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BIOLOGY

Production and essential oil quality of *Varronia curassavica* DC. submitted to different spacing between plants, harvest season and drying temperatures of leaves

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ABSTRACT: *Varronia curassavica* is a Brazilian native medicinal species. Among the critical points influencing the phytochemical quality of bioactive compounds is the spacing between plants, harvest and post-harvest. This research aimed evaluated the influence of plant distance, harvest season, and leaves drying temperature on the yield and phytochemical quality of *V. curassavica* essential oil. The organic cultivation was carried out in 2018/2019 using 0.6 x 1.0; 0.8 x 1.0; 1.0 x 1.0; 1.0 x 1.6 m spacing between plants. The macro and micronutrient contents of the leaves were evaluated and no considerable changes were observed. In 2018 the harvest was performed in summer, autumn, and winter, and the harvested leaves were immediately submitted to the drying process at 40 °C. In 2019 the harvest was performed in winter, and the leaves were submitted to the drying process at 40, 50, and 60 °C. The essential oil was extracted by hydrodistillation and the chemical constituents were evaluated using CG-MS. The essential oil yield was significantly higher in winter and used 0.8 x 1.0 m and 1.0 x 1.0 m spacing between plants. The alpha-humulene content remained within the recommended standards at all analyzed temperatures. Although, the drying temperatures tested did not compromise the alpha-humulene content, the increasing temperature caused a reduction in the essential oil yield. Thus, it is recommended for the organic cultivation of *V. currassavica* the spacing of 0.8 x 1.0 m and 1.0 m x 1.0 m, and the drying of its leaves between 40 and 50 °C to earn the highest essential oil yield and phytochemical quality.
Key words: medicinal plants, post-harvest, volatile compounds, erva-baleeira.

Produção e qualidade do óleo essencial de *Varronia curassavica* DC. submetida à diferentes espaçamentos entre plantas, épocas de colheita e temperaturas de secagem das folhas

RESUMO: *Varronia curassavica* DC. é uma espécie medicinal nativa do Brasil. Dentre os pontos críticos que influenciam a qualidade fitoquímica dos compostos bioativos destacam-se o espaçamento entre plantas, a colheita e a pós-colheita. Este trabalho teve como objetivo avaliar a influência do espaçamento entre plantas, da época de colheita e da temperatura de secagem das folhas na produtividade e na qualidade fitoquímica do óleo essencial de *V. curassavica*. O cultivo orgânico foi realizado em 2018/2019 utilizando-se os seguintes espaçamentos entre plantas: 0.6 x 1.0; 0.8 x 1.0; 1.0 x 1.0; 1.0 x 1.6 m. Os teores de macro e micronutrientes das folhas foram avaliados e não se observou alterações consideráveis. Em 2018, as colheitas foram realizadas no verão, outono e inverno, sendo as folhas colhidas imediatamente submetidas ao processo de secagem a 40 °C. Em 2019, a colheita foi realizada no inverno, sendo as folhas submetidas ao processo de secagem a 40, 50 e 60 °C. O óleo essencial foi extraído por hidrodestilação e os constituintes químicos identificados CG-MS. A produção de óleo essencial foi significativamente maior no inverno e nos espaçamentos de 0,8 x 1,0 m e 1,0 x 1,0 m entre plantas. O teor de alfa-humuleno manteve-se dentro dos padrões recomendados em todas as temperaturas analisadas. Embora as temperaturas de secagem testadas não tenham comprometido o teor de alfa-humuleno, o aumento da temperatura ocasionou redução no rendimento do óleo essencial. Assim, recomenda-se o cultivo orgânico de *V. currassavica* no espaçamento entre 0,8 x 1,0 m e 1,0 m x 1,0 m, e a secagem de suas folhas entre 40 e 50 °C visando obter o maior rendimento e melhor qualidade fitoquímica de óleo essencial.
Palavras-chave: plantas medicinais, pós-colheita, compostos voláteis, erva-baleeira.

INTRODUCTION

Varronia curassavica DC belongs to the Boraginaceae family and has as synonymous: *Cordia curassavica* (Jacq.) Roem. & Schult. and *Cordia verbenacea* Jacq. The plant features shrub architecture, is widely distributed throughout Brazil (STAPF, 2013), and is commonly known in Brazilian-

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Portuguese as: *erva-baleeira*, *catinga-de-barão*, *cordia*, *erva-balieira*, *balieira-cambará*, *erva-preta*, *maria-preta*, *maria-milagrosa*, *salicina*, *catinga-preta*, *maria-rezadeira*, *camarinha* e *camaramoneira-do-brejo* (MAGALHÃES, 2014).

V. curassavica is traditionally used as an anti-inflammatory (LORENZI & MATOS, 2008) due to its essential oil constituents stored in their leaves, highlighting the alpha-humulene (FERNANDES, 2007). This specie was included in the National Registry of Medicinal Plants of Interest to the Unified Health System (SUS) (RENISUS) considering its therapeutic potential (BRASIL, 2009).

The quality and yield of medicinal compounds in plants are directly related to the management or cultivation system, harvest season, drying process, and vegetal drug obtainment. With regard to the cultivation of medicinal plants, the spacing between plants is a factor that must be taken into account. This is due to the fact that it is directly related to the greater or lesser irradiance in the leaves, which influences the development of the plant as a whole, as well as the formation of glandular trichomes, structures where essential oils are secreted and stored (FEIJÓ et al., 2014).

Another important parameter for obtaining quality medicinal plants is the harvest season. CASTELLANI et al. (2006) reported that knowledge of the occurrence of seasonal variability in the production of active compounds is one of the main parameters to consider when planning crops from wild populations of native or domesticated medicinal plants to guarantee the quality of the raw material and; consequently, of the secondary metabolites present in the plant.

Medicinal plants have different stages of development during the seasons. These variations can generate changes in the chemical profile of the essential oil, compromising the quality of the bioactive compound, as well as impacting the biological activity of the species (PARKI et al., 2017; PRINSLOO & NOGEMANE, 2018). The influence of harvest season on chemical composition; and therefore, on bioactivity may be due to climatic changes such as temperature, soil, humidity, rainfall, as well as different stages of plant development (PARKI et al., 2017; PRINSLOO & NOGEMANE, 2018).

As previously described, the secondary metabolites production, responsible for medicinal plants' therapeutic activities, can suffer various factors such as seasonality, temperature, brightness, water availability, fertilization, pest or disease attack, and genotypes (GOBBO-NETO & LOPES, 2007; MATIAS et al., 2016; PRINSLOO & NOGEMANE, 2018). Besides, several factors can influence the phytochemistry quality and the essential oil yield in post-harvest processing (GOBBO-NETO & LOPES, 2007; MUJUMDAR & LAW, 2010; PARK et al., 2014). Among these factors, drying is a critical process to preserve the medicinal properties' quality, which is essential for the pharmaceutical industry in producing herbal medicines according to the standards of quality and therapeutic efficacy (MUJUMDAR & LAW, 2010; CHIN & LAW, 2012; PARK et al., 2014).

However, exposure of medicinal plants to high drying air temperatures can cause essential oils volatilization and change their chemical composition, resulting in significant losses in therapeutic properties (ARGYROPOULOS & MÜLLER, 2014; ORPHANIDES et al., 2015; JIN et al., 2017). Therefore, the final quality of the dry product is an important variable in the drying of medicinal plants.

Studies related to the cultivation of this medicinal species arouse great interest regarding agronomic aspects due to its influence on chemical composition and; consequently, on its therapeutic properties (MENDES, 2014). Therefore, this research evaluated the essential oil yield and alpha-humulene content in plants grown in organic system cultivation at the different spacing between plants, harvested seasons, and drying temperature.

MATERIALS AND METHODS

Plant material, spacing between plants, and harvest season

V. curassavica seedlings were obtained from CPQBA/Unicamp and transplanted in four distance between plants (0.6 x 1.0 m; 0.8 x 1.0 m; 1.0 x 1.0 m; 1.0 x 1.6 m) in organic system cultivation at the Experimental Research Station of EPAMIG, Oratórios-MG, Brazil (20°25'49" S; 42°48'20" W). The experiment was conducted in a factorial scheme, with 4 distance between plants x 3 seasons of the year x 3 replications per treatment (four useful plants per replication). Samples exsiccates of *V. curassavica* were deposited in the PAMG Herbarium of the Agricultural Research Agency of the state of Minas Gerais EPAMIG under voucher PAMG 57973.

Fertilization was carried out according to the soil analysis, using cattle manure, with the following chemical characteristics (%): N (1.4), P (0.39), K (0.88), Ca (1.54), Mg (0.27), S (0.23), CO (10.45) and C/N (7.46). The drip irrigation system was used, and the control of spontaneous plants by manual weeding was performed as required.

Branches of the same plants (four useful plants per repetition) were harvested on three seasons:

December (summer) (2018), April (autumn), and August (winter) (2019). The plants were harvested at 3 years, with harvest height of 1.5 m. The leaves were separated from their stems, weighed, and dried in a forced-air circulation oven at 40 °C until constant weight. Subsequently, 100 g of dried leaves were packed in sealed polyethylene bags until the extraction of essential oils.

Drying temperature

Another harvest was carried out in the winter (August-2019), the season with the highest yield of essential oil, to assess the appropriate drying temperature to obtain the highest oil yield and the alpha-humulene content. The experiment was conducted in a factorial scheme, with 3 drying temperature x 4 distance between plants x 3 replications per treatment. After harvesting, the leaves were detached from the branches (four useful plants per repetition), selected, and dried at temperatures of 40, 50, or 60 ºC in a forced-air circulation oven until reaching constant weight. Subsequently, 30 samples (100 g) of dry leaves were separated, packaged in polyethylene bags, sealed, and conditioned in kraft paper bag until the extraction of essential oils to evaluate the yield and chemical composition of oil.

Essential oil extraction

The extraction of essential oil from the leaves harvested at summer, autumn and winter was performed using the modified Clevenger apparatus adapted to a round-bottomed flask with a capacity of 1000 mL. In each extraction, 500 mL of distilled water and 100 g of dry leaves were added to the flask, beginning the hydrodistillation process. The extraction was carried out by dragging the essential oil through water vapor for 4 hours. After extraction, the oil yield was calculated by the difference between the bottle's final weight containing the oil and the initial weight of the bottle, without oil, obtaining the yield for 100 g of leaves. The oil samples were stored at 4 °C in an amber glass bottle with a screw cap until chromatographic analysis.

Analysis and identification of volatile chemical constituents

The identification and content of volatile constituents were performed using an Agilent gas chromatograph, model HP-6890, equipped with an Agilent mass selective detector, model HP-5975, and an HP-5MS capillary column (30 m x 0.25 mm id x 0.25 µm pore thickness). The splitless injection mode operated at the following temperatures: injector at 220 °C, a column at 60 °C, with a heating ramp of 3 °C/min and final temperature of 240 °C, and detector at 250 °C. Helium was used as carrier gas at a flow rate of 1 mL/min. A sample of essential oil was dissolved in ethyl acetate (20 mg/mL) for analysis. The identification of the constituents was carried out by comparing the calculated Kovatz Indexes (KI) obtained by the injection of hydrocarbon standards (C-8 to C-24), with the equipment's database (NIST-11 library) and with data from the literature (ADAMS, 2007).

Leaf nutrient analysis

The leaves were harvested at winter (2019) in the same four useful plants per repetition in all spacing between plants.

The analysis of macro and micronutrients, nitrate, ammoniacal nitrogen, boron, and phosphorus were obtained by colorimetry (BRAGA et al., 1983; CATALDO et al., 1975; JACKSON, 1958), sulfur by turbidimetry (CHESNIN & YIEN, 1950), potassium by flame photometry and calcium, magnesium, copper, iron, manganese, and zinc by atomic absorption spectrophotometry, after extraction with hot water, sulfuric digestion, incineration or nitro-perchloric digestion of the dry material, as required in each case.

2.6 Statistical analysis

The Tukey test compared the averages ($p = 0.05$) using the SAEG statistical analysis program (RIBEIRO JR, 2001). The authors test the normality of the data before ANOVA and Tukey.

RESULTS AND DISCUSSION

Spacing between plants: essential oil yield and alpha-humulene content

The essential oil yield reported in *V. curassavica* leaves varied with spacing between plants and was significantly higher ($P < 0.05$) (1% and 0.8%) using 0.8 x 1.0 m, 1.0 x 1.0 m spacing between plants, respectively (Figure 1). The alpha-humulene content was within the recommended standards (above 2.3 to 2.9%) (QUISPE-CONDORI et al., 2008) in all spacing between plants except in 1.0 x 1.0 m spacing in winter and summer (Table 1). The *V. curassavica* essential oil is secreted and stored in glandular trichomes present on leaves surface whose development can be influenced by irradiance and can affected the yield and composition of essential oil (FEIJÓ et al., 2014). Probably 0.8 x 1.0 m, 1.0 x 1.0 m plants contributed to better irradiance necessary to development of leaves trichomes. GOMES et al. (2010) obtained 0.6% of alpha-humulene in *C. verbenacea* plants collected and ZOTTI-SPEROTTO et al. (2020) obtained between

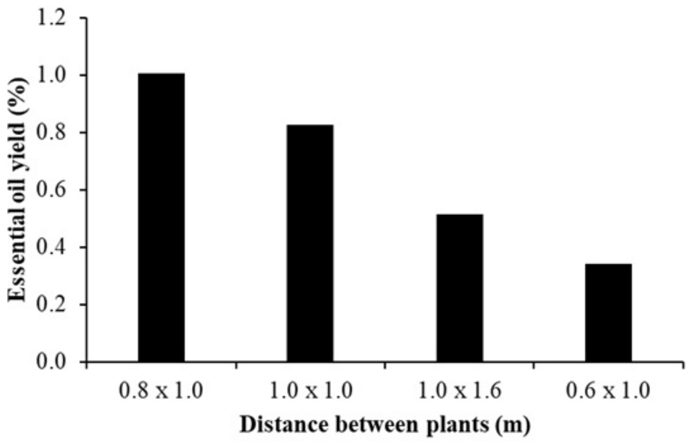

Figure 1 - Effect of distance between plants in essential oil yield of *V. curassavica.*

A, AB, BC and C: Results of Tukey test for comparison of the values of distance between plants in essential oil yield. Means followed by the same letter did not differ, by Tukey's test at 5% probability.

2.44 to 4.56% of alpha-humulene in *V. curassavica* plants cultivated. This difference is most probably because the species were being cultivated or collected in areas of natural occurrence.

Although, the alpha-humulene content is within the recommended standards in all distance between tested plants, varying according to the season in which the species was harvested. Larger (1.0 x 1.6 m) or smaller (0.6 x 1.0 m) spacings are not recommended for the cultivation of *V. curassavica*, due to the lower essential oil yield (Figure 1).

Season and alpha-humulene content

The essential oil yield found in the *V. curassavica* was higher in winter (Figure 2) when using the spacing 0.8 x 1.0 and 1.0 x 1.0 m. The essential oil yield variations are directly related to environmental factors, including seasonality and rainfall (ALMEIDA et al., 2016; SARRAZIN et al., 2015; PARKI, et al., 2017). The winter of experiment region is characterized by lower temperature and dry season which can contribute to reduce the volatilization of essential oil and major yield. The analyses verified that the alpha-humulene content was within the recommended standards (above 4.57%) by the pharmaceutical industry (HENRIQUES, 2009) in all seasons (Table 1): summer (4.91 %), autumn (4.89%), and winter (3.9%). Our results corroborated those presented by FERNANDES et al. (2019), who also reported higher levels of essential oil extracted from *V. curassavica* in July (winter), and the lowest content in November (summer). The results of HERNÁNDEZ et al. (2014) and Matias et al. (2016), also reported temporal variation in the chemical composition and biological properties of the essential oil of *C. curassavica*.

Table 1 - Effects of distance between plants and seasons on *V. curassavica* essential oil's alpha-humulene content.

Seasons	--Alpha-humulene yield (%)---			
	0.6 x 1.0 m	0.8 x 1.0 m	1.0 x 1.0 m	1.0 x 1.6 m
Autumn	5.93	4.72	4.09	4.89
Summer	7.32	5.18	1.13	4.91
Winter	3.53	3.47	1.12	3.90

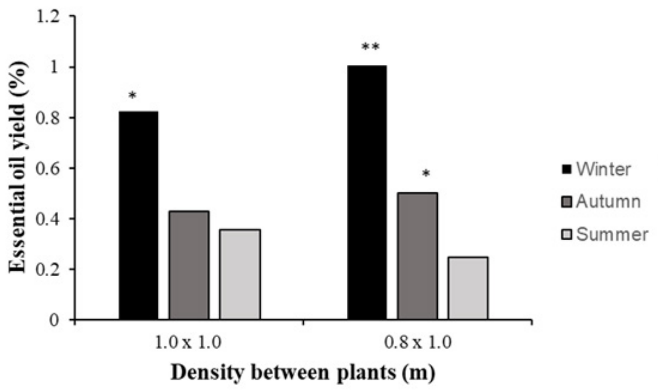

Figure 2 - Average yield of essential oil extraction from *V. curassavica* leaves as a function of harvest season and distance between plants.

* Significant by Tukey test at 5% probability and ** at 1% probability.

The monitoring of alpha-humulene yield during harvesting is important because it is the chemical constituent responsible for the species' anti-inflammatory effect (MEDEIROS et al., 2007).

Drying temperature: essential oil yield and phytochemical constituents

A mean of 83% of the chemical compound were identified, with 25 substances identified. The essential oil yield reduced significantly at the highest temperature tested (Figure 3). The majority compounds were: alpha-pinene, trans caryophyllene and alpha-humulene. Regarding these chemical constituents, the drying at 60 °C reduced the alpha-pinene content drastically because is a monoterpene that show high volatility. Wherever the drying temperature did not influence the major constituent trans-caryophyllene, and the chemical marker alpha-

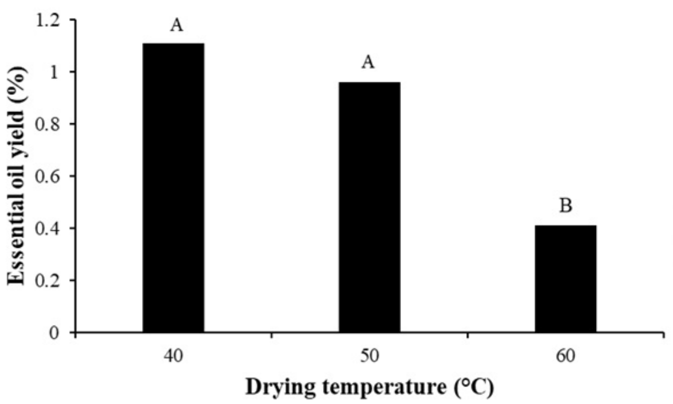

Figure 3 - Essential oil yield extracted from leaves of *V. curassavica,* submitted to drying in an oven with air circulation at 40, 50, and 60 ºC in the winter.

Notes: A and B: Results of Tukey test for comparison of the values of essential oil yield obtained in each drying procedure. Means followed by the same letter did not differ, by Tukey's test at 5% probability.

humulene because this sesquiterpenes are not volatile at the major tested temperature (Table 2). About the minority compounds, the drying at 60 °C reduced 11 compounds, did not affect 7 compounds and promote increase of 4 compounds probably due the reactions influenced by temperature (Table 2).

Essential oils are the most sensitive components of the drying process of medicinal plants and their volatilization depends mainly on the drying parameters and biological characteristics of the plants (ORPHANIDES et al., 2015; RAHIMMALEK & GOLI, 2013). Furthermore, essential oils are susceptible to air-drying temperature increases. Temperature increases favor their volatilization and, consequently, yield reduction (ARGYROPOULOS & MÜLLER, 2014; MUJUMDAR & LAW, 2010). Likewise, some chemical essential oils compounds are also lost during drying if performed at an inadequate temperature.

However, drying at higher temperatures can form a ‘‘partially dry surface layer’’, which limits the loss of volatile components (ORPHANIDES et al., 2015). Thus, in some species, conditions that increase the drying rate of plant materials (faster drying) also preserve the content of volatile compounds (ORPHANIDES et al., 2015). This factor may explain why some constituents were not affected by the increase in drying air temperature.

Leaf nutrients analysis

The macro and micronutrient levels quantified in the leaves of *V. curassavica* suggested no considerable changes in the absorption of these nutrients by the roots (Table 3). Thus, it can be

Table 2 - Chemical composition of the essential oil of *V. curassavica* leaves, submitted to drying in an oven with forced air circulation at 40, 50, and 60 ºC in the winter.

RT (min)	RI	Compound	-----------Drying air temperature----------		
			40 °C	50 °C	60 °C
		------Relative area (%)------			
5.40	933	alpha-pinene	23.36	19.63	6.04
6.40	972	beta-phellandrene	0.73	0.48	---
6.50	976	beta-pinene	1.35	1.06	0.54
6.86	990	beta-myrcene	0.52	---	---
8.08	1027	limonene	0.91	---	---
8.14	1029	1.8-cineole (eucalyptol)	1.05	1.11	0.49
18.14	1284	bornila acetate	0.57	0.67	0.58
21.09	1355	eugenol	0.58	---	0.48
22.55	1390	beta-elemene	1.40	1.44	1.41
23.16	1405	M = 204	1.99	2.47	1.28
23.53	1414	alpha-cis-bergamotene	1.45	1.90	1.88
23.69	1418	trans-caryophyllene	23.94	22.91	22.54
23.75	1419	alpha-santalene	---	10.2	8.68
25.02	1451	alpha-humulene	4.06	4.80	4.38
25.18	1455	trans-beta-farnesene	2.37	2.25	1.36
25.31	1458	beta-santalene	5.48	1.90	6.63
26.10	1478	germacrene D	1.80	1.77	1.79
27.25	1506	germacrene A	3.20	2.43	1.85
27.46	1512	cubebol	---	1.00	1.15
27.81	1521	delta-cadinene	1.22	1.22	1.12
28.14	1530	trans-gamma-bisabolene	1.25	1.22	0.81
28.98	1551	7-epi-cis-sesquisabinene hydrate	0.89	1.11	1.15
29.85	1574	spatulenol	0.91	1.32	1.49
30.05	1579	caryophyllene oxide	1.28	1.52	3.10
30.36	1587	7-epi-trans-sesquisabinene hydrate	0.94	1.15	1.51

Notes:
RT: Retention time.
RI: Retention index.

Table 3 - Nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), zinc (Zn), iron (Fe), manganese (Mn), copper (Cu), and boron (B) concentration in *V. curassavica* leaves grown in an organic system in 4 distance between plants.

-------------------Concentration of macro and micronutrients in *V. curassavica* leaves in function of distance between plants-----------------											
	N	P	K	Ca	Mg	S	Zn	Fe	Mn	Cu	B
Distance between plants	-------------------------dag/kg (%)-----------------------						------------------------mg/kg (ppm)--------------------				
0.6 x 1.0 m	2.21	0.49	1.61	1.50	0.27	0.27	27.33	368.00	112.33	6.33	37.23
0.8 x 1.0 m	2.07	0.45	1.36	1.27	0.25	0.28	18.67	353.00	102.00	6.00	35.70
1.0 x 1.0 m	2.04	0.46	1.52	1.19	0.22	0.27	16.67	359.00	85.67	5.33	34.43
1.6 x 1.0 m	2.20	0.44	1.52	1.54	0.24	0.28	19.33	401.00	131.67	5.00	40.77

inferred that differences in oil yield were associated with spacing between plants, a factor that directly influences the received solar radiation and; consequently, *V. curassavica* leaf trichomes development (COSTA et al., 2010; GOMES et al., 2010).

CONCLUSION

The 0.8 x 1.0 m or 1.0 m x 1.0 m distance between plants produced the highest essential oil yield and the highest concentration of phytochemicals, with plants harvested in winter and dried between 40 and 50 °C. The temperature increase reduced the essential oil yield of *Varronia curassavica* considerably. However, it did not compromise the alpha-humulene content.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest for this article. The founding sponsors had no role in the design of the study, in the collection, analyses, or interpretation of data, in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

The authors contributed equally to the manuscript.

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