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Seasonal variation in the essential oil from *Varronia curassavica* Jacq. accessions

[Variación estacional en el aceite esencial de accesiones de *Varronia curassavica* Jacq.]

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Abstract: *Varronia curassavica* has anti-inflammatory properties because of the terpenes, α-humulene and β-caryophyllene, present in the essential oil. The objective of this study was to evaluate the influence of seasonality on the essential oil of *V. curassavica* accessions. Leaves from six accessions were collected from the Germplasm Bank of the Federal University of Minas Gerais over 12 months. Correlations between the essential oil content and meteorological factors were determined. Gas chromatography analysis coupled with mass spectrometry was conducted to determine the chemical composition of the essential oil. The content and chemical composition of the oil varied throughout the year. Relative humidity was correlated with accessions ICA-VC2 (-0.64) and ICA-VC4 (0.68). β-bourbonene, β-elemene, spathulenol, germacrene, caryophyllene oxide, α-humulene, and β-caryophyllene occurred in all accessions. Accession ICA-VC3 exhibited lower variation (22.17%), higher average (0.97%) essential oil, and maintained an average abundance of α-humulene greater than 2.6%, which is the amount necessary for phytotherapeutics.

Keywords: Boraginaceae; Germplasm; Medicinal plants; α-humulene; β-caryophyllene

Resumen: *Varronia curassavica* tiene propiedades antiinflamatorias debido a los terpenos, α-humuleno y β-cariofileno, presentes en el aceite esencial. El objetivo de este estudio fue evaluar la influencia de la estacionalidad en el aceite esencial de las accesiones de *V. curassavica*. Se recolectaron hojas de seis accesiones del Banco de Germoplasma de la Universidad Federal de Minas Gerais durante 12 meses. Se determinaron las correlaciones entre el contenido de aceite esencial y los factores meteorológicos. Se realizó un análisis de cromatografía de gases junto con espectrometría de masas para determinar la composición química del aceite esencial. El contenido y la composición química del aceite varió a lo largo del año. La humedad relativa se correlacionó con las accesiones ICA-VC2 (-0,64) e ICA-VC4 (0,68). En todas las accesiones aparecieron β-bourboneno, β-elemeno, espatulenol, germacreno, óxido de cariofileno, α-humuleno y β-cariofileno. La accesión ICA-VC3 mostró una menor variación (22,17%), un promedio más alto (0,97%) de aceite esencial y mantuvo una abundancia media de α-humuleno superior al 2,6%, que es la cantidad necesaria para los fitoterápicos.

Palabras clave: Boraginaceae; Germoplasma; Plantas medicinales; α-humuleno; β-cariofileno.

INTRODUCTION

Varronia curassavica Jacq. is a valuable medicinal and aromatic species, popularly known in Brazil as "*erva-baleeira*" (Lorenzi & Matos, 2002; Souza & Lorenzi, 2012). Found in all biomes in the Brazilian national territory (HVFF, 2017), it has anti-inflammatory properties because of α-humulene and β-caryophyllene compounds (Fernandes *et al*., 2007). Thus, this plant was included in the development of the first Brazilian herbal medicines (Ryan, 2010).

For the manufacture of medicinal extracts, obtaining authentic and standardized raw materials is fundamental, because the quality of the raw materials is linked to the efficacy of herbal medicines. Additionally, there is no single international regulation for the manufacture of medicines from plants (Govindaraghavan *et al*., 2012). The Brazilian legislation regulates the registration and post-registration commercialization of phytotherapeutic products and drugs dynamized, via established standards available in ANVISA (2015). This institution describes and regulates each productive phase, from the quality of the raw material to the post-launch process. There is also the Brazilian Pharmacopeia that regulates the minimum quality standards for any health product (ANVISA, 2010).

Multidisciplinary studies are critical to understanding how plants relate to the environment and the possible consequences of these relationships. The origin of the target materials, growing environment, availability of water and nutrients, seasonality, and the temperature to which the plant is subjected influence the content and chemical composition of essential oils (Kutchan, 2001; Sales *et al*., 2009), as well as the time of collection of the vegetal material (Botrel *et al*., 2010; Gasparetto *et al*., 2017). Intrinsic factors associated with the plant also interfere in the behavior of essential oils and are related to the phenological stage of the plant (Bose *et al*., 2013). Thus, throughout the year, the composition and content of the essential oil can vary according to environmental factors and the phenological period of harvest.

For precise results, it is vital to understand where the essential oils are stored in the plant, such that the handling, harvesting, and storage of the material are done correctly. Furthermore, the analysis to determine the essential oil content and composition was performed seeking to represent an analysis performed directly on the plant. The storage location of the essential oils of *V. curassavica* has been reported by Ventrella & Marinho (2008), who observed two types of glandular trichomes. These include globular glandular trichomes, which are associated with the secretion of essential oils, and reniform glandular trichomes which are related to the flow of phenolic compounds such as flavonoids. Furthermore, research is needed to elucidate the effects of phototherapeutic quality on factors that might interfere in the production process of raw materials. Studies whose goals have been to monitor the composition and content of the essential oils in accessions have increased our understanding of the behavior of the essential oil, concerning the effects of its origin, environment, and seasonality on the process. Therefore, it is possible to define what materials may be used in genetic improvement and what to expect from them.

With the ultimate aim to obtain superior quality raw materials for the manufacture of phytotherapeutics with stable chemical composition and components of interest to the industry, the objective of this study was to evaluate the influence of seasonality on the content and chemical composition of essential oils in accessions of *V. curassavica*.

MATERIAL AND METHODS

Study area and plant material

The experiment was conducted at the Instituto de Ciências Agrárias - ICA (Agrarian Sciences Institute) of the Federal University of Minas Gerais (UFMG), Montes Claros Campus, Minas Gerais, Brazil. The in vivo germplasm collection with 12 accessions (ICA-VC1, ICA-VC2, ICA-VC3, ICA-VC4, ICA-VC5 and ICA-VC6) is located on the Montes Claros Campus, at the north of the state of Minas Gerais (16°42′16″S, 43°49′13″W, average altitude of 638 masl). All plants were assembled under the same conditions, with a spacing of 1.0 × 0.5 m and with the application of organic fertilization and drip irrigation. For the experiment, six accessions from different locations (Table No. 1) were chosen, having at least three replicates.

The meteorological factors obtained for the correlation analysis with the essential oil content of the accessions are listed in Table No. 2.

Extraction and content of essential oils

For the extraction of essential oils, the leaves of *V.*

Table No. 1
Corresponding locations and identification of *Varronia curassavica* Jacq. accessions that belonged to the Germplasm Bank from the Institute of Agrarian Sciences of the Federal University of Minas Gerais, Montes Claros Campus, Minas Gerais, Brazil

Accessions	Locations	Biomes****	CC***	R (mm)	T(Cº)
ICA-VC1	Grão Mogol – MG	Cerrado	Cwa	969**	21.0**
ICA-VC2	Taquaraçu de Minas – MG	Cerrado	Cwa	1350.50*	20.13*
ICA-VC3	Santo Antônio do Monte - MG	Cerrado and Atlantic forest	Cwa	1346.85*	21.15*
ICA-VC4	Itacambira – MG	Cerrado	Cwa	1092**	19.9**
ICA-VC5	Cristália – MG	Cerrado	Aw	954**	21.6**
ICA-VC6	Serro – MG	Cerrado and Atlantic forest	Cwa	1471**	20.5**

CC: Climatic Classification; R: Rainfall; T: Temperature; Aw: Tropical weather; Cwa: Subtropical dry winter climate. Source: ****IBGE, 2016; ***Alvares *et al*., 2013; **INMET, 2018; * NASA POWER, 2020

Table No. 2
Data on the average meteorological factors during the reference months collected at the weather station at Montes Claros, Minas Gerais, Brazil

Months	I (h)	R (mm)	T_H (ºC)	T_M (ºC)	T_L (ºC)	R_H (%)
July/13	287,20	0,00	29,37	22,01	15,55	53,09
Ago/13	289,10	0,00	30,10	22,84	16,31	46,52
Sep/13	226,80	37,80	31,49	24,66	19,01	48,88
Oct/13	219,10	72,50	30,34	24,16	19,07	59,60
Nov/13	221,40	196,30	31,09	24,96	20,31	61,53
Dez/13	128,70	414,70	28,55	23,90	20,84	81,01
Jan/14	271,80	25,10	30,82	24,87	19,75	62,04
Feb/14	267,60	12,70	31,79	25,44	20,05	55,84
Mar/14	214,20	76,80	30,58	24,90	20,70	67,53
Apr/14	261,60	35,40	31,18	24,68	19,48	63,08
May/14	281,80	2,00	30,13	22,96	16,76	56,92
June/14	234,80	1,80	28,31	21,44	15,82	60,01

I: Insolation; R: Rainfall; T_H: higher temperature; T_M: Mean temperature; T_L: Lowest temperature; R_H: Relative humidity. Source: INMET, 2018

curassavica. Boraginaceae were collected from three replicates of six accessions, totaling 18 leaves. The collections were conducted at 6 pm, as proposed by Souza *et al*. (2009) in a study conducted at the same location. Next, the samples were stored in plastic bags at -20°C until the extraction of the essential oil. The experiment was conducted for 12 months, from August 2013 to July 2014, with sample collection and removal of the essential oil during the first week of each month.

The samples were first weighed (100 g of fresh material), transferred to 1000 mL volumetric flasks containing 500 mL of distilled water, and subjected to hydrodistillation in a Clevenger system for 160 min (Vaz *et al*., 2006; Souza *et al.*, 2009). The remaining vegetable material from the hydrodistillation was placed in a forced circulation oven at a temperature of 65°C until constant weight. The oil content per plant was calculated considering the dry matter of the sample using the formula below:

$$\text{Essential oil content (\%)} = 100 * \frac{\text{Essential oil matter (g)}}{\text{Dry matter leaves (g)}} \quad (1)$$

Meteorological data were obtained from the local station for correlation with essential oil content.

Chemical characterization of the essential oil

The chemical analysis of the oil was performed at the campus Laboratory of Chemistry by gas chromatography coupled with mass spectrometry (GC-MS). Essential oils of one repetition per accession were used. The analyses were conducted using samples collected in odd months from November 2013 to July 2014, from a total of 27 samples.

The essential oil samples were weighed, diluted in dichloromethane, and transferred to 2 mL vials. Subsequently, they were individually submitted to the GC-MS, in a molten silica capillary column DB-5 MS (30 m × 0.25 mm × 0.25 µm) and helium (flux 1 mL min^{-1}) as a carrier gas. The temperature setting was 60°C to 240°C, with an escalation of 3°C min^{-1}. The system was operated in scan mode (monitoring) with electronic impact (70 eV), in a range of 45 to 550 *m/z*. The standard series of *n*-alkanes was injected under the same conditions for the calculation of the retention index (IR).

Data were analyzed, and the compounds were identified using MSD Chemstation software, together with the library NIST 62. Lib (2012) and by comparing with the information from the literature (Adams, 2017) and the IR. The relative abundance (%) of the total ions corresponding to the compounds was calculated from the peak area of the chromatogram and organized according to the order of elution. The IR was calculated according to Van Den Dool & Kratz (1963).

Statistical analyses

Descriptive statistics were used to monitor variation in inter- and intra-chemical composition. Pearson's correlation analysis was performed between the meteorological variables and essential oil content using means from the months used. A comparison of averages was conducted for the measurements of essential oil content of the accessions (primary factor), across the months (secondary factor), in a completely randomized design and subdivided plot scheme. The means were compared by the Scott-Knott test at p<0.05. The statistical program used was the R software (2013).

For the estimation of the coefficient of variation (CV) for the treatments, the following formula was used:

$$CV_i = \frac{\sqrt{\frac{\sum_{j=1}^{J}\left(\mathrm{M}_{ij} - \mathrm{M}_i\right)^2}{J-1}}}{\bar{M}_i} \quad (2)$$

Where CV_i is the CV associated with the ith genotype (accession), $\bar{M}_{ij}$ is the average obtained for accession i in evaluation j, and $\bar{M}_i$ is the accession average:

$$\bar{M}_i = \frac{\sum_{j=1}^{J}(\bar{M}_{ij})}{J} \quad (3)$$

For the demonstration of the variation between accessions within each month, we used the equation below:

$$CV_i = \frac{\sqrt{\frac{\sum_{j=1}^{I}\left(\bar{M}_{ij} - \bar{M}_J\right)^2}{J}}}{\bar{M}_J} \quad (4)$$

Where CV_i is the CV for jth month, $\bar{M}_{ij}$ is the average obtained for accession i in month j, $\bar{M}_J$ is the average of the jth evaluation:

$$\bar{M}_{ij} = \frac{\sum_i^I (\bar{M}_{ij})}{I} \quad (5)$$

RESULTS AND DISCUSSION

The results of the evaluation of essential oil content of the accessions during the study months are displayed in Table N° 3 and Figure N° 1. A statistically significant difference was observed in the interaction between months and accessions. No accession revealed an annual average of more than 1% for essential oil content.

Table No. 3
Content of essential oil, from August 2013 to July 2014, of six accessions of *Varronia curassavica J*acq. from the Germplasm Bank of the Institute of Agrarian Sciences of the Federal University of Minas Gerais, Montes Claros Campus, Minas Gerais, Brazil

	ICA-VC1	ICA-VC2	ICA-VC3	ICA-VC4	ICA-VC5	ICA-VC6		
Months	Essential oil content (%)						Average	CV (%)
aug/13	0.74bB	0.61bA	1.22aA	0.43bA	0.32bC	0.74bA	0.68	42.48
set/13	1.53aA	1.19aA	0.70bA	0.58bA	0.83bB	0.14cA	0.83	53.43
out/13	1.02aA	1.10aA	0.94aA	0.30bA	0.84aB	0.47bA	0.77	39.95
nov/13	1.16aA	0.65aA	0.80aA	0.54aA	0.36aC	0.50aA	0.67	38.60
dec/13	1.09aA	0.66aA	0.79aA	0.82aA	0.49aC	0.72aA	0.76	23.84
jan/14	1.23aA	0.47aA	0.71aA	0.78aA	0.79aB	0.56aA	0.76	31.86
fev/14	0.44aB	0.88aA	0.82aA	0.79aA	0.68aB	0.68aA	0.72	19.94
mar/14	1.01aA	0.96aA	1.26aA	0.57bA	0.05bC	0.81aA	0.78	49.69
abr/14	0.55aB	0.99aA	1.13aA	0.94aA	1.32aA	1.18aA	0.85	36.69
may/14	0.82aB	0.58aA	0.99aA	0.75aA	0.89aB	0.70aA	0.79	16.76
jun/14	0.71bB	1.03aA	1.35aA	0.69bA	1.40aA	1.11aA	1.05	26.46
jul/14	1.18aA	0.81aA	0.88aA	0.81aA	1.02aB	0.89aA	0.93	14.10
Average	0.96	0.83	0.97	0.67	0.67	0.70		
CV (%)	31.33	26.79	22.17	26.61	53.64	39.25		

Means followed by the same letter, lowercase in rows and upper case in columns, indicate statistically non-significant values according to the Skott-Knott test at *p*<0.05. CV: Coefficient of Variation

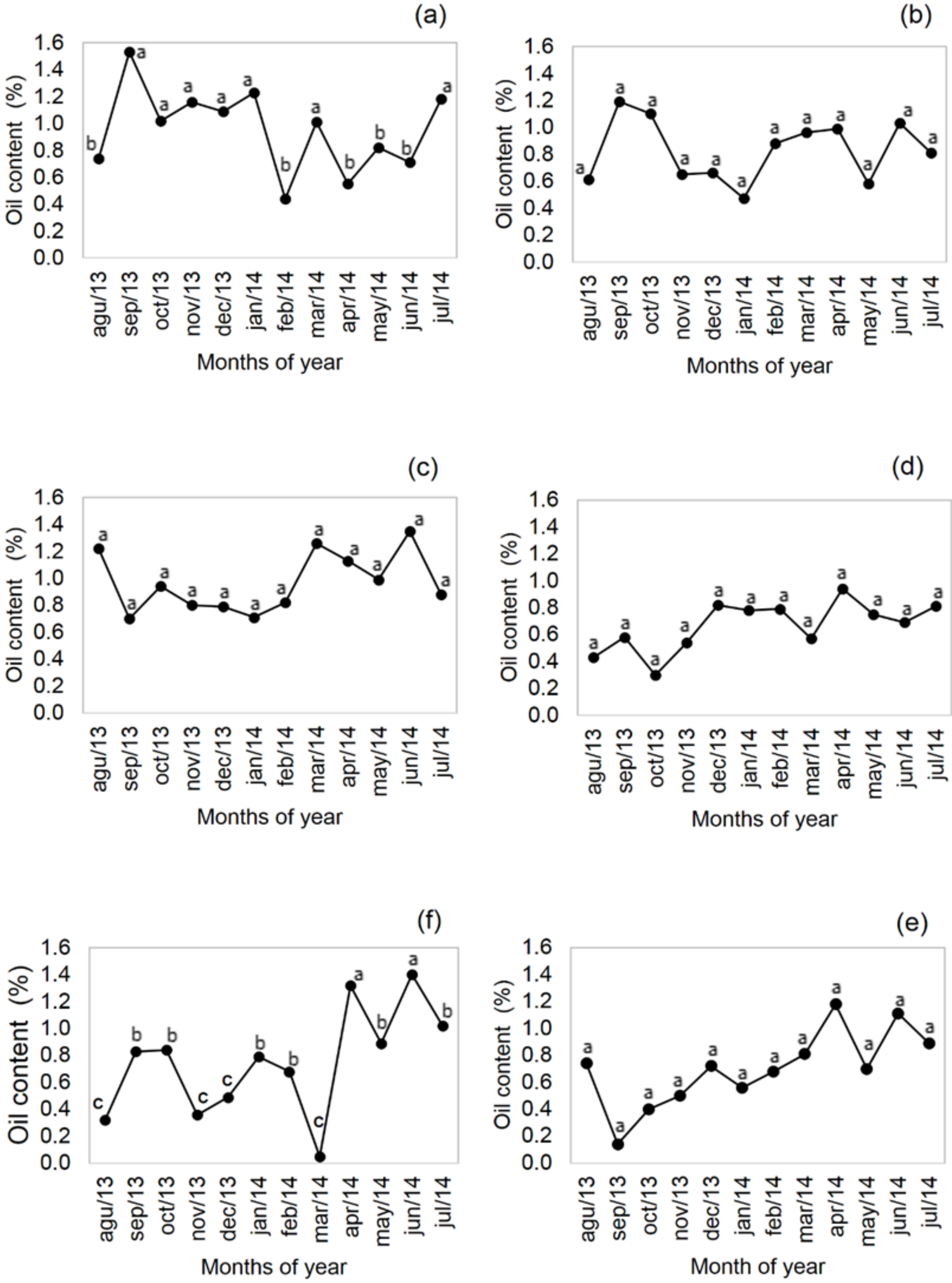

Figure No. 1
Content of essential oil, from August 2013 to July 2014, of six accessions of V*arronia curassavica* Jacq. from the Germplasm Bank of the Institute of Agrarian Sciences of the Federal University of Minas Gerais, Montes Claros Campus, Minas Gerais, Brazil
Coefficient of Variation: 39.14%. Means followed by the same letter indicate statistically non-significant values according to the Skott-Knott test at *p*<0.05

The evaluation of the essential oil content among the accessions showed insignificant differences among the essential oil contents of ICA–VC2, ICA–VC3, and ICA–VC6 extracted during the months in which the samples were analyzed. The analysis during the months of November and December 2013 and January, February, April, May, and July 2014 exhibited non-significant statistical differences regarding the oil content among accessions.

The accession ICA–VC3 presented the highest mean (0.97%) and the lowest annual variation (22.17%) regarding essential oil content. This was the accession with the highest annual stability. On the other hand, the ICA-VC5 accession exhibited the highest annual variation (53.64%), while presenting the lowest annual average (0.67%) among all accessions.

The evaluation of the essential oil content across months showed that the lowest variation (14.10%) and the second-highest average (0.93%) was occurred in July 2014. The highest variation (53.43%) occurred in September 2013, whereas the lowest essential oil content (0.67%) was recorded in November 2013. The averages of essential oil content (0.96–1.53%) were close to those observed by Queiroz *et al*. (2016) (1.04–1.36%) in a study on the ICA-VC4 accession from the same germplasm bank.

The standardized production of secondary metabolites is not typical, and an understanding of variation is essential in recognizing the ecological interactions between plants and their environment. Thus, control of secondary metabolites is desirable when industrial production is targeted (Gobbo-Neto & Lopes, 2007).

Table N° 4 shows the correlations of meteorological factors with the essential oil content of the accessionsof *V. curassavica* from the Germplasm Bank of ICA/UFMG.

Table No. 4
Correlations between meteorological factors and the essential oil content of six accessions of *Varronia curassavica* Jacq. from the Germplasm Bank from the Institute of Agricultural Sciences of the Federal University of Minas Gerais, Montes Claros Campus, Minas Gerais, Brazil

Meteorological factors	ICA-VC1	ICA-VC2	ICA-VC3	ICA-VC4	ICA-VC5	ICA-VC6
I	-0.24	0.48	0.45	-0.3	-0.08	0.02
R	0.25	-0.57	-0.46	0.33	-0.03	-0.13
T_H	-0.27	0.36	0.23	-0.22	-0.26	-0.03
T_M	-0.28	0.03	-0.03	0.13	-0.23	0.03
T_L	-0.21	-0.23	-0.19	0.37	-0.12	0.09
R_H	-0.17	-0.64*	-0.21	0.68*	0.18	0.31

***Significant mean by t-test at p<0.05. I: Insolation; R: Rainfall; T_H: Higher temperature; T_M: Mean temperature; T_L: Lowest temperature; R_H: Relative humidity**

The correlations were significant only between relative humidity and the ICA-VC2 (-0.64) and ICA-VC4 (0.68) accessions. Therefore, although the essential oil content varied during the year, no correlations could be attributed to the monsoon or other meteorological factors observed. This corroborates the seasonality study of *V. curassavica* from the area of Crato – CE, as the essential oil content and chemical compounds differed, and the correlation between essential oil content and precipitation was significant (Matias *et al*., 2016).

Correlation with rainfall may not have been significant because the Germplasm Bank from ICA/UFMG is irrigated and the amount of water supplied was enough to maintain the accessions at an optimal condition for metabolic activity. However, even the water abundance provided by actual rainfall was insufficient to change the behavior of the species.

Low relative humidity and high temperatures could increase volatilization of the essential oil present in the leaves (Brant *et al*., 2008). Under these conditions, at the time of the collection, there may have been loss of oil to the air and the variation was not significant, because there were few chemical compounds and less oil in the leaves. Moreover, according to Brant *et al*. (2008), meteorological factors can, together, induce more substantial changes in the active principles of plants.

A baixa umidade relativa do area alta

temperatura podem aumentar a volatilização do óleo essencial das folhas (Brant *et al.*, 2008). Nessas condições, na hora da coleta, pode ter ocorrido perda do óleo para o ar e a variação não ter sido significativa pelo fato de ter poucos compostos químicos e pouco óleo presente nas folhas. Ainda, segundo esses autores, os fatores meteorológicos, em conjunto, podem induzir a mudanças mais expressivas nos princípios ativos das plantas do que eles isoladamente.

Consequently, even when correlations with individual elements were not statistically different, there was a seasonality influence.

Table No. 5 shows the compounds present and those that are non-existent in the accessions throughout the year. A total of 46 compounds were detected. The two primary chemical markers of the species, α-humulene and β-caryophyllene, were detected in all samples analyzed. Additionally, the compounds β-bourbonene, β-elemene, spathulenol, germacrene, and caryophyllene oxide were detected in all accessions. In the ICA-VC1 accession, 21 compounds were identified; in ICA-VC2, 19 compounds; in ICA-VC3, 31 compounds; in ICA-VC4, 15 compounds; in ICA-VC5, 23 compounds, and in ICA-VC6 accession, 24 compounds.

Table No. 5

Chemical compounds detected from November 2013 to July 2014 in the essential oil from *Varronia curassavica* Jacq. accessions from the Germplasm Bank of the Institute of Agrarian Sciences of the Federal University of Minas Gerais, Montes Claros Campus, Minas Gerais, Brazil

	Compounds	CC	RI_{Cal}	RI_{l}	ICA-VC1	ICA-VC2	ICA-VC3	ICA-VC4	ICA-VC5	ICA-VC6
1	α-Tujene	Mo	925	924	-	-	+	-	-	-
2	α-Pinene	Mo	933	932	+	-	+	+	+	+
3	Sabinene	Mo	971	969	-	-	+	+	+	+
4	β-Pinene	Mo	979	974	-	-	+	+	+	+
5	Mircene	Mo	988	988	-	-	-	-	-	+
6	o-Cymene	Mo	1024	1022	-	-	-	-	+	-
7	Limonene	Mo	1028	1024	-	-	-	-	+	-
8	Eucalyptol	Omo	1031	1026	-	-	+	+	-	-
9	γ-Terpinene	Mo	1057	1054	-	-	-	-	+	-
10	Bornyl acetate	Omo	1282	1284	-	-	-	-	+	-
11	δ-Elemene	Se	1329	1335	+	+	-	-	-	-
12	α-Copaene	Se	1372	1374	+	+	+	-	+	+
13	β-Bourbonene	Se	1380	1375	+	+	+	+	+	+
14	β-Elemene	Se	1387	1389	+	+	+	+	+	+
15	α-Gurjunene	Se	1409	1408	-	-	-	-	-	+
16	β-Caryophyllene	Se	1419	1417	+	+	+	+	+	+
17	Unknown 1		1438			-	+	+	+	-
18	α-Humulene	Se	1451	1452	+	+	+	+	+	+
19	Aromadendrene	Se	1457	1460	+	+	-	-	+	+
20	γ-Muurolene	Se	1471	1478	+	+	-	-	+	-
21	Unknown 2		1478			-	-	-	-	+
22	Germacrene	Se	1478	1484	+	+	+	+	+	+
23	Unknown 3		1484			-	+	-	-	+
24	Unknown 4		1487		-	-	+	-	-	+
25	α-Selinene	Se	1492	1498	-	-	+	-	-	-
26	Unknown 5		1495		+	+	+	+	+	+
27	β-Bisabolene	Se	1504	1505	+	+	+	+	-	-
28	Unknown 6		1511		-	-	-	-	+	-
29	γ-Cadinene	Se	1516	1513	+	+	+	-	+	+
30	Unknown 7		1537		+	+	+	-	-	-
31	Unknown 8		1540		+	+	-	-	-	-

32	Unknown 9		1570		-	-	+	-	+	+
33	Spathulenol	Sea	1572	1577	+	+	+	+	+	+
34	Caryophyllene oxide	Ose	1576	1582	+	+	+	+	+	+
35	Unknown 10		1580		+	+	+	+	-	+
36	Unknown 11		1612		-	-	+	-	-	+
37	Unknown 12		1618		-	-	+	-	-	+
38	Unknown 13		1622		-	-	+	-	+	-
39	Unknown 14		1626		+	-	-	-	-	-
40	α-Cadinol	Sea	1651	1652	+	-	+	-	-	+
41	Unknown 15		1651		-	-	-	-	-	+
42	Unknown 16		1677		-	-	+	-	-	-
43	Unknown 17		1689		-	+	-	-	-	-
44	α-Bisabolol	Sea	1690	1685	+	+	+	-	-	-
45	Unknown 18		1694		-	-	+	-	-	-
46	Unknown 19		1777		-	-	+	-	-	-

Caption: +: present compound. -: absent compound. CC: Chemical Class. RI_{Cal}: Calculated Retention Index. RI_{I}: Retention Index from Adams (2012). Mo: Monoterpenes. Se: Sesquiterpenes. Sea: Sesquiterpene alcohols. Ose: Oxygenated sesquiterpenes. Omo: Oxygenated Monoterpenes. -: Compounds not detected. * Retention index was calculated for all the samples analyzed; however, the value expressed in the table refers to the index derived from the longest retention time obtained

Hernández *et al.* (2014) compared the chemical composition and biological activity of the essential oil of “erva-baleeira” obtained in the dry and rainy seasons using gas chromatography. They found, among other compounds, the presence of α-pinene, germacrene, α-humulene, limonene, α-bisabolol, and myrcene. Feijó *et al.* (2014), when evaluating the effect of solar radiation on essential oil composition of V. curassavica, also reported the presence of the compounds β-pinene, β-elemene, δ-elemene, aromadendrene, β-caryophyllene, α-humulene, spathulenol, and caryophyllene oxide. Matias *et al.* (2016), who studied the seasonality of “erva-baleeira” essential oils, noted compounds, noted the presence of sabinene. Queiroz *et al.* (2016) characterized the essential oil of “erva-baleeira” in samples collected at different intervals and determined chemical markers of the species and other compounds.

The results of the studies cited corroborate those of this research because the same compounds were observed in the samples, but not all of them were detected in all six accessions. It is important to emphasize that the compounds acknowledged as “identified” were those for which the abundance represented at least 1% of the chromatogram peak area. The variation in the content and chemical composition of the essential oil and the correlations presented can be explained by the geographic origin of the material (Sales *et al.*, 2009), because the possible genetic variability, causing individuals of the same species to have different responses, although subjected to the same conditions.

In a study on the species Tournefortia paniculata (Boraginaceae), Moraes & Souza (2007) suggested that meteorological factors influenced the biosynthesis of secondary metabolites and that the routes of biosynthesis of these metabolites could be diverted from the physiological demands of the plants. Bose *et al.* (2013) reported that the phenological stage of the plant interfered with the chemical composition of the essential oils because of the demands of vegetative and reproductive growth.

Temperature can induce variation in the chemical composition of essential oils because of its relationship with the metabolic activities of the plant (Carvalho Filho *et al.*, 2006), whereas precipitation may influence the production of certain compounds (Cerqueira *et al.*, 2009). Therefore, temperature and precipitation may have, together, affected the variation found in the data obtained in this study.

Table No. 6 and Figure No. 2 indicate the behavior of the main chemical compounds that were identified in all accessions, including the primary chemical markers of the species and α-bisabolol, which has anti-inflammatory action (Kamatou & Viljoen, 2010; Rocha *et al.*, 2011).

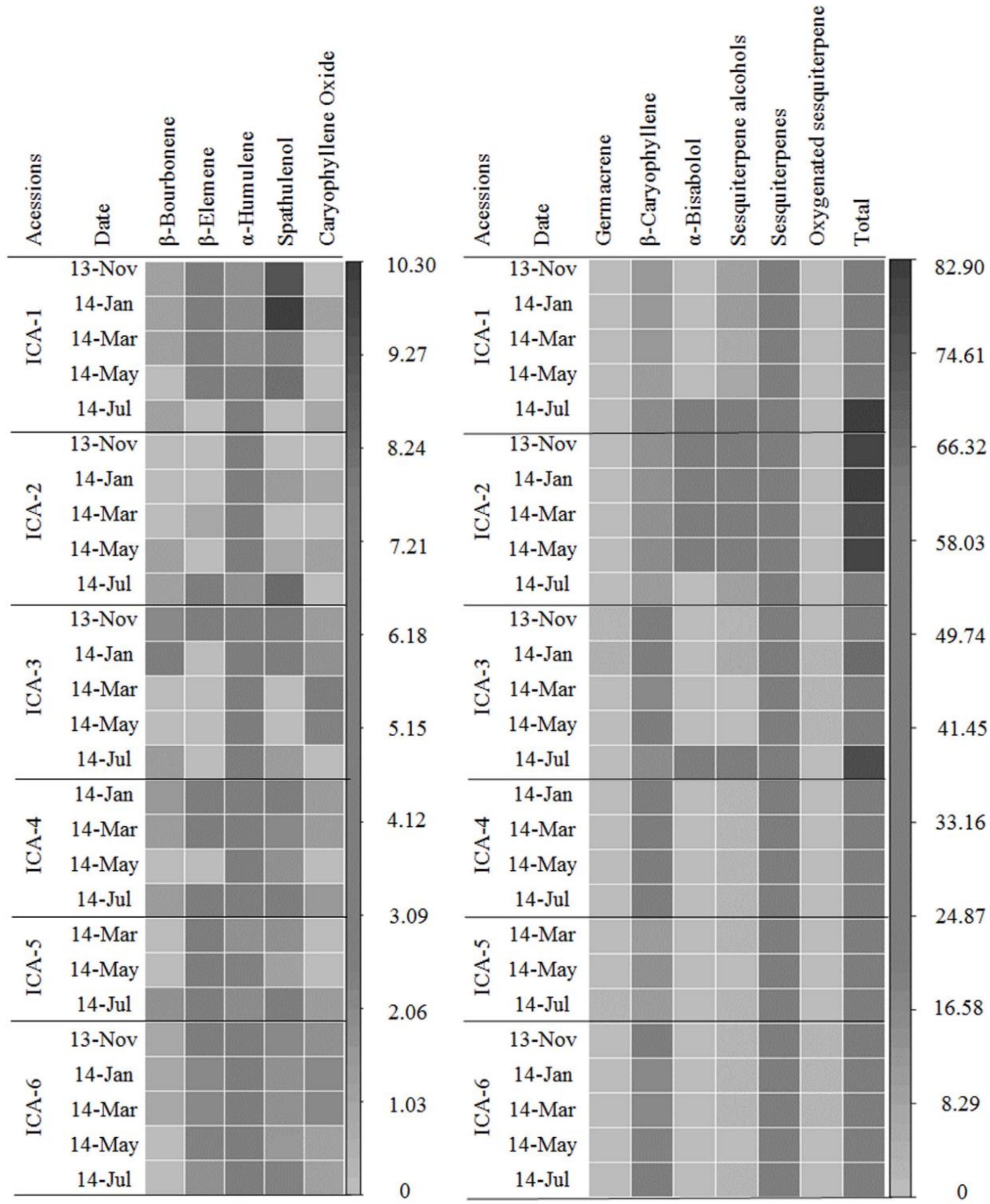

Figure No. 2
Relative abundance (%) of the main chemical compounds detected from November 2013 to July 2014 in the essential oil present in the leaves of *Varronia curassavica* Jacq. from the accessions from the Germplasm Bank from the Institute of Agrarian Sciences of the Federal University of Minas Gerais, Montes Claros Campus, Minas Gerais, Brazil

Table No. 6
Chemical characteristics of the main compounds detected from November 2013 to July 2014 in the essential oil present in the leaves of *Varronia curassavica* Jacq. of the accessions from the Germplasm Bank of the Institute of Agrarian Sciences of the Federal University of Minas Gerais, Montes Claros Campus, Minas Gerais, Brazil

Compounds				ICA- VC1	ICA-VC2	ICA-VC3	ICA-VC4	ICA-VC5	ICA-VC6
		CC	RI_l	RI^*_{Cal}	RI^*_{Cal}	RI^*_{Cal}	RI^*_{Cal}	RI^*_{Cal}	RI^*_{Cal}
1.	β-Bourbonene	Se	1375	1380	1380	1380	1380	1380	1380
2.	β-Elemene	Se	1389	1387	1387	1386	1386	1387	1386
3.	β-Cariofilene	Se	1417	1416	1417	1416	1418	1417	1419
4.	α-Humulene	Se	1452	1451	1451	1451	1451	1451	1451
5.	Germacrene	Se	1484	1477	1477	1478	1477	1477	1478
6.	Spathulenol	Sea	1577	1572	1572	1571	1571	1571	1572
7.	Caryophyllene Oxide	Ose	1582	1576	1575	1576	1575	1576	1576
8.	α-Bisabolol	Sea	1685	1687	1690	1686	-	-	-

CC: Chemical Class. RI_{Cal}: Calculated Retention Index. RI_l: Retention Index from Adams (2017). Se: Sesquiterpenes. Sea: Sesquiterpene alcohols. Ose: Oxygenated sesquiterpenes. -: Compounds not detected. * The retention index was calculated for all samples analyzed; however, the value expressed in the table refers to the index calculated from the longest retention time obtained

β-bourbonene values showed little variation across the months and between accessions, maintaining an abundance between 1.0–1.7%. Only in the ICA-VC3 accession was the compound abundance different, reaching 3.7% in October 2013.

The relative abundance of β-elemene varied between months and accessions. The ICA-VC1 accession presented the highest abundance for this compound in November 2013 (6.5%). A high abundance was also detected in January (6.0%), March (6.2%), and May 2014 (5.4%). In the ICA-VC2 accession, β-elemene was detected in March (1.0%) and July 2014 (5.5%), and in the ICA-VC3 accession, it was detected in November 2013 (2.9%). In the ICA-VC4 accession, β-elemene detection occurred in January (5.5%), March (4.7%), and July 2014 (3.5%). β-elemene was detected in all months analyzed and its abundance varied between 2.7–4.0%. In the accession ICA-VC6, the β-elemene annual variation was between 1.7–2.7%. In the ICA-VC6 accession, germacrene was detected in November 2013 (21.0%), and May (20.3%) and July 2014 (5.7%).

The abundance of caryophyllene oxide remained stable between months and accessions. In the ICA-VC1 accession, this compound was detected in January (1.2%) and July 2014 (1.0%). In the ICA-VC2 accession, it was detected in January (1.0%) and May 2014 (1.2%). In the ICA-VC3 accession, the highest abundance was detected in March 2014 (2.6%). Additionally, in the ICA-VC3 accession, caryophyllene oxide was detected in November 2013 (1.4%), and January (1.8%) and May 2014 (2.3%). In the ICA-VC4 accession, this compound was detected in January (1.4%), March (1.3%), and July 2014 (1.5%). In the ICA-VC5 accession, it was detected in July 2014 (1.4%). Finally, in the ICA-VC6 accession, the compound was detected in all months and the abundance varied between 1.1–2.1%.

α-bisabolol was detected in the ICA-VC1 and ICA-VC3 accessions in July 2014 with abundances of 52.2% and 41.47%, respectively. For the accession ICA-VC2 in 2013, the greatest abundance of the compound was detected in November (57.0%). Additionally, for the ICA-VC2 accession in 2014, the compound was detected in January (57.2%), March (50.1%), and May (47.7%).

The main chemical markers of the species were detected in all the analyzed accessions. The accessions ICA-VC1, ICA-VC2, ICA-VC3, ICA-

VC4, and ICA-VC6 presented an annual average higher than 2.6% of α-humulene, which is the same amount found in the essential oil of the herbal medicine Acheflan (Ache, 2016). Additionally, β-caryophyllene was one of the two most abundant compounds detected in all accessions.

β-caryophyllene, in the ICA-VC1 accession, presented greater abundance (16.2%) in July 2014 and an annual average of 12.9%. α-humulene in the ICA-VC1 accession had a higher relative abundance (5.3%) in May 2014 and an annual average of 2.9%. However, in November 2013 (1.8%) and January (1.9%) and March 2014 (1.9%), the abundance was less than 2.6%. The ICA-VC1 accession exhibited the highest seasonal variation for α-humulene (53.3%) among the accessions.

In the ICA–VC2 accession, β-caryophyllene presented a greater abundance (13.9%) in March 2014 and an annual average of 13.44%. α-humulene displayed a higher abundance (3.9%) in May 2014, and a yearly average of 3.1%. In July 2014 (1.7%), it presented a lower abundance than 2.6%.

In the ICA–VC3 accession, β-caryophyllene presented a greater abundance (22.0%) in January 2014 and an annual average of 19.5%. α-humulene showed a higher abundance (3.4%) in January 2014 and a yearly average of 3.0%. However, in March 2014 (2.5%), abundance was lower than 2.6%.

The ICA–VC4 accession displayed the highest annual average abundance among accessions for β-caryophyllene (27.3%) and α-humulene (5.5%) and the highest significance in annual stability, due to the lower variation in abundance. Furthermore, β-caryophyllene presented a variation of 6.2% and α-humulene, of 5.5%. β-caryophyllene and α-humulene exhibited their highest abundances of 29.4% and 5.8%, respectively, in March 2014.

For the ICA–VC5 accession, the lowest annual average for abundance was observed between accessions for the compounds β-caryophyllene (11.8%) and α-humulene (2.1%). β-caryophyllene and α-humulene exhibited their highest abundance in May 2014 of 14.6% and 2.4%, respectively. The abundance of α-humulene in all months was less than 2.6%.

In the ICA–VC6 accession, β-caryophyllene demonstrated the highest abundance (27.5%) in November 2013, with an annual average of 21.5% and the highest annual variation (21.0%) in abundance among the accessions. Additionally, in November 2013, α-humulene exhibited the highest abundance (3.7%) with an average abundance of 3.3%.

In a study conducted by Vaz *et al*. (2006) on essential oils and chemical markers of improved genotypes of medicinal plants in Altinópolis, São Carlos, Campinas, and Jales, all of which are in the state of São Paulo, 2.38–4.42% α-humulene content in the essential oil of "*erva-baleeira*" were observed. This observation agrees with the values found in the present study, characterizing the essential oil of "*erva-baleeira*" from the *Horto de Plantas Medicinais* (Orchard of Medicinal Plants) of the Regional University of Cariri, Crato – CE. Rodrigues *et al*., (2012) also observed β-caryophyllene, with an abundance of 25.4%, making it one of the most plentiful compounds.

It is important to note that the compound α-bisabolol has been reported to have anti-inflammatory activity, from a reduction in the levels of mediators involved in its inflammatory activity or inhibition of the activation and migration of polymorphonuclear neutrophils that are part of the anti-inflammatory process (Rocha *et al.*, 2011). α-bisabolol can act together with the current chemical markers in the anti-inflammatory action of the species.

For the pharmaceutical industry, it is crucial to maintain constancy in the production of "*erva-baleeira*" because prices of a kilogram of essential oil with at least 2% α-humulene was reported to have reached values over U$ 3000.00 and gained profits between 10–20% (Magalhães, 2010). In addition to the main compounds, it is necessary to evaluate the behavior of the other chemical compounds because they may be related to the quality of the raw material. The maintenance of the quality of the raw material obtained from a herbal source ensures the efficacy of the product (Govindaraghavan *et al.*, 2012) and the generation of higher income for producers.

To be competitive in the international market, the producers of essential oil in Brazil must consider innovative cultivation techniques and select the best plants (Bizzo & Rezende, 2009). Therefore, it is important to monitor the chemical composition of the essential oil of accessions.

CONCLUSIONS

It is reasonable to conclude that seasonality influenced the content and chemical composition of

V. curassavica Jacq. essential oil because parameter variation was observed across months for all accessions. The ICA-VC3 accession was found to be the most stable regarding essential oil content and maintaining an average annual abundance of α-humulene that was greater than 2.6%, which is the amount required for the production of the phytotherapeutic. β-caryophyllene was the only compound that was abundant in all samples. These results support future genetic improvement research on the evaluated accessions, with special emphasis on ICA-VC3. This study demonstrated that it is possible to select materials that improve the production of essential oil and its chemical markers as a function of seasonality, making the production of the species raw materials more efficient and standardized.

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