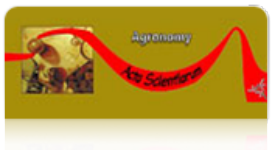

Acta Scientiarum. Agronomy
ISSN: 1807-8621
Editora da Universidade Estadual de Maringá - EDUEM

Chemical diversity of accessions of the *in vivo* germplasm bank of *Varronia curassavica* (Jacq.)

Queiroz, Túlio Barroso; Alves da Fonseca, Francine Souza; Resende Mendes, Aretusa Daniela; Azevedo, Alcinei Mistico; Martins, Ernane Ronie

Chemical diversity of accessions of the *in vivo* germplasm bank of *Varronia curassavica* (Jacq.)
Acta Scientiarum. Agronomy, vol. 42, 2020
Editora da Universidade Estadual de Maringá - EDUEM
Available in: http://www.redalyc.org/articulo.oa?id=303062597002
DOI: 10.4025/actasciagron.v42i1.42726

Chemical diversity of accessions of the *in vivo* germplasm bank of *Varronia curassavica* (Jacq.)

Túlio Barroso Queiroz [1*]
Universidade Estadual Paulista, Brazil
ORCID: http://orcid.org/0000-0002-0208-1225
Francine Souza Alves da Fonseca [2]
Universidade Estadual de Campinas, Brazil
Aretusa Daniela Resende Mendes [3]
Universidade Federal de Minas Gerais, Brazil
Alcinei Mistico Azevedo [3]
Universidade Federal de Minas Gerais, Brazil
Ernane Ronie Martins [3]
Universidade Federal de Minas Gerais, Brazil

Acta Scientiarum. Agronomy, vol. 42, 2020

Editora da Universidade Estadual de Maringá - EDUEM

Received: 28 December 2017
Accepted: 16 March 2018

DOI: 10.4025/actasciagron.v42i1.42726

ABSTRACT. : *Varronia curassavica* (Jacq.) is a shrub and perennial plant native to Brazil that has therapeutic, anti-inflammatory and cicatrizing properties. The objective of this work was to study the chemical diversity of the native populations of this species maintained in the *in vivo* germplasm bank from Montes Claros, Minas Gerais, Brazil. The essential oils of 10 accessions analyzed in triplicate were obtained by hydrodistillation. Fifty-five compounds were detected, 46 of which were identified by GC-MS analysis. Essential oil yield ranged from 0.1 (VC-9) to 1.22% (VC-3) among the studied accessions. Three groups were formed by cluster analysis. Group A presented lower relative abundance of the compounds α-humulene (1.4%) and β-caryophyllene (8.5%). Conversely, in Group B, the most abundant compound was α-humulene (31.6%). Group C, with the greatest number of accessions, presented values of up to 6.1 and 41.2% for α-humulene and β-caryophyllene, respectively. Only two accessions (VC-1 and VC-5) presented α-humulene content lower than that recommended for phytotherapeutic production. These results, along with consolidating breeding programs, contribute to the conservation of the species.

Keywords: essential oil, medicinal plant, chemotype, α-humulene, β-caryophyllene.

Introduction

Varronia curassavica (Jacq.) [(synonym *Cordia verbenaceae* DC and *Cordia curassavica* (Jacq.) Roem. & Schult (Miller & Gottschling, 2007; Passos et al., 2007)] belongs to the family Boraginaceae and is not endemic to Brazil; however, it is observed in the phytogeographical domains of the Amazon, Caatinga, Cerrado and Atlantic Forest, with confirmed occurrences in 20 Brazilian states (Stapf, 2015).

This shrub species presents tannins, flavonoids and essential oils in the shoots (Fernandes et al., 2007). The major compounds of the essential oils are α-pinene, β-caryophyllene, β-elemene, α-

humulene, bicyclogermacrene and δ-cadinene (Carvalho, Rodrigues, Sawaya, Marques, & Shimizu, 2004). The economic and pharmacological potential of these essential oils is due to the production of the chemical markers β-caryophyllene and α-humulene (Figueira, Risterucci, Zucchi, Cavallari, & Noyer, 2009), which have spicy and woody tastes (Hampel, Monsandl, & Wüst, 2005) and which are associated with cytotoxicity and anti-proliferative, pro-apoptotic and anti-tumor activities (Parisotto et al., 2012).

Within the same population, essential oil presents differences in chemical composition due to biological activities of the plant (Eisenman, Juliani, Struwe, & Simon, 2013). This variation within the same species has been reported by several researchers (Rao, Rajput, & Mallavarapu, 2011; Karousou, Hanlidou, & Lazari, 2012; Verma, Padalia, Chauhan, & Thul, 2013).

Genetic and chemical diversities between populations are explained by geographic isolation, polymorphisms and environmental conditions (Djabou et al., 2012; Xie, Huang, Yang, & Lei, 2012). Abiotic factors, such as climate, influence secondary metabolism, especially in sesquiterpenes and monoterpenes (Feijó, Oliveira, & Costa, 2014).

Thus, conservation of species in germplasm banks can capture a large proportion of the common allelic variation present within the species of interest (Whitlock, Hipperson, Thompson, Butlin, & Burke, 2016). Therefore, the broad genetic base allows for the development of new varieties (Marfil, Hidalgo, & Masuelli, 2015), in addition to contributing to the conservation of the species. Moreover, wild plant cultivation under human selection boosts the transition to domestication by selecting genotypes adapted to human needs (Martínez-Ainsworth & Tenaillon, 2016).

The objective of this study was to analyze the chemical diversity between accessions from native populations of an *in vivo* germplasm bank of *V. curassavica* and to select plants with higher content of α-humulene and β-caryophyllene.

Material and methods

Establishment of the germplasm bank

For the collection of *Varronia curassavica* accessions, expeditions occurred between October 2011 and January 2012 in the central, north and high Jequitinhonha regions of the state of Minas Gerais and in the south region of the state of Bahia, Brazil (Figure 1). Plant material (10 accessions) was collected and propagated by cutting and remained in the greenhouse until seedling formation. Seedlings were transplanted into a planting pit (30 x 30 x 30 cm), spaced 1 x 0.5 m apart, with applications of natural phosphate and bovine manure. The collection was maintained *in vivo* under drip irrigation, and the experimental area (16º 40' 58.5" S, 43º 50' 25.6" W, at 626 m a.s.l., in Montes Claros, Minas Gerais, Brazil) was subsequently micro-sprayed.

Exsiccates of *V. curassavica* are maintained in the herbarium of ICA/UFMG. In addition, exsiccates were sent to the Institute of Biosciences, Languages, and Exact Sciences of the Sao Paulo State University (Ibilce/Unesp) for taxonomic identification by Prof. Dr. Neusa Taroda Ranga.

Extraction and analysis of essential oil

Leaves of 10 *Varronia curassavica* accessions (40 g each) were collected in triplicate and at standardized times (18 hours), since essential oil content is not influenced by collection time (Queiroz, Mendes, Silva, Fonseca, & Martins, 2016). Samples were kept in the refrigerator (8°C) until the extraction of essential oil by hydrodistillation in the Clevenger apparatus (2 hours) in a round bottom flask (1,000 mL) containing 500 mL of distilled water. At the end of the extraction process, the essential oil was collected, weighed and dried with anhydrous sodium sulfate, and it was stored in a freezer (-20°C) in amber flasks.

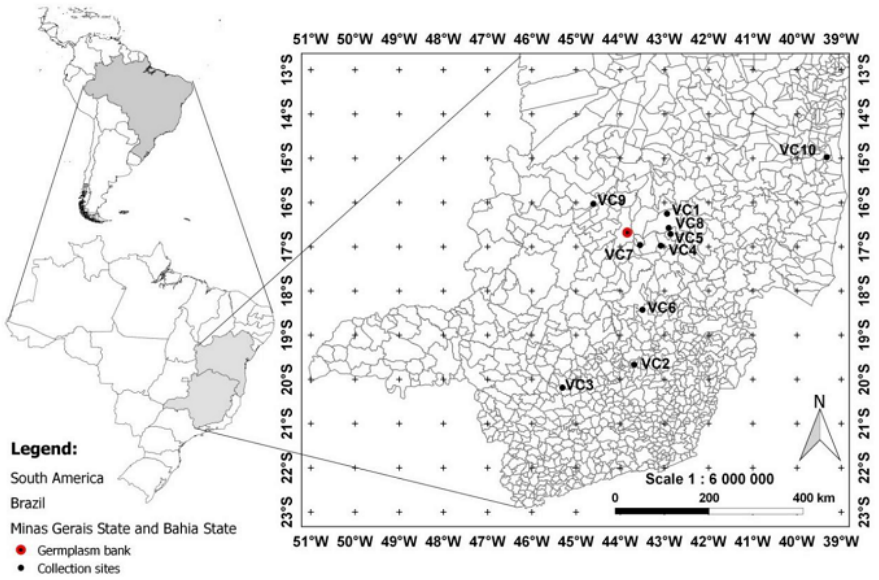

Figure 1

Location of the *Varronia curassavica in vivo* germplasm bank of the Institute of Agricultural Sciences of Federal University of Minas Gerais (ICA/UFMG). Identification of collection sites: Minas Gerais State = VC 1 - Grão Mogol (Americana Settlement), VC 2 - Taquaraçu de Minas, VC 3 - Santo Antônio do Monte, VC 4 - Itacambira, VC 5 - Cristália, VC 6 - São Gonçalo do Rio das Pedras, VC 7 - Juramento, VC 8 - Grão Mogol, VC 9 - São Francisco and Bahia State, VC 10 - São José da Vitória.

For the analysis of the chemical composition of the essential oil, samples were diluted in dichloromethane (1 mg mL^{-1}), transferred to vials (2 mL) and analyzed individually by gas chromatography coupled to mass spectrometry (GC-MS). The Agilent Technologies system (7890A) was coupled to a mass spectrometer (MS 5975C) with an HP-5 MS capillary column (30 m x 0.25 mm x 0.25 μm) and helium (1 mL $min.^{-1}$) as the drag gas. The injector temperature was 220°C, with a split ratio of 1:5. The oven temperature was programmed to 60 - 240°C (3°C $min.^{-1}$) for 10 min. The

interface temperature was 240°C. The MS data (total ion chromatogram, TIC) were acquired in full scan mode (m z^{-1} of 40-350) at a scan rate of 0.3 scan/s using electron ionization (EI) with an electron energy of 70 eV. The retention index was calculated from the retention time of a mixture of n-alkanes (C7-C40, Sigma USA) at 20 ppm, with a flow rate of 1:100.

Data were analyzed using the MSD ChemStation software together with the NIST library (National Institute of Standards and Technology [NIST], 2011). The relative abundance (%) of the compounds was calculated from the peak area of the chromatogram and organized according to the order of elution. The percentage of each compound was obtained from the normalized area of each chromatogram, with mean values for each compound. The identification of β-caryophyllene and α-humulene was performed by co-injection of a synthetic standard (Sigma Aldrich), and other compounds were identified by comparison of the mass spectra with that of the NIST library. The relative retention index (RI) of each compound was calculated according to Van Den Dool and Kratz (1963) and compared with information in the literature (Adams, 2007).

The chromatograms obtained in the experiment were evaluated individually to verify normal deviations, such as contaminants, excessively broad peaks and low abundances. Afterward, chromatograms were subjected to automatic integration in the MSD ChemStation software (E.02.02.1431, Agilent Technologies), which considered all peaks with abundance greater than 0.1%. The compounds were then adjusted based on retention time, and relative abundance of each chromatographic peak was used in the chemometric analyses after calculating the logarithm and centering on the mean. The logarithms of the absolute areas were used in the construction of the statistical models in order to reduce the overvaluation of the major compounds.

Statistical analysis

All statistical analyses were carried out using the free software R Project (R Core Team, 2014). Data correspond to the mean area of total ion chromatogram obtained by the GC-MS (in triplicate) of the volatile compounds extracted by hydrodistillation. These data were subjected to Pearson's correlation analyses using the 'color' function of the 'stats' package. For the multivariate analysis, the Euclidean distance was estimated by means of the 'dist' function; the hierarchical cluster analysis (HCA) was performed by the UPGMA method (Unweighted Pair Group with Arithmetic Mean) by means of the 'hclust' function; and the principal component analysis was performed by the 'princomp' function. All of the functions belong to the 'stats' package.

Results and discussion

The yields of the essential oil obtained from the leaves of *V. curassavica* varied from 0.1 (CV-9) to 1.22% (CV-3) among the accessions. This variation in the essential oil content of plants of different origins is related to their geographical origins, which in turn is also associated with their chemical compositions (Mechergui et al., 2010).

According to the chemical analyses, 55 compounds were detected by GC-MS in the essential oil samples from the 10 *V. curassavica* accessions, of which 46 compounds were identified. The compounds α-pinene (2), β-caryophyllene (17), α-humulene (20), caryophyllene oxide (42), and humulene epoxide II (45) were common to all the accessions analyzed in the experiment. Table 1 shows the variation in the chemical composition of the essential oil of the accessions from *V. curassavica* germplasm bank, highlighting the chemical diversity within the same species.

The analysis of the chemical composition of the essential oils presented β-caryophyllene and α-humulene in all the accessions, but with variations between them. β-Caryophyllene in the accessions VC-3 (26.2 ± 1.5%), VC-4 (27.3 ± 2.3%), VC-6 (22.9 ± 3.1), VC-7 (27.8 ± 0.1), and VC-9 (41.2 ± 0.2) presented higher area values than in the other accessions. VC-8 differed from the other accessions in relation to α-humulene (31.6 ± 0.1%). In addition to the two major chemical compound characteristics of the species (β-caryophyllene and α-humulene), other compounds such as bicyclogermacrene (VC-1, 39.6 ± 1.3%), bisabolol (VC-2, 33.1 ± 28,4%), α-pinene (VC-5, 14.5 ± 3.2%), and caryophyllene oxide (CV-10, 26.8 ± 0.2%) also were evident in the analysis.

Table 1

Chemical composition of essential oil extracted from the leaves of *Varronia curassavica* from the germplasm bank of the Institute of Agricultural Sciences of UFMG (ICA-UFMG).

N°	Compounds	RT	RI[1]	RI[2]	% area (TIC) of the accession in the germplasm bank										
					VC-1	VC-2	VC-3	VC-4	VC-5	VC-6	VC-7	VC-8	VC-9	VC-10	TI
					Monoterpenes										
01	α-thujene	5.3	950	925	-	-	0.6 ± 0.8	-	0.4 ± 0.1	-	-	-	-	1.0 ± 1.0	a,b
02	α-pinene	5.5	959	932	-	0.1 ± 0.0	5.0 ± 0.6	7.4 ± 1.5	14.5 ± 3.2	6.2 ± 1.1	11.1 ± 0.0	2.3 ± 0.0	0.5 ± 0.0	0.4 ± 0.0	a,b
03	sabinene	6.6	975	972	-	-	-	0.8 ± 0.1	1.3 ± 0.4	0.3 ± 0.1	0.1 ± 0.0	-	0.3 ± 0.0	0.4 ± 0.0	a,b
04	β-pinene	6.7	979	978	-	0.2 ± 0.1	-	3.4 ± 0.6	4.7 ± 1.0	5.9 ± 2.8	3.2 ± 0.0	1.2 ± 0.0	1.3 ± 0.0	0.2 ± 0.0	a,b
05	β-myrcene	7.0	991	989	-	-	8.8 ± 6.5	0.3 ± 0.1	0.9 ± 0.2	0.7 ± 0.4	1.1 ± 0.0	-	2.0 ± 0.0	-	a,b
06	p-cymene	8.2	1026	1024	-	0.2 ± 0.3	1.2 ± 0.5	-	6.8 ± 1.4	-	-	-	-	0.3 ± 0.0	a,b
07	limonene	8.3	1029	1028	-	-	0.4 ± 0.5	0.2 ± 0.0	3.0 ± 0.8	0.3 ± 0.1	0.2 ± 0.0	-	-	0.3 ± 0.0	a,b
					Oxygenated Monoterpenes										
08	eucalyptol	8.4	1031	1032	-	-	0.5 ± 0.1	1.0 ± 0.3	1.5 ± 0.4	0.2 ± 0.1	0.3 ± 0.0	0.2 ± 0.0	0.8 ± 0.0	1.2 ± 0.0	a,b
09	γ-terpinene	9.3	1062	1056	-	-	0.2 ± 0.2	-	7.0 ± 2.1	-	-	-	-	-	a,b
					Sesquiterpenes										
10	δ-elemene	20.5	1338	1328	1.5 ± 0.1	-	0.1 ± 0.0	0.5 ± 0.0	-	0.1 ± 0.0	-	-	-	-	a,b
11	α-cubebene	21.2	1351	1343	-	-	0.1 ± 0.1	-	0.1 ± 0.0	0.1 ± 0.1	-	-	-	0.2 ± 0.0	a,b
12	α-copaene	22.4	1377	1372	2.6 ± 0.0	1.7 ± 0.9	0.9 ± 0.9	1.0 ± 0.1	3.4 ± 0.2	1.8 ± 0.5	0.6 ± 0.0	-	0.3 ± 0.3	11.6 ± 0.3	a,b
13	β-bourbonene	22.7	1380	1379	1.4 ± 0.1	1.9 ± 1.2	1.3 ± 1.2	1.8 ± 0.4	1.7 ± 0.3	1.3 ± 0.4	1.0 ± 0.0	0.2 ± 0.0	0.4 ± 0.0	1.7 ± 0.0	a,b
14	β-cubebene	22.9	1390	1384	-	-	0.9 ± 1.1	0.1 ± 0.0	0.9 ± 0.1	0.3 ± 0.0	0.1 ± 0.1	-	-	2.2 ± 0.0	a,b
15	β-elemene	23	1391	1386	4.81 ± 0.4	1.5 ± 0.8	1.5 ± 0.8	3.7 ± 0.4	2.4 ± 0.1	1.8 ± 0.0	1.1 ± 0.1	3.2 ± 0.1	2.1 ± 0.0	1.5 ± 0.0	a,b
16	α-cedrene	23.6	1412	1403	-	-	-	-	-	0.1 ± 0.2	0.2 ± 0.2	3.1 ± 0.0	-	0.2 ± 0.0	a,b
17	β-caryophyllene	24.2	1419	1416	8.5 ± 0.8	21.0 ± 13.5	26.2 ± 1.5	27.3 ± 2.3	10.8 ± 1.8	22.9 ± 3.1	27.8 ± 0.1	15.3 ± 0.0	41.2 ± 0.2	9.3 ± 0.2	a,b,c
18	β-gurjunene	24.6	1432	1425	0.2 ± 0.0	0.5 ± 0.4	0.4 ± 0.3	0.4 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.3 ± 0.0	0.8 ± 0.0	0.2 ± 0.0	1.5 ± 0.0	a,b
19	β-farnesene	25.1	1446	1437	-	0.1 ± 0.0	1.0 ± 0.8	4.5 ± 0.2	1.8 ± 0.9	0.4 ± 0.1	-	-	-	0.2 ± 0.0	a,b
20	α-humulene	25.6	1455	1451	1.4 ± 0.1	4.6 ± 2.2	4.1 ± 0.4	5.1 ± 0.5	1.8 ± 0.4	3.4 ± 0.5	3.9 ± 0.0	31.6 ± 0.1	6.1 ± 0.0	4.1 ± 0.1	a,b,c
21	alloaromadendrene	25.8	1460	1455	2.7 ± 0.0	1.8 ± 2.9	0.2 ± 0.1	0.8 ± 0.0	1.2 ± 0.2	5.3 ± 3.4	10.5 ± 0.1	0.6 ± 0.0	0.4 ± 0.0	0.5 ± 0.0	a,b
22	γ-gurjunene	26.5	1477	1471	1.1 ± 0.0	0.1 ± 0.0	0.6 ± 0.3	0.1 ± 0.0	2.0 ± 0.2	0.5 ± 0.2	0.7 ± 0.0	0.1 ± 0.0	3.0 ± 0.0	9.0 ± 0.0	a,b
23	γ-muurolene	26.7	1477	1476	9.9 ± 0.1	9.9 ± 3.5	2.5 ± 2.2	10.8 ± 0.5	4.6 ± 1.2	15.3 ± 4.8	2.7 ± 0.0	1.3 ± 0.0	5.3 ± 0.1	0.2 ± 0.0	a,b
24	unknown	26.8	-	1480	-	-	-	-	-	3.1 ± 1.2	-	0.4 ± 0.0	2.9 ± 0.1	-	-
25	δ-selinene	27	1485	1483	-	-	8.5 ± 6.5	-	-	-	-	-	-	-	a,b
26	β-selinene	27.0	1490	1484	0.2 ± 0.0	0.3 ± 0.2	6.6 ± 2.7	0.4 ± 0.0	0.2 ± 0.0	3.3 ± 1.0	1.1 ± 0.0	0.2 ± 0.0	7.0 ± 0.1	2.2 ± 0.0	a,b
27	α-alasken	27.3	1498	1491	-	4.8 ± 0.7	5.9 ± 6.5	16.2 ± 1.1	4.6 ± 1.4	6.1 ± 0.2	2.4 ± 0.0	3.4 ± 0.0	3.4 ± 0.0	1.3 ± 0.0	a,b
28	bicyclogermacrene	27.4	1500	1494	39.6 ± 1.3	0.9 ± 0.9	0.4 ± 0.2	0.2 ± 0.0	0.4 ± 0.0	0.3 ± 0.0	1.0 ± 0.0	-	0.3 ± 0.0	1.3 ± 0.0	a,b
29	β-bisabolene	27.8	1506	1505	-	1.7 ± 0.6	0.4 ± 0.2	0.9 ± 0.1	-	0.4 ± 0.1	0.2 ± 0.0	4.6 ± 0.0	0.5 ± 0.0	-	a,b
30	sesquicineol	28.0	1516	1509	0.1 ± 0.0	0.7 ± 0.0	0.2 ± 0.1	0.1 ± 0.0	-	0.2 ± 0.1	2.4 ± 0.0	0.6 ± 0.0	0.2 ± 0.0	-	a,b
31	cubebol	28.1	1518	1511	0.6 ± 0.0	-	0.5 ± 0.4	0.4 ± 0.0	4.7 ± 0.3	0.6 ± 0.2	2.5 ± 0.0	0.9 ± 0.0	1.1 ± 0.0	1.9 ± 0.0	a,b
32	δ-cadinene	28.2	1523	1515	8.3 ± 0.3	1.9 ± 1.8	1.1 ± 0.5	0.6 ± 0.0	7.9 ± 0.8	1.2 ± 0.2	2.9 ± 0.0	-	1.3 ± 0.0	0.5 ± 0.0	a,b
33	γ-bisabolene	28.5	1531	1523	-	0.3 ± 0.0	-	-	-	-	0.2 ± 0.0	2.1 ± 0.0	-	-	a,b
34	cadina-1(2),4-diene	28.7	1535	1528	0.1 ± 0.0	-	-	-	-	-	0.8 ± 0.1	-	-	-	a,b
35	1, 4,7, -cyclododecatriene,1,5,9,9-tetramethyl	29.1	1567	1536	-	1.9 ± 0.6	0.2 ± 0.2	-	-	-	0.2 ± 0.0	-	-	0.6 ± 0.0	a,b
					Oxygenated Sesquiterpenes										
36	sesquisabinene hydrate	29.2	1544	1541	-	1.2 ± 0.4	0.1 ± 0.2	-	-	-	0.5 ± 0.0	-	-	0.2 ± 0.0	a,b
37	unknown	29.4	-	1545	-	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.0	-	0.1 ± 0.0	0.6 ± 0.0	-	0.1 ± 0.1	1.4 ± 0.0	-
38	unknown	29.7	-	1551	-	0.3 ± 0.0	0.8 ± 0.5	-	-	0.4 ± 0.1	0.3 ± 0.0	1.0 ± 0.0	-	0.3 ± 0.0	-
39	unknown	29.9	-	1557	-	0.1 ± 0.1	0.2 ± 0.2	0.1 ± 0.0	-	0.3 ± 0.1	0.2 ± 0.0	1.5 ± 0.0	-	0.5 ± 0.0	-
40	unknown	30.3		1567	-	0.1 ± 0.1	3.5 ± 3.2	-	2.5 ± 0.2	1.5 ± 0.5	0.2 ± 0.0	-	0.1 ± 0.0	0.2 ± 0.0	-
41	spathulenol	30.4	1578	1571	12.5 ± 1.1	0.9 ± 0.4	1.8 ± 2.1	3.4 ± 1.5	2.6 ± 0.8	2.5 ± 0.8	5.3 ± 0.1	5.8 ± 0.1	2.6 ± 0.0	3.0 ± 01	a,b
42	caryophyllene oxide	30.6	1585	1577	0.4 ± 0.1	2.0 ± 1.6	3.4 ± 1.1	1.9 ± 0.7	1.9 ± 0.5	2.7 ± 1.1	10.2 ± 0.0	7.0 ± 0.1	9.8 ± 0.1	26.8 ± 0.2	a,b
43	unknown	30.8		1580	2.9 ± 0.1	0.3 ± 0.1	0.9 ± 0.6	1.7 ± 0.2	0.7 ± 0.1	0.6 ± 0.5	1.3 ± 0.0	0.5 ± 0.0	-	1.0 ± 0.0	-
44	epiglobulol	30.9	1585	1583	-	0.2 ± 0.1	0.3 ± 0.1	-	0.2 ± 0.1	0.3 ± 0.1	0.1 ± 0.0	3.1 ± 0.1	-	0.4 ± 0.2	a,b
45	humulene epoxide II	31.7	1609	1603	0.5 ± 0.0	0.7 ± 0.2	0.5 ± 0.1	0.6 ± 0.2	0.4 ± 0.1	0.5 ± 0.1	1.0 ± 0.0	0.9 ± 0.0	1.0 ± 0.0	0.5 ± 0.1	a,b
46	humulane-1,6-dien-3-ol	32.0	1619	1612	-	0.3 ± 0.1	0.3 ± 0.3	0.1 ± 0.1	0.3 ± 0.1	3.1 ± 1.2	0.5 ± 0.0	0.9 ± 0.0	3.3 ± 0.0	0.5 ± 0.1	a,b
47	selina-6-en-4-ol	32.2	-	1617	-	-	0.1 ± 0.1	0.2 ± 0.0	0.2 ± 0.3	1.9 ± 0.8	0.1 ± 0.0	0.3 ± 0.0	-	0.3 ± 0.1	a,b
48	unknown	32.3	-	1621	1.1 ± 0.0	0.5 ± 0.0	1.5 ± 1.3	0.2 ± 0.0	1.5 ± 1.3	0.3 ± 0.1	0.7 ± 0.0	0.4 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	-
49	unknown	32.7	-	1631	-	0.7 ± 0.6	0.2 ± 0.2	0.8 ± 0.3	0.7 ± 0.2	0.2 ± 0.1	1.4 ± 0.0	0.3 ± 0.0	1.2 ± 0.0	1.8 ± 0.1	-
50	cubenol	32.9	1640	1637	0.2 ± 0.0	0.1 ± 0.1	1.4 ± 2.2	0.7 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	2.4 ± 0.0	0.4 ± 0.0	0.8 ± 0.0	0.5 ± 0.0	a,b
51	unknown	33.1	-	1642	0.2 ± 0.0	0.9 ± 0.3	0.7 ± 0.1	0.5 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	1.3 ± 0.0	2.7 ± 0.0	0.5 ± 0.0	2.0 ± 0.1	-
52	α-cadinol	33.4	1654	1650	1.1 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.5 ± 0.1	0.2 ± 0.2	0.6 ± 0.1	3.9 ± 0.1	0.5 ± 0.0	0.6 ± 0.0	0.5 ± 0.0	a,b
53	eudesm-7(11)-en-4-ol	33.6	1676	1654	-	0.5 ± 0.1	2.1 ± 1.4	0.2 ± 0.1	-	1.1 ± 0.3	0.2 ± 0.0	0.4 ± 0.0	0.8 ± 0.0	0.5 ± 0.3	a,b
54	calamene-10-ol	33.8	1661	1659	0.3 ± 0.0	0.4 ± 0.1	0.2 ± 0.2	0.4 ± 0.1	0.4 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.5 ± 0.0	0.2 ± 0.0	1.5 ± 0.1	a,b
55	unknown	34.1	-	1668	0.3 ± 0.0	0.3 ± 0.1	0.3 ± 0.3	0.1 ± 0.1	0.4 ± 0.1	0.1 ± 0.0	0.5 ± 0.0	0.9 ± 0.0	-	2.3 ± 0.1	-
56	bisabolene	34.8	1689	1687	0.4 ± 0.0	33.1 ± 28.4	0.2 ± 0.1	0.3 ± 0.0	-	0.2 ± 0.1	0.3 ± 0.0	0.2 ± 0.0	0.3 ± 0.1	0.1 ± 0.2	a,b
57	unknown	34.9	-	1691	0.2 ± 0.0	0.8 ± 0.1	-	-	-	-	-	-	-	-	-
58	unknown	36.3	-	1727	-	-	0.3 ± 0.4	0.1 ± 0.1	-	-	0.2 ± 0.1	-	-	2.2 ± 0.2	-
59	unknown	39.1	-	1808	-	-	-	-	-	-	-	-	-	1.7 ± 0.1	-
	identified compounds (%)				95.3	95.9	91.5	96.3	94.0	92.8	93.4	91.5	94.8	90.2	
	unknown compounds (%)				4.7	4.1	8.5	3.7	6.0	7.2	6.6	8.5	5.2	9.8	
	monoterpenes (%)				-	0.5	16	12.1	31	13.,5	5.7	3.5	4.1	2.6	
	oxygenated monoterpenes (%)				-	-	0.7	1.0	8.5	0.2	0.3	0.2	0.8	1.2	
	sesquiterpenes (%)				80	55.6	63.6	74.9	48.2	65.8	62.9	67.9	73.5	41.8	
	oxygenated sesquiterpenes (%)				15.3	40.4	11.1	8.3	6.3	13.3	24.7	19.9	16.4	44.6	
	Total				100	100	100	100	100	100	100	100	100	100	

IT: identification type; (-): compound not detected; a: identification based on the retention index, b: identification based on the mass spectrum (70 eV), c: substance identified by the co-injection of the commercial synthetic standard; RT: retention time in minutes; RI1: retention index of the literature, RI2: retention index calculated. Mean values of the area of the total ion chromatogram (TIC), followed by the respective standard deviation (±).

Such behavior can be explained by changes in secondary metabolism, which favors the biosynthesis of volatile compounds due to the induction of specific enzymes (Venditti et al., 2015). Although essential oil biosynthesis is determined by genetics, the content and proportion of chemical constituents of essential oils are intensely affected by geographical origin (Santos, Pinto, Santos, Cruz, & Arrigoni-Blank, 2016).

Five native populations of *V. curassavica* from the northeast of Brazil present differences in the relative abundances of chemical compounds of the essential oil (Farhat, Jordán, Chaouch-Hamada, Landoulsi, & Sotomayor, 2016). Differences in β-caryophyllene (1.67 to 22.25%) and α-humulene (0.00 to 10.32%) were found in this research and are the result of the influence of genetic factors and environmental conditions. These findings occur due to polymorphisms and geographic locations, which are responsible for the chemical diversity of the essential oil, since it is associated with adaptations as a survival strategy.

Although climate, soil orders (Rahimmalek, Heidari, Ehtemam, & Mohammadi, 2017) and water availability (Maatallah, Nasri, Hajlaoui, Albuch, & Elaissi, 2016) alter the secondary metabolism of plants, the effects of genetic factors on essential oil composition are greater than the effects of environmental factors (Moghaddam & Farhadi, 2015). In this study, the chemical composition of the essential oil of leaves of different *V. curassavica* genotypes subjected to the same culture conditions was analyzed. Researchers have evaluated the content and chemical composition of twenty-one accessions of *Acorus calamus* from different geographic origins grown under the same environmental conditions and have also noted a variation in the number of compounds present in the essential oil (Kumar et al., 2016).

Figure 2 shows the correlation for the 15 most abundant compounds in all accessions; green and red lines are positive and negative correlations, respectively, and their correlation intensity is expressed by the thickness of the line that connects each compound. Thus, copaene (C10), bicyclogermacrene (C11), δ-cadinene (C04) and α-humulene (C09) are strongly associated, which may be a consequence of a common metabolic pathway.

The dendrogram of Figure 3 classifies the essential oils of 10 *Varronia curassavica* populations based on the Euclidian distance, presenting the groups similar to each other in relation to the chemical compositions of the essential oil. The hierarchical cluster analysis revealed three principal groups, classified as A, B, and C. Group A was formed by accession VC-1; group B was formed by the accession VC-8; and group C was formed by the other accessions (VC-2, VC-3, VC-4, VC-5, VC-6, VC-7, VC-9, VC-10).

The identification of several chemotypes in a single species is valuable for the commercial propagation and cultivation for domestication and

breeding programs, since it allows for identification of divergent groups by the chemical composition (Fattahi, Nazeri, Kalantari, Bonfil, & Fattahi, 2016). For this species, five groups were characterized with the following major compounds: turmerone (8.96-30.15%) and β-caryophyllene (4.6-20.17%) for group 1; tricyclene (22.20-35.95%) and camphene (16.62-27.38%) for group 2; α-azingiberene (24.81-35.83%) and β-sesquiphellandrene for group 3; E-caryophyllene (3.90-31.06%) and 7-cyclodecen-1-ona,7-methyl-3-methylene-10-(1-propyl) for group 4; and finally 7-cyclodecen-1-ona,7-methyl-3-methylene-10-(1-propil) (24.68-50.20%) as the major compound for group 5 (Nízio et al., 2015). These differences are possibly related to survival strategies for the different cultivation conditions, since the studied genotypes are in constant evolution. The state of Sergipe is similar to the north of the state of Minas Gerais; however, the latter is a complex region, due to regional topography and maximum altitudes between 700 and 800 m asl (Alvares, Sentelhas, Gonçalves, & Sparovek, 2013).

The principal component analysis notes the most important characteristics in the data set. This technique revealed that four principal components explain 82.58% of the variation in the chemical composition of the essential oil from *V. curassavica* (Table 2). The first principal component (PC1) explained 28.71% of the variation. The compounds of this group presented significant positive correlation for α-humulene (0.79) and negative correlation for copaene (-0.92) and bicyclogermacrene (-0.95). The second principal component (PC2) explained 24.45% of the variation among the studied accessions, with positive correlation for spathulenol (0.77) and negative correlation for α-pinene (-0.66), terpinene (-0.66), cymene (-0.8), and α-alaskene (-0.63).

The distribution of the 10 *V. curassavica* accessions analyzed is presented in Figure 4. PCA confirmed the relationships between the accessions; the interrelationship between the chemical compounds grouped those that presented similarities. This figure shows the same tendency of dendrogram grouping, since VC-1 and VC-8 accessions formed groups isolated from the other groups. In addition, VC-3 and VC-10 are almost overlapping, indicating that they are quite similar. The arrows indicate the compounds with the highest correlation with PC1 and PC2, which are the most important components in the study.

Table 3 shows the similarity between the three groups formed by the dendrogram. *V. curassavica* accessions were highlighted in Group A for 6 chemical compounds: muurolene (C01), α-pinene (C02), β-caryophyllene (C03), δ-cadinene (C04), cymene (C06), and alloaromadendrene (C07). Group B presented α-pinene (C02), caryophyllene (C03) and δ-cadinene (C04), with absence of bicyclogermacrene (C11). Group C had α-pinene (C02), β-caryophyllene (C03), and δ-cadinene (C04), while α-humulene (C9) and myrcene (C14) were absent.

Monoterpenes, diterpenes and sesquiterpenes are produced by terpene synthases (TPs) that use geranyl diphosphate (GDP) farnesyl diphosphate (FDP) and geranylgeranyl diphosphate (GGDP),

respectively (Cheng et al., 2007). The chemical diversity among these compounds may be related to the ability of a single TP protein to form several terpenes from a single substrate. Furthermore, these authors note that the formation of several products could be a consequence of the greater conformational flexibility in the enzyme's active site, allowing the formation of reaction intermediates (Degenhardt, Jöllner, & Gershenzon, 2009).

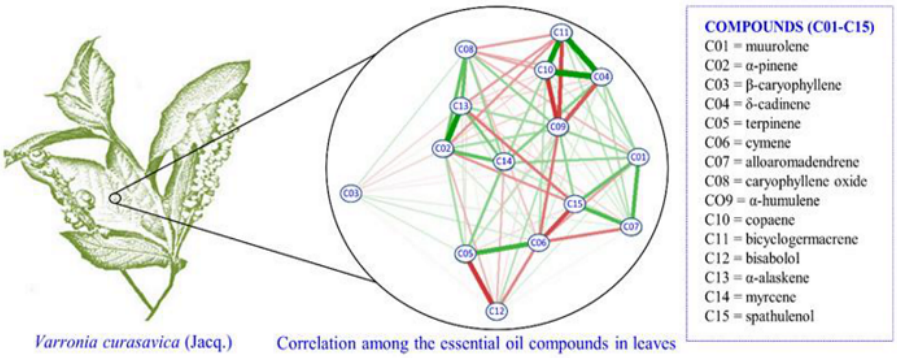

Figure 2
Diagram of Pearson's correlation coefficients matrix among the fifteen major essential oil compounds (C01-C15) of the analyzed *Varronia curassavica* populations.

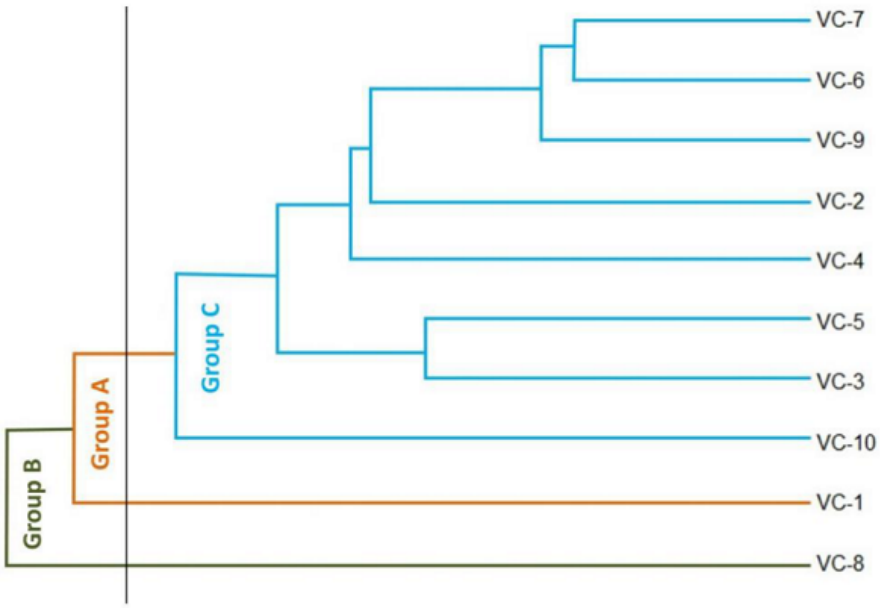

Figure 3
Dendrogram representing the similarity between 10 *Varronia curassavica* accessions based on the chemical composition of the essential oils obtained by hydrodistillation and analyzed by gas chromatography coupled to mass spectrometry.

Table 2

Correlation between component loadings for the first four principal components and 15 major essential oil compounds (C01-C15) of the analyzed *Varronia curassavica* populations.

Compound	N°	Principal component			
		PCA1	PCA2	PCA3	PCA4
muurolene	C01	-0.22^{ns}	0.45^{ns}	0.70*	0.13^{ns}
α-pinene	C02	0.47^{ns}	-0.66*	0.55^{ns}	-0.13^{ns}
β-caryophyllene	C03	-0.01^{ns}	-0.03^{ns}	-0.15^{ns}	0.42^{ns}
δ-cadinene	C04	-0.91*	-0.06^{ns}	0.33^{ns}	-0.18^{ns}
terpinene	C05	-0.27^{ns}	-0.66*	0.21^{ns}	0.62^{ns}
cymene	C06	-0.32^{ns}	-0.80*	-0.29^{ns}	0.21^{ns}
alloaromadendrene	C07	-0.21^{ns}	0.59^{ns}	0.56^{ns}	0.16^{ns}
caryophyllene oxide	C08	0.59^{ns}	-0.26^{ns}	0.33^{ns}	-0.24^{ns}
α-humulene	C09	0.79*	0.33^{ns}	0.39^{ns}	0.14^{ns}
copaene	C10	-0.92*	-0.17^{ns}	0.19^{ns}	-0.3^{ns}
bicyclogermacrene	C11	-0.95*	0.06^{ns}	0.15^{ns}	-0.23^{ns}
bisabolol	C12	0.19^{ns}	0.55^{ns}	-0.13^{ns}	-0.62^{ns}
α-alasken	C13	0.48^{ns}	-0.63*	0.49^{ns}	-0.29^{ns}
myrcene	C14	-0.13^{ns}	-0.32^{ns}	0.88*	-0.03^{ns}
spathulenol	C15	-0.01^{ns}	0.77*	0.35^{ns}	0.45^{ns}
Eigenvalue		4.31	3.67	2.83	1.58
% of Variance		28.71	24.45	18.86	10.56

Significance is indicated as follows: *significance at 5% nominal level; **significance at 1% nominal level; nsnot significant by the t-test.

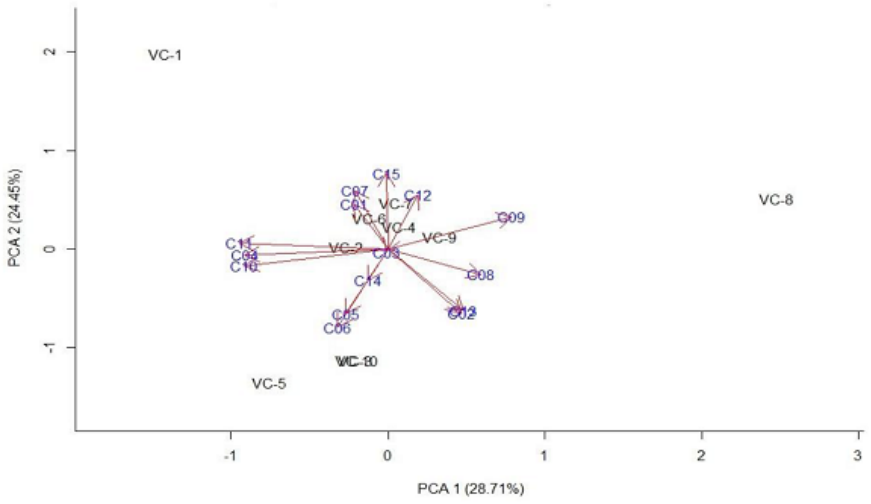

Figure 4

Distribution of the chemical components of the essential oil of the *V. curassavica* germplasm in relation to the two principal components by the principal component analysis (PCA).

Table 3
Content of the 15 principal components of essential oil (C01-C15) for the three groups (A, B, C) found in 10 *Varronia curassavica* accessions.

Compound	N°	Content [%]		
		A	B	C
muurolene	C01	8.74 ± 1.69	8.63 ± 0.69	8.44 ± 0.35
α-pinene	C02	8.16 ± 3.43	9.36 ± 0.83	9.32 ± 0.39
β-caryophyllene	C03	8.16 ± 3.43	9.36 ± 0.83	9.32 ± 0.39
δ-cadinene	C04	8.16 ± 3.43	9.36 ± 0.83	9.32 ± 0.39
terpinene	C05	6.68 ± 3.2	8.12 ± 0.69	4.74 ± 4.11
cymeno	C06	8.06 ± 1.3	7.61 ± 1.44	7.17 ± 0.3
alloaromadendrene	C07	8.06 ± 1.3	7.61 ± 1.44	7.17 ± 0.3
caryophyllene oxide	C08	7.1 ± 3.47	8.68 ± 0.52	8.16 ± 0.34
α-humulene	C09	2.72 ± 3.59	6.07 ± 1.92	0 ± 0.00
copaene	C10	7.1 ± 3.47	8.68 ± 0.52	8.16 ± 0.34
bicyclogermacrene	C11	4.57 ± 3.64	0 ± 0.00	7.93 ± 0.33
bisabolol	C12	5.89 ± 3.27	8.98 ± 0.9	7.09 ± 0.41
α-alasken	C13	7.1 ± 3.47	8.68 ± 0.52	8.16 ± 0.34
myrceno	C14	2.72 ± 3.59	6.07 ± 1.92	0 ± 0.00
spathulenol	C15	5.89 ± 3.27	8.98 ± 0.9	7.09 ± 0.41

Contents of the main essential oil components are represented as the means ± standard deviations.

The biosynthesis of isoprenoids (terpenoids) is important for the production of precursors, such as triose phosphate and pyruvate, which may have favored methylerritol phosphate (MEP) to assume functions of both the photosynthetic tissues and secondary metabolism under stress conditions (Hemmerlin, Harwood, & Bach, 2012). Little information is found in genomic databases regarding the specific metabolic pathways of the synthesis of these compounds in trichomes and the genes involved in their developmental stages (Tiwari, 2016).

In general, metabolic pathways control the production of compounds synthesized for cell growth and survival, as well as interactions with the environment, including responses to biotic and abiotic factors. The reduction or absence of the major compounds of interest (β-caryophyllene/α-humulene) is an example of such a situation. The significant decline in β-caryophyllene expression and α-humulene synthesis activity is significantly influenced by heat stress conditions (Pazouki et al., 2016).

Conclusion

The present study reveals chemical diversity in the composition of the essential oil obtained from leaves of the *in vivo* germplasm bank of *V. curassavica*. These differences enabled the separation of the accessions into three groups, where the biosynthesis of compounds influenced the relative abundance of the essential oil compounds. Knowledge of the chemical diversity of *V. curassavica* plants in Minas Gerais State and neighboring regions allow for breeding programs enhancement and generation of pharmaceutical products, with consequent improvement in the raw material production chain.

Acknowledgements

The authors thank the Laboratory of Instrumental Chemistry and the Laboratory of Medicinal and Aromatic Plants of the ICA-UFMG; the *Programa de Educação Tutorial* (PET-Agronomia of the ICA-UFMG); and the Minas Gerais State Foundation for Research Support (Fapemig) for providing scholarships and resources to develop this study

References

Adams, R. P. (2007). *Identification of essential oils components by gas chromatography: quadrupole mass spectroscopy* (4nd ed). Chicago, IL: Allured Pub Corp.

Alvares, C. A., Sentelhas, P. C., Gonçalves, J. L. M., & Sparovek, G. (2013). Köppen's climate classification map for Brazil. *Meteorologische Zeitschrift*, *22*(6), 1-18. DOI: 10.1127/0941-2948/2013/0507

Carvalho Jr, P. M., Rodrigues, R. F. O., Sawaya, A. C. H. F., Marques, M. O. M., & Shimizu, M. T. (2004). Chemical composition and antimicrobial activity of the essential oil of *Cordia verbenacea* D.C. *Journal of Ethnopharmacology*, *95*(2-3), 297-301. DOI: 10.4103/0974-8490.99080

Cheng, A.-X., Xiang, C.-Y., Yang, C.-Q., Hu, W.-L., Wang, L.-J., Lou, Y.-G., & Chen, X.-Y. (2007). The rice (E)-β-caryophyllene synthase (OsTPS3) accounts for the major inducible volatile sesquiterpenes. *Phytochemistry*, *68*(12), 1632-1641. DOI: 10.1016/j.phytochem.2007.04.008

Degenhardt, J., Jöllner, T. G., & Gershenzon, J. (2009). Monoterpene and sesquiterpene synthases and the origin of terpene skeletal diversity in plants. *Phytochemistry*, *70*(15-16), 1621-1637. DOI: 10.1016/j.phytochem.2009.07.030

Djabou, N., Muselli, A., Allali, H., Dib, M. E. A., Tabjti, B., Varesi, L., & Costa, J. (2012). Chemical a genetic diversity of two Mediterranean subspecies of *Teucriumpolium* L. *Phytochemistry*, *83*, 51-62. DOI: 10.1016/j.phytochem.2012.05.019

Eisenman, S. W., Juliani, H. R., Struwe, L., & Simon, J. E. (2013). Essential oil diversity in North American wild tarragon (*Artemisia dracunculus* L.) wich comparison to French and Kyrgyz tarragon. *Industrial Crops and Products*, *49*, 220-232. DOI: 10.1016/j.indcrop.2013.04.037

Farhat, M. B., Jordán, M. J., Chaouch-Hamada, R., Landoulsi, A., & Sotomayor, J. A. (2016). Phenophase effects on sage (*Salvia officinalis* L.) yield and composition of essential oil. *Journal of Applied Research on Medicinal and Aromatic Plants*, 3(3), 1-7. DOI: 10.1016/j.jarmap.2016.02.001

Fattahi, B., Nazeri, V., Kalantari, S., Bonfil, M., & Fattahi, M. (2016). Essential oil variation in wild-growing populations of Salvia reuterana Boiss. collected from Iran: using GC-MS and multivariate analysis. *Industrial Crops and Products*, *81*, 180-190. DOI: 10.1016/j.indcrop.2015.11.061

Feijó, E. V. R. S., Oliveira, R. A., & Costa, L. C. F. B. (2014). Light affects *Varronia curassavica* essential oil yield by increasing trichomes frequency. *Revista Brasileira de Farmacognosia*, *24*(5), 516-523. DOI: 10.1016/j.bjp.2014.10.005

Fernandes, E. S., Passos, G. F., Medeiros, R., Cunha, F. M., Ferreira, J., Campos, M. M., ... Calixto, J. B. (2007). Anti-inflammatory effects of compounds alpha-humulene and (−)-*trans*-caryophyllene isolated from the essential oil of *Cordia verbenacea*. *European Journal of Pharmacology*, *569*(3), 228-236. DOI: 10.1016/j.ejphar.2007.04.059

Figueira, G.-M., Risterucci, A.-M., Zucchi, M. I., Cavallari, M. M., & Noyer, J.-L. (2009). Development and characterization of microsatellite markers for *Cordia verbenacea* (Boraginaceae), an important medicinal species from the Brazilian coast. *Conservation Genetics*, *11*(3), 1127-1129. DOI: 10.1007/s10592-009-9897-x

Hampel, D., Monsandl, A., & Wüst, M. (2005). Biosynthesis of mono- and sesquiterpenes in carrot roots and leaves (*Daucus carota* L.): metabolic cross talk of cytosolic mevalonate and plastidial methylerythritol phosphate pathways. *Phytochemistry*, *66*(3), 305-311. DOI: 10.1016/j.phytochem.2004.12.010

Hemmerlin, A., Harwood, J., & Bach, T. J. (2012). A raison *d'être* for two distinct pathways in the early steps of plant isoprenoid biosynthesis? *Progress in Lipid Research*, *51*(2), 95-148. DOI: 10.1016/j.plipres.2011.12.001

Karousou, R., Hanlidou, E., & Lazari, D. (2012). Essential-oil diversity of three *Calamintha Species* from greece. *Chemistry & Biodiversity*, 9(7), 1364-1374. DOI: 10.1002/cbdv.201100262

Kumar, R., Sharma, S., Sharma, S., Kumari, A., Kumar, D., Nadda, G., ... Kumar, N. (2016). Chemical composition, cytotoxicity and insecticidal activities of Acorus calamus accessions from the western Himalayas. *Industrial Crops and Products*, *94*, 520-527. DOI: 10.1016/j.indcrop.2016.09.024

Maatallah, S., Nasri, N., Hajlaoui, H., Albuch, A., & Elaissi, A. (2016). Evaluation changing of essential oil of laurel (*Laurus nobilis* L.) under water deficit stress conditions. *Industrial Crops and Products*, *91*, 170-178. DOI: 10.1016/j.indcrop.2016.07.001

Marfil, C. F., Hidalgo, V., & Masuelli, R. W. (2015). In situ conservation of wild potato germplasm in Argentina: Example and possibilities. *Global Ecology and Conservation*, 3, 461-476. DOI: 10.1016/j.gecco.2015.01.009

Martínez-Ainsworth, N., & Tenaillon, M. I. (2016). Superheroes and masterminds of plant domestication La domestication des plantes : une affaire de super-héros et demasterminds. *Comptes Rendus Biologies*, *339*(7-8), 268-273. DOI: 10.1016/j.crvi.2016.05.005

Mechergui, K., Coelho, J. A., Serra, M. C., Lamine, S. B., Boukhchina, S., & Khouja, M. (2010). Essential oils of *Origanum vulgare* L. subsp. glandulosum (Desf.) Ietswaart from Tunisia: chemical composition and antioxidant activity. *Journal of the Science of Food and Agriculture*, *90*(10), 1745-1749. DOI: 10.1002/jsfa.4011

Miller, J. S., & Gottschling, M. (2007). Generic classification in the Cordiaceae (Boraginales): resurrection of the genus *Varronia* P. Br. *Táxon*, *56*(1), 163-169. DOI: 10.2307/25065747

Moghaddam, M., & Farhadi, N. (2015). Influence of environmental and genetic factors on resin yield, essential oil content and chemical composition of Ferula assa-foetida L. populations. *Journal of Applied Research on Medicinal and Aromatic Plants*, 2(3), 69-76. DOI: 10.1016/j.jarmap.2015.04.001

National Institute of Standards and Technology [NIST]. (2011). *NIST/ EPA/NIH Mass Spectral Library with Search Program (Data Version: NIST 11, Software Version 2.0g.)*. Gaithersburg, MD: NIST. Retrieved from November 10, 2017 on Retrieved from November 10, 2017 on https://www.nist.gov/sites/default/files/documents/srd/NIST1a11Ver2-0Man.pdf

Nízio, D. A. C., Brito, F. A., Sampaio, T. S., Melo, J. O., Silva, F. L. S., GagliardI, P. R., ... Blank, A. F. (2015). Chemical diversity of native populations of *Varronia curassavica* Jacq. and antifungal activity against *Lasiodoplodia theobromae*. *Industrial Crops and Products*, *76*, 347-448. DOI: 10.1016/j.indcrop.2015.07.026

Parisotto, E. B., Michielin, E. M. Z., Biscaro, F., Ferreira, S. R. S., Wilhelm Filho, D., & Pedrosa, R. C. (2012). The antitumor activity of extracts from *Cordia verbenacea* D.C. obtained by supercritical fluid extraction. *The Journal of Supercritical Fluids*, *61*, 101-107. DOI: 10.1016/j.supflu.2011.08.016

Passos, G. F., Fernades, E. S., Cunha, F. M., Ferreira, J., Pianowski, L. F., Campos, M. M., & Calixto, J. B. (2007). Anti-inflammatory and anti-allergic properties of the essential oil and active compounds from *Cordia verbenacea*. *Journal of Ethnopharmacology*, *110*(2), 323-333. DOI: 10.1016/j.jep.2006.09.032

Pazouki, L., Kanagendran, A., Li, S., Kännaste, A., Memari, H. R., Bichele, R., & Niinemets, U. (2016). Mono- and sesquiterpene release from tomato (*Solanum lycopersicum*) leaves upon mild and severe heat stress and through recovery: From gene expression to emission responses. *Environmental and Experimental Botany*, *132*, 1-15. DOI: 10.1016/j.envexpbot.2016.08.003

Queiroz, T. B., Mendes, A. D. R., Silva, J. C. R. L., Fonseca, F. S. A., & Martins, E. R. (2016). Teor e composição química do óleo essencial de erva-baleeira (Varronia curassavica Jacq) em função dos horários de coleta. *Revista Brasileira de Plantas Medicinais*, *18*(1), 356-362. DOI: 10.1590/1983-084X/15_116

R Core Team. (2014). *A language and environment for statistical computung, 3.3*. Vienna, AT: R Core Team.

Rahimmalek, M., Heidari, E. F., Ehtemam, M. H., & Mohammadi, S. (2017). Essential oil variation in Iranian Ajowan (*Trachyspermum ammi* (L.) Sprague) populations collected from different geographical regions inrelation to climatic factors. *Industrial Crops and Products*, *95*, 591-598. DOI: 10.1016/j.indcrop.2016.11.017

Rao, B. R. R., Rajput, D. K., & Mallavarapu, G. R. (2011). Chemical diversity in curry leaf (*Murrayan koeningii*) essential oils. *Food Chemistry*, *126*(3), 989-994. DOI: 10.1016/j.foodchem.2010.11.106

Santos, C. P., Pinto, J. A. O., Santos, C. A., Cruz, E. M. O., & Arrigoni-Blank, M. F. (2016). Harvest time and geographical origin affect the essential oil of *Lippia gracilis* Schauer. *Industrial Crops and Products*, *79*, 205-210. DOI: 10.1016/j.indcrop.2015.11.015

Stapf, M. N. S. (2015). *Varronia in lista de espécies da flora do Brasil*. Rio de Janeiro, RJ: Jardim Botânico do Rio de Janeiro. Retrieved on November 1, 2017 from Retrieved on November 1, 2017 from http://floradobrasil.jbrj.gov.br/jabot/floradobrasil/FB105435

Tiwari, P. (2016). Recent advances and challenges in trichome research and essential oil biosynthesis in *Mentha arvensis* L. *Industrial Crops and Products*, *82*, 141-148. DOI: 10.1016/j.indcrop.2015.11.069

Van Den Dool, H., & Kratz, D. J. (1963). A generalization of the retention index system incluing linear temperature programmed gas-liquid partition chromatography. *Journal of Chromatography*, *11*, 463-467. DOI: 10.1016/S0021-9673(01)80947-X

Venditti, A., Blanco, A., Frezza, C., Conti, F., Bini, L. M., Giuliani, C., ... Maggi, F. (2015). Essential oil composition, polar compounds, glandular trichomes and biological activity of *Hyssopus* officinalis subsp.*aristatus* (Godr.) Nyman from central. *Industrial Crops and Products*, *77*(C), 353-363. DOI: 10.1016/j.indcrop.2015.09.002

Verma, R. S., Padalia, R. C., Chauhan, A., & Thul, S. T. (2013). Exploring compositional diversity in the essential oils of 34 *Ocimum* taxa from Indian flora. *Industrial Crops and Products*, *45*, 7-19. DOI: 10.1016/j.indcrop.2012.12.005

Whitlock, R., Hipperson, H., Thompson, D. B. A., Butlin, R. K., & Burke, T. (2016). Consequences of in-situ strategies for the conservation of plant genetic diversity. *Biological Conservation*, *203*, 134-142. DOI: 10.1016/j.biocon.2016.08.006

Xie, Y., Huang, Q., Yang, F., & Lei, C. (2012). Chemical variation in essential oil of Cryptomeria fortune form various areas of China. *Industrial Crops and Products*, *36*(1), 308-312. DOI: 10.1016/j.indcrop.2011.10.023

Author notes

*

Author for correspondence. E-mail: tulio-bq@hotmail.com