool	1.2	5.2	0.8	3.7	1.9	5.7	0.6	1.1	tr	2.8	0.7	4.1	1.6	1100	1096	
borneol	nd	1.2	tr	nd	tr	nd	0.5	0.3	tr	nd	nd	nd	0.6	1165	1169	
terpinen-4-ol	nd	0.6	nd	nd	nd	nd	1.8	tr	nd	nd	nd	nd	2.4	1177	1177	
$\alpha$ -terpineol	nd	1.0	nd	nd	nd	nd	6.7	tr	nd	nd	nd	nd	8.6	1190	1188	
Hydrogenated Sesquiterpenes																
$\alpha$ -copaene	5.3	5.1	3.4	5.4	3.7	1.0	tr	8.5	4.2	4.6	8.5	1.4	nd	1378	1376	
$\beta$ -elemene	4.2	1.6	1.4	1.7	3.3	10.2	0.5	nd	nd	nd	tr	0.5	nd	1394	1390	
$\alpha$ -gurjunene	nd	0.3	tr	0.2	nd	nd	nd	0.6	nd	0.2	nd	nd	nd	1411	1409	
<i>trans</i> -caryophyllene	63.4	34.2	12.7	39.3	38.6	11.2	3.9	33.7	9.5	24.9	60.6	9.5	2.5	1422	1419	
$\alpha$ -guaiene	nd	0.9	nd	nd	nd	nd	0.3	1.8	nd	nd	nd	tr	0.2	1440	1439	
aromadendrene	nd	nd	nd	nd	nd	0.6	nd	nd	nd	nd	nd	0.6	nd	1446	1441	
$\alpha$ -humulene	3.7	2.3	58.7	35.0	2.2	8.3	0.3	2.7	66.3	40.0	3.7	10.3	tr	1455	1554	
<i>allo</i> -aromadendrene	0.6	nd	tr	0.8	0.2	tr	tr	0.3	0.3	0.7	0.5	tr	tr	1462	1460	
$\gamma$ -muurolene	1.0	0.4	0.5	1.0	nd	1.3	nd	0.9	nd	nd	nd	1.2	nd	1475	1479	
germacrene D	1.6	0.6	0.3	0.9	0.7	0.7	0.6	1.0	0.6	0.9	1.2	nd	nd	1478	1485	
$\gamma$ -selinene	nd	nd	nd	nd	nd	14.9	nd	tr	nd	nd	nd	16.7	nd	1481	1498	
$\beta$ -selinene	0.9	0.7	1.3	0.5	1.1	3.7	nd	tr	nd	nd	tr	3.1	nd	1487	1492	
valencene	nd	nd	nd	nd	nd	3.0	nd	nd	nd	nd	nd	3.6	nd	1490	1496	
$\alpha$ -selinene	nd	nd	nd	nd	nd	6.6	nd	0.9	0.3	nd	tr	6.0	nd	1496	1498	
bicyclogermacrene	0.2	0.5	tr	tr	4.1	nd	5.3	nd	nd	nd	nd	nd	2.2	1497	1500	
$\alpha$ -muurolene	nd	nd	nd	1.7	nd	nd	nd	1.6	0.7	0.9	1.4	nd	nd	1501	1500	
germacrene A	1.8	2.4	tr	nd	nd	1.0	nd	0.4	nd	nd	nd	nd	nd	1506	1509	
7- <i>epi</i> - $\alpha$ -selinene	nd	nd	nd	nd	nd	10.5	nd	nd	nd	nd	nd	13.5	nd	1514	1522	
$\delta$ -cadinene	2.2	5.4	1.0	2.2	0.8	0.2	tr	10.3	1.5	2.5	2.9	0.4	nd	1525	1513	
<i>trans</i> -cadin-1,4-diene	nd	tr	nd	nd	nd	nd	nd	0.3	nd	nd	nd	nd	nd	1534	1534	
germacrene B	tr	nd	tr	nd	nd	0.5	nd	nd	nd	nd	nd	0.5	nd	1553	1561	

Oxygenated Sesquiterpenes															
spathulenol	nd	tr	nd	0.3	1.0	nd	3.4	nd	nd	0.3	nd	nd	2.0	1578	1578
caryophyllene oxide	2.1	3.6	0.5	2.3	5.8	1.4	2.3	6.0	0.7	3.9	6.2	1.0	2.5	1583	1583
humulene epoxide II	tr	nd	nd	nd	tr	1.1	nd	tr	5.0	4.6	2.8	1.2	nd	1610	1608
1- <i>epi</i> -cubenol	0.3	nd	1.2	1.5	nd	0.8	nd	4.5	1.7	2.2	nd	1.7	nd	1629	1628
cubenol	0.6	1.4	tr	0.5	0.6	nd	0.7	0.3	0.3	2.4	1.1	2.0	tr	1643	1646
$\alpha$ -muurolol	tr	0.8	tr	0.5	nd	0.7	nd	5.5	0.2	1.0	0.7	0.6	nd	1646	1646
selin-11-en-4- $\alpha$ -ol	nd	nd	nd	nd	nd	5.8	nd	tr	nd	nd	nd	8.1	nd	1654	1652***
$\alpha$ -cadinol	2.6	0.5	0.6	1.6	2.0	5.9	nd	1.1	1.3	2.8	nd	8.6	tr	1655	1654
Hydrocarbon Monoterpenes	6.3	21.9	16.9	0.4	33.7	2.0	18.9	9.1	1.0	0.4	3.0	1.8	18.2		
Oxygenated Monoterpenes	1.8	16.9	1.7	4.1	2.1	6.0	61.8	6.6	1.3	3.2	0.7	4.1	71.3		
Hydrogenated Sesquiterpenes	84.8	54.2	79.1	88.5	54.6	73.6	10.9	62.9	83.3	74.7	78.8	67.1	4.9		
Oxygenated Sesquiterpenes	5.5	6.2	2.3	6.5	9.4	15.6	6.3	17.4	9.2	16.9	10.7	23.2	4.5		
Total identified	98.4	99.2	100.0	99.5	99.7	97.2	97.9	95.9	94.9	95.2	93.3	96.0	98.9		

RI\*= Retention index calculated; IR\*\*= Retention index (Adams 2007); nd = not detected; tr = trace (tr  $\leq$  0.13). \*\*\* Retention index obtained on the website: [www.pherobase.com](http://www.pherobase.com)

about the influence of exogenous factors on the origin of secretory structures in plants. Studies have reported on the effects of light, temperature, altitude, and availability of nutrients, among others, on the glandular density in different plant species (Gianfagna et al. 1992, Horgan et al. 2009, Pérez-Estrada et al. 2000). In drier environments, the density of trichomes on leaves can be higher than in plants of the same species growing in wetter situations (Pérez-Estrada et al. 2000). Similarly, light intensity can positively influence the glandular trichomes (Pérez-Estrada et al. 2000, Yamaura et al. 1989). According to Pérez-Estrada et al. (2000), in addition to playing a defensive role against biotic agents, glandular trichomes can help to reflect sunlight and minimize water loss.

Although the PPFD was similar between the areas, plant density in the cerrado *stricto sensu* is higher, and at certain periods of the day the individuals can be shaded. This does not occur in the campo cerrado, where shorter herbaceous species predominate. The shading of *L. origanoides* plants in the cerrado *stricto sensu* may be related to the lower glandular density of their leaves, since plants under more intense radiation tend to have higher glandular

density as a protective mechanism (Gianfagna et al. 1992, Pérez-Estrada et al. 2000). In addition, the higher density of glandular trichomes on leaves of *L. origanoides* plants in the campo cerrado may be related to the higher altitude of this area.

The influence of altitude on the abundance of glandular structures was evaluated by Sheue et al. (2003), who found a higher density of internal glands in individuals of *Pinus taiwanensis* in middle altitudes (1000 to 2500 m), in comparison with plants at lower (700 m) or higher (3000 m) altitudes. According to the authors, variation in the number of glands can result from genetic and environmental interactions. This suggests that variations in the glandular density in individuals of *L. origanoides* growing in the campo cerrado and the cerrado *stricto sensu* may result from interactions between environmental or micro-environmental factors and genetic factors.

Although the glandular density differed in leaves from individuals of *L. origanoides* living in the campo cerrado and in the cerrado *stricto sensu*, the yields of essential oil from plants living in the two environments were similar. This may be related to the mixed chemical nature of the



secretion produced by the glandular trichomes of *L. origanoides*. Besides lipid substances, Tozin et al. (2015), using histochemical methods, detected the presence of polysaccharides, phenolic compounds, proteins, and alkaloids, among others, in the glandular trichomes of this species. The mixed nature of the secretion produced by the trichomes is an aspect common to other *Lippia* species (Argyropoulou et al. 2010, Combrinck et al. 2007). Tozin et al. (2015) demonstrated that not all morphotypes of glandular trichomes of *L. origanoides* produce essential oils. Therefore, the more-abundant glandular trichomes in leaves in individuals from the campo cerrado are probably not secretors of essential oils. We can still suggest that under the environmental conditions of the campo cerrado there is a higher investment in the production of other types of substances by the glandular trichomes, and this provides greater protection against high light intensity and lower relative humidity. Hydrophilic substances can act as a lubricant in dry environments and facilitate leaf expansion (Ascensão et al. 1999). In *Lippia* species, phenolic compounds are abundant and variable in constitution (Pascual et al. 2001), and may help to protect against intense UV-B radiation resulting from greater exposure to sunlight (Liakoura et al. 1997).

Our results also showed that *L. origanoides* inflorescences had a higher density of glandular trichomes and produced a higher yield of essential oils in comparison to the leaf blades of individuals in the same environment. These data contrast with studies on other species, which reported a low yield of essential oils in flowers and inflorescences in comparison to leaves (Bassole et al. 2005, Parra-Gárces et al. 2010), and can be related to the different roles of these glands in reproductive organs (Ascensão et al. 1999, Paiva and Martins 2011). We suggest that in *L. origanoides*, the trichomes in the inflorescences are mainly involved in the production of large amounts of essential oils that play an important role in protecting the reproductive organs against

herbivores and pathogens (Stökl et al. 2010). In addition, several terpenes can be reused in the plant metabolism (Harborne 1988), acting as precursors in the synthesis of hormones such as flowering hormones (Danilova and Kashina 1987, Roshchina and Roshchina 1993).

Chemical analysis of the essential oils in leaves and inflorescences of *L. origanoides* revealed a wide variation in the chemical composition of the essential oil among the individuals in each area. This may be related to genetic variability among individuals from these populations. Catalan and Lampasona (2002) found that intraspecific variations in the chemical composition of essential oils are common in species of *Lippia*.

Studies with *L. origanoides* (*sensu lato*) individuals have presented widely divergent chemical profiles. Thymol, carvacrol and p-cymene have been reported as the majority compounds (Cavalcanti et al. 2010, Oliveira et al. 2007, Stashenko et al. 2010, 2013). However, in this study, thymol and carvacrol were not found, while p-cymene was detected in small quantities. These findings are in accordance with the information reported by Rodrigues et al. (2011) and Stashenko et al. (2010), who demonstrated very divergent chemical profiles in this species. The wide variety of chemotypes found in *L. origanoides* may be a factor of the synonymization proposed by O'Leary et al. (2012); in a revision of the *Goniostachyum* section of *Lippia*, the authors synonymized 41 species into only four. The taxon that is presently known as *L. origanoides* results from the synonymization of 28 taxa that were formerly considered to be separate species (O'Leary et al. 2012).

In some individuals of *L. origanoides*,  $\alpha$ -pinene was found in large quantities in the inflorescences. This may be associated with the known role of this substance in attracting pollinators (Stökl et al. 2010). However, the inflorescences of some *L. origanoides* individuals did not contain this compound in large quantities, perhaps because of

phenological differences among the individuals. Studies indicate that some compounds, such as carvone, limonene and  $\beta$ -pinene (Bicas et al. 2008) can volatilize or be utilized as precursors in the formation of other substances after the anthesis (Parra-Garcés et al. 2010, Stökl et al. 2010).

*Trans*-caryophyllene and  $\alpha$ -humulene were among the major compounds in leaves and inflorescences from several individuals. These substances show several important biological activities, including anti-inflammatory, analgesic, and others (Fernandes et al. 2007, Sabulal et al. 2006), and they are used to manufacture soaps, detergents and food products (Standen et al. 2006).

Individual number 3 from the campo cerrado, situated on an ant colony, diverged most widely from the others in oil yield and chemical composition; only this individual contained 1,8-cineole as the major compound, in both the inflorescences (52.2%) and the leaves (58.2%). The abundant production of this substance can be related to ant attacks on this individual, since 1,8-cineole is toxic to herbivorous insects (Prates et al. 1998, Sukontason et al. 2004). This toxic substance penetrates the insect body via the respiratory or digestive system, and is lethal to several animal species (Sukontason et al. 2004).

These findings evidenced the occurrence of variability in the glandular density in leaves of *L. origanoides* plants living in different areas. Our data demonstrated the chemical plasticity of this species, which can produce essential oils with differential chemical composition in distinct environments and evidenced the differential answers between leaves and inflorescences in a same individual. In view of this plasticity, the use of this species should be better monitored, particularly in popular medicine.

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#### RESUMO

Os óleos essenciais de folhas e inflorescências de *Lippia origanoides* Kunth apresentam potencial aromático e medicinal e têm sido utilizados no tratamento de diversas doenças, incluindo melanoma. No Brasil, *L. origanoides* é comumente encontrada no campo cerrado e no cerrado *stricto sensu*, fisionomias caracterizadas principalmente pelas diferentes condições de luminosidade às quais as plantas de médio e pequeno porte estão sujeitas. Nosso objetivo foi investigar a densidade de tricomas glandulares e o rendimento e a composição química do óleo essencial em folhas e inflorescências de *L. origanoides* do campo cerrado e do cerrado *stricto sensu*. Para análise de densidade glandular, folhas e inflorescências foram processadas segundo técnicas convencionais em microscopia eletrônica de varredura. Os óleos essenciais de folhas e inflorescências foram obtidos por hidrodestilação e identificados com cromatografia gasosa. Brácteas e sépalas mostraram a maior densidade glandular, seguidas por pétalas e folhas. A densidade glandular na face abaxial do limbo foliar foi maior em indivíduos do campo cerrado. Em ambas as fisionomias, o rendimento de óleos essenciais foi maior nas inflorescências em comparação com folhas. A composição química dos óleos essenciais variou entre os indivíduos de diferentes áreas e entre indivíduos da mesma população. Nossos resultados demonstraram a plasticidade química de *L. origanoides* sugerindo a importância do monitoramento de seu uso popular.

**Palavras-chave:** campo cerrado, cerrado *stricto sensu*, glândulas externas, terpenos.

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