DISTILLATION METHODS AFFECT THE CHEMICAL COMPOSITION OF *Varronia curassavica* Jacq. ESSENTIAL OIL?

MÉTODOS DE DESTILAÇÃO AFETAM A COMPOSIÇÃO QUÍMICA DO ÓLEO ESSENCIAL DE Varronia curassavica *Jacq?*

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ABSTRACT: The objective of this work was to evaluate the chemical composition of essential oil from *Varronia curassavica* Jacq. obtained by microwave (MI) and hydrodistillation (HD) extraction methods. The MI method tested three powers (500, 600, and 700W), three distillation times (20, 30, and 40 min.), and three water volumes (0, 25, and 50 mL per sample). The HD method tested three distillation times (100, 120, and 140 min.) and three water volumes (1.0, 1.5, and 2.0 L per 3-liter flask). The essential oils were analyzed by GC/MS-FID. The optimal condition for the essential oil extraction by the MI method was 700W for 40 min. (3.28%), regardless of the volume of water. In its turn, the best condition for essential oil extraction by the HD method was 120 min. with 1.0 L of water per flask (3.34%). The most abundant compounds for MI (700 W for 40 min. without water) were shyobunol (26.53%) and bicyclogermacrene (4.96%); and the most abundant compounds for HD (120 min. with 1.0 L of water/flask) were shyobunol (24.00%) and germacrene D-4-ol (10.23%). Methyl farnesoate (2*E*, 6*E*) and farnesyl acetate (2*Z*, 6*E*) were not detected in the essential oil extracted by HD; however, they were identified by the MI method. By increasing the distillation time and/or volume of water in HD, a reduction was observed for the content of the chemical compounds β-elemene (from 1.23 to 0.97%), *E*-caryophyllene (from 5.49 to 4.35%), α-humulene (from 1.80 to 1.43%), alloaromadendrene (from 1.78 to 1.44%), bicyclogermacrene (from 5.63 to 4.55%), and germacrene D-4-ol (from 11.40 to 9.86%). Power, extraction time, and their interactions influenced the content of essential oil obtained by microwave extraction (MI). Within each power, the highest essential oil content was extracted at the longest distillation time (40 min.), except for 600W, where no significant difference was detected between 30 and 40 min. The optimal essential oil contents for both extraction methods were statically similar by the t-test for dependent samples. However, the MI method presents advantages, such as shorter distillation time and less energy and water consumption.

KEYWORDS: Microwave distillation/ Volatile oil/ Chemical compounds

INTRODUCTION

The medicinal and aromatic plant *Varronia curassavica* Jacq. (syn. *Cordia verbenacea* DC.), known as "erva-baleeira" in Brazil, belongs to the family Cordiaceae (GASPARINO; BARROS, 2009) and is largely distributed in Brazil. Several properties of the species, such as anti-inflammatory, antiucer, antiallergic, antitumor, and antioxidant are mainly attributed to the compounds α-humulene and *E*-caryophyllene, present in its essential oil (FERNANDES et al., 2007; PASSOS et al., 2007; MEDEIROS et al., 2007; ROGÉRIO et al., 2009; OLIVEIRA et al., 2011; MICHIELIN et al., 2011; PARISSOTO et al., 2012; PIMENTEL et al., 2012). Other biological activities, such as antibacterial (MECCIA et al., 2009; MATIAS et al., 2010; PINHO et al., 2012; RODRIGUES et al., 2012) and antifungal activities (SILVA et al., 2012; HOYOS et al., 2012; SILVA et al., 2014) were reported in studies involving the essential oil of *V. curassavica*. In the pharmaceutical industry, the essential oil of *V. curassavica* is used to make a phytotherapeutic medicine for inflammation treatment.

Essential oils consist of a complex mixture of several chemical compounds with wide variation in their composition, and most of the compounds are terpenes. The chemical composition of essential oils is influenced by plants genetics, environmental factors (irrigation, harvest time, season, and others), genotype x environment interaction, and processing parameters (e.g., temperature, drying time of the plant material, and essential oil extraction method) (VERMA et al., 2010; GONZÁLEZ-RIVERA et al., 2016). The extraction method is one of the primary factors to determine the quality of an essential oil. The improper extraction process can result in changes in the chemical composition of the essential oil, leading to the loss of its bioactivity and natural

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characteristics, such as color, flavor, and viscosity (TONGNUANCHAN; BENJAKUL, 2014).

Among the laboratory methods used to extract essential oils from plants, hydrodistillation (HD) is the most frequently applied. However, it presents disadvantages, such as long extraction time, high demand for energy and water, and overheating of plant material, causing the loss of bioactive compounds of the essential oil by thermal degradations (RANITHA et al., 2014; CHEN et al., 2016).

New techniques are currently available, such as microwave-assisted extraction (MI). This technique allows the plant material to reach its boiling point quickly, resulting in a shorter extraction time and with less energy expenditure (KHANAVI et al., 2013; LI et al., 2014). In this sense, several works have been carried out aiming to improve the conditions of microwave-assisted extraction for several medicinal and commercial species, such as *Mentha* spp. (COSTA et al., 2014; GAVAHIAN et al., 2015); *Rosmarinus officinalis* (KARAKAYA et al., 2014); *Eucalyptus camaldulensis* (LI et al., 2016); *Cinnamomum cassia* (CHEN et al., 2016), among others.

The objective of this work was to evaluate the content (%) and chemical composition of essential oil from *V. curassavica* obtained by the hydrodistillation (HD) and microwave (MI) extraction methods.

MATERIAL AND METHODS

For essential oil extraction, leaves were harvested from plants of *Varronia curassavica* Jacq. (accession VCUR-201), maintained at the Active Germplasm Bank (AGB) of aromatic plants of the Federal University of Sergipe, located at the Research Farm "Campus Rural da UFS". Fresh leaves from the entire plants were collected, weighted, separated for essential oil extraction, maintained in a freezer at -20°C, until the beginning of the experiments.

The hydrodistillation was performed in a modified Clevenger apparatus using 3-liter flasks and samples of 100g of fresh leaves. The experiment consisted of a completely randomized design, in a 3x3 factorial scheme, using three replications. Three distillation times (100, 120, and 140 minutes of boiling) and three volumes of filtered water (using osmose reverse filter system) (1.0, 1.5, and 2.0 L per flask) were tested. The essential oils were dried over anhydrous sodium sulfate and stored in amber vials at -20^{o}C, until chemical composition analysis.

The essential oil content was calculated using the mean dry weight of six samples of 100 g of fresh leaves at 100^{o}C in a forced-air-circulation oven until constant weight, for 48 hours. The essential oil content was calculated according to the following equation:

Content (%, v/w) = (volume of essential oil extracted from the sample/mean of dry weight of leaves) x 100.

The microwave-accelerated reaction system (NEOS, Milestone, Italy) equipped with a circulating water-cooling system was utilized for the experiment. Samples of 50 g of fresh leaves were used. The experiment consisted of a completely randomized design, in a 3x3x3 factorial scheme, with three replications. Three powers (500, 600, and 700 W), three extraction times (20, 30, and 40 minutes), and three volumes of filtered water (using osmose reverse filter system) (0, 25, and 50 mL per sample) were tested. The essential oils were dried using anhydrous sodium sulfate and stored in amber vials at -20 °C until chemical composition analysis. The same procedure described for hydrodistillation was applied to calculate the essential oil content.

The analysis of the chemical composition of the essential oils was performed using a GC-MS/FID (QP2010 Ultra, Shimadzu Corporation, Kyoto, Japan) equipped with an autosampler AOC-20i (Shimadzu). Separations were accomplished using an Rtx®-5MS Restek fused silica capillary column (5%-diphenyl–95%-dimethyl polysiloxane) of 30 m × 0.25 mm i.d., 0.25 mm film thickness, at a constant helium (99.999%) flow rate of 1.2 mL.min^{-1}. An injection volume of 0.5 µL (5 mg.mL^{-1}) was employed, with a split ratio of 1:10. The oven temperature was programmed from 50^{o}C (isothermal for 1.5 min), with an increase of 4 ^{o}C.min^{-1}, to 200 ^{o}C, then 10 ^{o}C.min^{-1} to 250 ^{o}C, ending with a 5 min isothermal at 250 ^{o}C.

The MS and FID data were simultaneously acquired by a Detector Splitting System; the split flow ratio was 4:1 (MS:FID). A 0.62 m x 0.15 mm i.d. restrictor tube (capillary column) was used to connect the splitter to the MS detector; a 0.74 m x 0.22 mm i.d. restrictor tube was used to connect the splitter to the FID detector. The MS data (total ion chromatogram, TIC) were obtained in the full scan mode (m/z of 40–350) at a 0.3 scan/s scan rate, using the electron ionization (EI) with an electron energy of 70 eV. The injector temperature was 250 ^{o}C, and the ion-source temperature was 250 ^{o}C. The FID temperature was set to 250 ^{o}C, and the gas supplies for the FID were hydrogen, air, and helium at flow rates of 30, 300, and 30 mL.min^{-1}, respectively. Quantification of each constituent was

estimated by FID peak-area normalization (%). Compound concentrations were calculated from the GC peak areas and arranged in order of GC elution.

The identification of individual components of the essential oil was performed by computerized matching of the acquired mass spectra with those stored in the NIST21, NIST107, and WILEY8 mass spectral library of the GC-MS data system. A mixture of hydrocarbons (C_9H_{20}–$C_{19}H_{40}$) was injected under these same conditions. Compounds were identified by comparing the spectra obtained with those of the equipment's data bank and by the Kovats index, calculated for each compound, as previously described (ADAMS, 2007). Retention indices were obtained using the equation proposed by Van den Dool and Kratz (1963).

Essential oil content and chemical composition were subject to analysis of variance (ANOVA), and the means were compared by the Scott-Knott test ($p<0.05$), using the Sisvar® software. The means of the content of the best hydrodistillation (HD) and microwave (MI) treatments were compared by the t-test for dependent samples ($p<0.05$), using the Statistica 7.0 software.

RESULTS

The optimal condition for essential oil extraction by the MI method was 700W for 40 min. (3.28%), regardless of the volume of water. In its turn, the best condition for essential oil extraction by the HD method was 120 min. with 1.0 L of water per flask (3.34%). (Tables 1 and 2). The most abundant compounds for MI (700W for 40 min. without water) were shyobunol (26.53%) and bicyclogermacrene (4.96%); and the most abundant compounds for HD (120 min. with 1.0 L of water/flask) were shyobunol (24.00%) and germacrene D-4-ol (10.23%) (Tables 1 and 2).

Most of the compounds presented significant distillation time x water volume interaction for HD. The increase in the distillation time and/or volume of water reduced the content of the chemical compounds β-elemene (from 1.23 to 0.97%), *E*-caryophyllene (from 5.49 to 4.35%), α-humulene (from 1.80 to 1.43%), alloaromadendrene (from 1.78 to 1.44%), bicyclogermacrene (from 5.63 to 4.55%), and germacrene D-4-ol (from 11.40 to 9.86%) (Table 1). The content of germacrene D-4-ol reduced when distillation time was increased. However, the largest volume of water per flask resulted in higher contents, reaching the maximum (12.55%) with 100 minutes distillation time and 2.0 L of water per flask. The contents of the compounds spathulenol, ledol, epi-α-murulol, and shyobunol increased with the increase in the volume of water and/or distillation time (Table 1).

Power, extraction time, and their interactions influenced the essential oil content obtained by microwave extraction (MI). Within each power, the highest essential oil content was obtained at the longest extraction time (40 min.), except for 600W, where no significant difference was recorded between 30 and 40 minutes. The maximum essential oil content (3.28%) was observed using the power of 700W for 40 min., regardless of the volume of water/sample (Table 2).

Methyl farnesoate (2*E*, 6*E*) and farnesyl acetate (2*Z*, 6*E*) were not detected in the essential oil extracted by HD. However, they were detected by the MI extraction method.

The extraction time of 40 min. used in the solvent-free MI distillation together with the highest power (700W) resulted in an increased content of β-elemene (0.99%), E-caryophyllene (3.97%), α-humulene (1.44%), alloaromadendrene (1.65%), bicyclogermacrene (4.96%), ledol (4.00%), epi-α-murulol (4.06%), and farnesyl acetate (*2Z, 6E*) (3.59%) (Table 2). Only the content of alloaromadendrene (1.34%), ledol (4.10%), and farnesyl acetate (*2Z, 6E*) (3.60%) increased with the addition of 50 mL of water per sample to the MI extraction system (Table 2).

The extraction time of 40 min. in the solvent-free MI distillation together with the lowest power (500W) resulted in an increased content of germacrene D-4-ol (4.24%), caryophyllene oxide (2.24%), cubenol (2.17%), shyobunol (27.35%), and methyl farnesoate (*2E, 6E*) (3.51%) (Table 2). Only the content of germacrene D-4-ol (5.51%) and methyl farnesoate (*2E, 6E*) (3.11%) increased with the addition of 50 mL of water per sample to the MI extraction system (Table 2).

The means of essential oil contents obtained in the best HD (120 min. with 1.0 L of water per flask) and MI (700W for 40 min) treatments were statistically similar ($p<0.05$) when compared by the t-test for dependent samples.

Table 1. Content and chemical composition of the essential oil from *Varronia curassavica* extracted by hydrodistillation (HD), according to the extraction time and water volume.

Compounds	RRI-o	RRI-l	Time (min.)	Water (L/flask)		
				1.0	1.5	2.0
				Content (%) of chemical constituents		
β-elemene	1389	1389	100	1.23aA	1.15aA	1.18aA
			120	1.21aA	1.11aB	1.10bB
			140	1.14bA	1.09aA	0.97cB
E-caryophyllene	1422	1417	100	5.49aA	5.14aB	5.20aB
			120	5.44aA	4.98aB	4.87bB
			140	4.98aA	4.92aA	4.35cB
α-humulene	1457	1452	100	1.80aA	1.71aB	1.7aB
			120	1.76aA	1.64aB	1.61bB
			140	1.63bA	1.61bA	1.43cB
alloaromadendrene	1465	1458	100	1.78aA	1.66aB	1.67aB
			120	1.74aA	1.63aB	1.56bB
			140	1.69aA	1.69aA	1.44cB
bicyclogermacrene	1499	1500	100	5.63aA	5.27aB	5.44aB
			120	5.31bA	5.20aA	4.91bB
			140	5.01cA	5.00aA	4.55cB
germacrene D-4-ol	1580	1574	100	11.40aB	11.70aB	12.55aA
			120	10.23bC	11.09bB	11.84bA
			140	9.86bB	9.90cB	10.36cA
sphatulenol	1583	1577	100	1.16aB	1.20aB	1.32aA
			120	1.10aA	1.19aA	1.21bA
			140	1.11aA	1.14aA	1.13bA
caryophyllene oxide	1592	1582	100	1.34aA	1.34aA	0.97bB
			120	1.36aA	1.34aA	1.34aA
			140	1.32aA	1.35aA	1.34aA
ledol	1612	1602	100	3.71aB	3.65bB	4.50aA
			120	3.69aB	3.88aA	3.93bA
			140	3.67aB	3.67bB	3.88bA
epi-α-murulol	1646	1640	100	2.76bB	2.90bB	3.90aA
			120	3.00bB	4.10aA	3.97aA
			140	3.63aB	3.94aA	3.92aA
cubenol	1650	1645	100	1.82aA	1.86aA	1.75bA
			120	1.85aA	2.00aA	1.89bA
			140	1.98aA	2.06aA	2.22aA
α-cadinol	1660	1652	100	4.56aA	4.68aA	4.83bA
			120	4.91aA	4.93aA	5.33aA
			140	4.94aA	5.41aA	5.63aA
shyobunol	1705	1709	100	24.24bA	23.90bA	22.95cB
			120	24.00bA	24.14bA	24.24bA
			140	24.93aB	24.81aB	25.58aA
methyl farnesoate (2*E*, 6*E*)	1776	1783	100	2.95aA	3.10aA	1.67cB
			120	2.25bB	2.91aA	2.88aA
			140	2.86aA	2.77aA	2.24bB
essential oil content (%)	---	---	100	2.93bA	2.93bA	2.43cB
			120	3.34aA	3.03bB	2.93bB
			140	3.54aA	3.44aA	3.23aA

RRI-o: Relative Retention Index - observed; RRI-l: Relative Retention Index - literature. Means followed by the same lowercase letter in the column and uppercase letter in the row do not differ by the Scott Knott test ($P < 0.05$).

Table 2. Content and chemical composition of the essential oil from *Varronia curassavica* obtained by solvent-free microwave extraction (MI), according to the tested power, extraction time, and water volume.

Compounds	RRI-o	RRI-l	Power (W)	Water (mL/sample)								
				0			25			50		
				Time (min.)								
				20	30	40	20	30	40	20	30	40
				Content (%) of chemical compounds								
			500	1.05bAβ	1.01bAα	0.96aBα	1.00bAβ	0.89cBβ	0.87cBβ	1.20aAα	0.83cBβ	0.87aBβ
β-elemene	1389	1389	600	1.21aAα	1.12aBα	0.87bCβ	1.19aAα	1.13aAα	1.03aBα	1.08bAβ	1.02aAβ	0.93aBβ
			700	1.25aAα	1.02bBα	0.99aBα	1.03bAβ	1.03bAα	0.94bBα	1.00cAβ	0.93bAβ	0.93aAα
			500	4.40aAγ	3.55cBα	3.43bBα	3.78bAβ	2.87bBβ	2.92bBβ	5.24aAα	2.60bCγ	3.08aBβ
E-caryophyllene	1422	1417	600	4.45aAα	4.19aBα	2.99cCβ	4.25aAα	3.82aBβ	3.59aCα	3.82bAβ	3.51aBγ	3.19aCβ
			700	4.59aAα	3.91bBα	3.97aBα	3.96bAβ	3.68aBβ	3.46aBβ	3.67bAγ	3.35aBγ	3.12aCγ
			500	1.49bAβ	1.30cBα	1.29bBα	1.36cCγ	1.14bBβ	1.15bBβ	1.75aAα	1.06bCγ	1.19aBβ
α-humulene	1457	1452	600	1.58bAα	1.51aBα	1.19cCβ	1.56aAα	1.45aBα	1.36aCα	1.42bAβ	1.37aAβ	1.25aBβ
			700	1.68aAα	1.39bBα	1.44aBα	1.44bAβ	1.40aAα	1.30aBβ	1.35cAγ	1.31aAβ	1.21aBγ
			500	1.65cAα	1.43bBα	1.45bBα	1.44cAβ	1.25bBβ	1.25bBβ	1.71aAα	1.11cCγ	1.26bBβ
alloaromadendrene	1465	1458	600	1.75bAα	1.65aBα	1.35cCβ	1.71aAα	1.54aBβ	1.52aBα	1.52bAβ	1.51aAβ	1.40aBβ
			700	1.92aAα	1.59aBα	1.65aBα	1.62bAβ	1.57aAα	1.45aBβ	1.45cAγ	1.44bAβ	1.34aBγ
			500	5.08cAβ	4.52bBα	4.59bBα	4.70cAγ	4.25bBβ	4.20cBβ	5.31aAα	4.04cCγ	4.22bBβ
bicyclogermacrene	1499	1500	600	5.41bAα	5.07aBα	4.41cCβ	5.31aAα	4.88aBβ	4.79aBα	4.88bAβ	4.71aBγ	4.43aCβ
			700	5.67aAα	4.94aBα	4.96aBα	5.07bAβ	4.82aBα	4.50bCβ	4.78bAγ	4.50bBβ	4.21bCγ
			500	4.09aBγ	4.29aAγ	4.24aAγ	4.31bCβ	4.48aBβ	4.77aAβ	11.86aAα	5.09aCα	5.51aBα
germacrene D-4-ol	1580	1574	600	4.03aAγ	3.89bBγ	4.02bAγ	4.56aAβ	4.34bBβ	4.53bAβ	4.83bAα	4.75bAα	4.77bAα
			700	3.93aAγ	3.91bAγ	3.60cBγ	4.03cBβ	4.21cAβ	4.03cBβ	4.70cAα	4.62cBα	4.73bAα
			500	2.45aAα	2.34aBα	2.24aCα	2.49aAα	2.14bBβ	2.20aBα	1.39bCβ	2.09aBβ	2.20aAα
caryophyllene oxide	1592	1582	600	2.46aAα	2.18bCα	2.28aBα	2.37bAβ	1.96cCβ	2.12bBβ	2.14aAγ	2.12aAα	2.02bBγ
			700	2.28bAα	2.22bAβ	1.97bBβ	2.10cBγ	2.30aAα	2.04cBβ	2.17aAβ	2.06aAγ	2.17aAα
			500	3.78bAβ	3.38bBβ	3.47bBα	3.71cAβ	3.79bAα	3.63aAα	4.01bAα	3.72bBα	3.62bBα
ledol	1612	1602	600	3.62bBβ	4.03aAβ	3.98aAα	4.01bAα	3.93bAβ	3.86aAα	3.83bBα	4.30aAα	4.11aAα
			700	4.22aAα	3.95aBβ	4.00aBα	4.26aAα	4.30aAα	3.87aBα	4.33aAα	4.10aAβ	4.10aAα
			500	2.79bBα	2.88bBγ	3.50bAβ	2.86bCα	4.16aAα	3.82aBα	2.98bBα	3.89aAβ	3.80aAα
epi-α-murulol	1646	1640	600	3.41aBα	3.67aAα	3.89aAα	3.58aAα	3.73bAα	3.68aAα	3.71aAα	3.69aAα	3.75aAα
			700	3.60aBα	3.55aBα	4.06aAα	3.64aAα	3.79bAα	3.57aAβ	3.67aAα	3.75aAα	3.87aAβ

Continuation of table 2:

			500	1.63aBα	1.99aAα	2.17aAα	1.87aAα	1.77aAα	2.10aAα	1.93aAα	1.96aAα	1.57bBβ
cubenol	1650	1645	600	1.90aAα	1.68bAα	2.02aAα	1.52bBβ	1.78aAα	1.89aAα	1.64aAβ	1.74aAα	1.86aAα
			700	1.67aAα	2.07aAα	1.69bBβ	1.53bBα	1.70aBβ	2.09aAα	1.78aAα	1.71aAβ	1.90aAα
			500	4.54aAα	4.51aAα	4.54aAα	4.17aAα	4.55aAα	4.26aAα	4.67aAα	4.62aAα	4.79aAα
α-cadinol	1660	1652	600	3.84aBβ	4.49aAα	4.93aAα	4.55aAα	4.44aAα	4.31aAβ	4.01aBβ	4.62aAα	4.82aAα
			700	4.50aAα	4.59aAα	4.28aAα	4.79aAα	4.24aAα	4.48aAα	4.19aAα	4.49aAα	4.60aAα
			500	26.47aBβ	26.92aAβ	27.35aAα	27.30aBα	28.43aAα	27.84aBα	23.53bCγ	29.07aAα	27.45aBα
shyobunol	1705	1709	600	26.11aBβ	25.88bBβ	27.22aAα	25.50bBβ	26.68cAα	26.91bAα	26.98aAα	26.40cBα	27.45aAα
			700	25.20bBβ	26.11bAβ	26.53bAβ	27.34aAα	27.49bAα	27.12bAα	27.03aAα	27.71bAα	27.46aAα
			500	3.94aBα	4.55aAα	3.51aCα	3.33aBβ	3.67aAβ	3.19aBβ	1.79cCγ	3.77aAβ	3.11aBβ
methyl farnesoate (2*E*, 6*E*)	1776	1783	600	3.63aAα	3.83bAα	2.85bBα	2.44bBγ	3.28bAβ	2.51bBβ	2.85aAβ	1.70bBγ	1.84bBγ
			700	1.63bAβ	1.54cAα	1.78cAα	1.38cBβ	1.80cAα	2.10cAα	2.14bAα	1.63bBα	1.77bBα
			500	2.50cAβ	2.16cBα	2.81bAα	2.83cAα	2.47bAα	2.72cAα	0.00cCγ	2.03bBβ	2.40bAβ
farnesyl acetate (2*Z*, 6*E*)	1824	1821	600	2.82bBβ	2.69bBβ	3.56aAα	3.19bAα	2.48bBβ	3.24bAβ	2.70bBβ	3.64aAα	3.69aAα
			700	4.34aAα	3.83aBα	3.59aBα	4.14aAα	3.61aBα	3.65aBα	3.33aBβ	3.83aAα	3.60aAα
			500	1.65bCα	2.05bBα	2.69bAα	1.45cCα	2.07bBα	2.71bAα	1.52cCα	2.09cBα	2.58cAα
essential oil content (%)	---	---	600	2.20aBα	2.86aAα	2.48bBβ	2.21bBα	2.88aAα	2.90bAα	2.08bCα	2.50bBα	3.00bAα
			700	2.42aBα	2.90aBα	3.28aAα	2.71aBα	2.90aBα	3.36aAα	2.71aBα	2.95aBα	3.43aAα

RRI-o: Relative Retention Index - observed; RRI-l: Relative Retention Index - literature. Means followed by the same lowercase letter in the column, uppercase letter in the row and Greek letter between water volumes (for the same time and power) do not differ by the Scott Knott test (P <0.05).

DISCUSSION

The use of smaller volumes of water in the extraction by HD provided the highest essential oil content. The addition of smaller volumes of water to the flasks allows more space for the movement of water vapor and essential oil during distillation, which favors higher essential oil condensing in the modified Clevenger apparatus. The compounds sesquiterpene hydrocarbons were observed in higher contents at a shorter distillation time and/or smaller volumes of water in the HD method. The increase in the extraction time led to a greater loss of sesquiterpene hydrocarbons compounds by volatilization and degradation due to the high temperature or hydrolysis (SOZMEN et al., 2011; LI et al., 2012; QI et al., 2014). Conversely, the oxygenated sesquiterpenes presented the highest contents when increasing the extraction time and/or volumes of water. Due to the lower volatility of these compounds, they are lost in a lesser amount when compared with the sesquiterpenes hydrocarbons during the extraction process.

The extraction time and power were the most important factors in the essential oil extraction of *V. curassavica* by MI. Similarly, Shah and Garg (2014) reported that the longest time (30 min.) and higher power (640W) provided greater essential content in a study performed with ginger. According to Chen et al. (2011), the increase of power accelerates the transfer of mass and increases the essential oil content.

For the species *Ocimum basilicum* and *Chenopodium ambrosioides*, time and water factors were more significant than power for the essential oil content obtained by MI. Similarly to the present study for HD, Cardoso-Ugarte et al. (2013) reported that the longer the time of extraction and the lower the volumes of water, the greater were the essential oil contents. In their work, the authors used an adapted household microwave and larger volumes of water, which explains the resemblance of their results with that observed for the HD method in the current study.

A higher number of compounds was detected in the essential oil of *V. curassavica* extracted by the MI method when compared with that revealed by the HD method. The same was observed in *Ferulago campestres* (RIELA et al., 2011). The plant material heated by microwaves overheat inside the cell, which causes the expansion and rupture of the cell walls and consequently allows extracting the essential oil and less volatile compounds more efficiently (CHEN et al., 2011).

The significant interaction between the studied factors when using extraction by MI, for most compounds, showed that these factors cause quantitative variation in the chemical composition of the essential oil of *V. curassavica*. Similar germacrene D-4-ol content was observed between the essential oils extracted by HD and MI, where higher contents were reached when applying the shortest time and greater volumes of water. *E*-caryophyllene and α-humulene presented similar behavior regarding the time, where higher contents were observed at shorter extraction times when using both methods. In a study carried out with clove (*Syzygium aromaticum* L.), a higher content of *E*-caryophyllene (24.8%) and α-humulene (3.1%) were obtained by the MI method when compared with the hydrodistillation method (5.1 and 0.6%, respectively) (GONZÁLEZ-RIVERA et al., 2016), probably due to the shorter time and lower volume of water used in the microwave-assisted extraction.

This study revealed that both methods (HD and MI) were effective in extracting the essential oil of *V. curassavica*. The chemical composition and essential oil content of this species are influenced by the extraction method used. The optimal essential oil contents for the two extraction methods were similar. However, the new technique of microwave-assisted extraction (MI) has some advantages, such as shorter distillation time and lower energy and water consumption. The use of 700 W for 40 min. (without water) for MI extraction and 120 min. with 1.0 L of water per flask for HD can be recommended to obtain high essential oil content of *V. curassavica*.

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RESUMO: O objetivo deste trabalho foi avaliar a composição química do óleo essencial de *Varronia curassavica* Jacq. obtido pelos métodos de extração micro-ondas (MI) e hidrodestilação (HD). Para MI, foram testadas três potências (500, 600 e 700W), três tempos de destilação (20, 30 e 40 min.) e três volumes de água (0, 25 e 50 mL por amostra). Para HD, foram testados três tempos de destilação (100, 120 e 140 min.) e três volumes de água (1,0; 1,5 e 2,0 L por balão de 3 litros). Os óleos essenciais foram analisados por CG/EM-FID. Maiores teores de óleo essencial foram obtidos nas condições de 700 W por 40 min. (3.28%), independente do volume de água para MI, e 120 min. com 1,0 L de

água por balão para HD (3,34%). Os compostos mais abundantes para MI (700W, por 40 min., sem água) foram o shyobunol (26,53%) e biciclogermacreno (4,96%) e para HD (120 min. com 1,0 L de água /balão) foram shyobunol (24,00%) e germacreno D -4 -ol (10,23%). Metil farnesoato (2*E*, 6*E*) e farnesil acetato (2*Z*, 6*E*) não foram detectados no óleo essencial extraído por HD, porém, foram detectados nas amostras extraídas por MI. Com o aumento do tempo de destilação e/ou do volume de água em HD, houve redução no conteúdo dos constituintes químicos β-elemeno (de 1,23 para 0,97%), *E*-cariofileno (de 5,49 para 4,35%), α-humuleno (1,80 para 1,43%), aloaromadendreno (de 1,78 para 1,44%), biciclogermacreno (de 5,63 para 4,55%) e germacreno D-4-ol (de 11,40 para 9,86%). A potência, o tempo de extração e suas interações influenciaram no teor de óleo essencial obtido na extração por micro-ondas (MI). Dentro de cada potência, o maior teor de óleo essencial foi obtido no tempo mais longo de extração (40 min.), exceto para 600 W, que não apresentou diferença significativa entre 30 e 40 min. Nas condições ótimas de extração, os teores de óleo essencial obtidos foram estatisticamente semelhantes pelo teste t para amostras dependentes. No entanto, a extração por micro-ondas apresenta algumas vantagens em relação a HD, como menor tempo de destilação e menor consumo de energia e água.

PALAVRAS-CHAVE: *Varronia curassavica*. Óleos voláteis. Destilação por micro-ondas.

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