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Microwave-assisted extraction: an alternative to extract *Piper aduncum* essential oil

Extração assistida por micro-ondas: uma alternativa para a obtenção do óleo essencial de *Piper aduncum*

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ABSTRACT
Hydrodistillation and steam distillation have been widely described as methods of obtaining essential oils, however, green technologies with lower levels of energy consumption, time, and solvent have gained increasing prominence. *Piper aduncum* has confirmed occurrences throughout the Brazilian territory and its essential oil has been described with antifungal, anti-helminthic, antioxidant, and repellent activities. In the present study, we compared the essential oil yield and the dillapiole content from the essential oils obtained using hydrodistillation (Clevenger apparatus) and microwave-assisted extraction (using a prototype extractor). A factorial design was applied to evaluate the influence of the entrance variables fraction diameter (F_R) and load of material plant/water volume (LW) on the response variables essential oil yield (Y_d) and dillapiole content (DC). Via statistical analysis, a polynomial model was obtained, as well as, the response surface. Hydrodistillation provided higher essential oil yields and the dillapiole content was not statistically influenced by the extraction methods.

Keywords: dillapiole; essential oil yield; microwave extraction.

RESUMO
Os processos de hidrodestilação e destilação a vapor tem sido amplamente descritos como métodos de obtenção de óleos essenciais, no entanto, tecnologias verdes com menores níveis de consumo de energia, tempo e solvente têm ganhado cada vez mais destaque. *Piper aduncum* tem ocorrência confirmada em todo o território brasileiro e seu óleo essencial é descrito por suas propriedades antifúngicas, anti-helmíntica, citotóxica, antioxidante e repelente. No presente estudo, foi comparado o rendimento de óleo essencial e o teor de dilapiol dos óleos essenciais obtidos por hidrodestilação (em sistema Clevenger) e extração assistida por micro-ondas (utilizando um protótipo de extrator). Um planejamento fatorial foi utilizado para avaliar a influência das variáveis de entrada diâmetro da fração (F_R) e relação carga de material/volume de água (LW) nas variáveis de resposta rendimento de óleo essencial (Y_d) e teor de dilapiol (DC). Por meio da análise estatística foi obtido um modelo polinomial e sua respectiva superfície de resposta. A hidrodestilação forneceu os maiores rendimentos de óleo essencial e o teor de dilapiol não foi estatisticamente influenciado pelo método de extração.

Palavras-chave: dilapiol; rendimento de óleo essencial; extração com micro-ondas.

1 INTRODUCTION

Plants can synthesize two types of oils: fixed oils and essential oils. Fixed oils consist of glycerol esters and fatty acids (triglycerides or triacylglycerols), while essential oils are mixtures of volatile organic compounds and contribute to the taste and fragrance of plants (Tisserand and Young, 2014). Essential oils are secondary metabolites synthesized in different plant organs, such as flowers, flower buds, leaves, fruits, branches, bark, seeds, wood, resins, rhizomes, and roots. They are stored in secretory cells, cavities, channels, epidermal cells, or glandular trichomes (Asbahani et al., 2015;

Nazzaro et al., 2017; Pandey et al., 2017). Essential oils are complex mixtures composed of low molecular weight aliphatic compounds (acids, alcohols, aldehydes, alkanes, esters, and ketones), monoterpenes, sesquiterpenes, and phenylpropanoids (Conde-Hernández et al., 2017). They have gained increasing prominence due to their aromatic and preservative properties, which are interesting for the cosmetic and food industries (Beraldo et al., 2013; Fonseca Júnior et al., 2019; Singh Chouhan et al., 2019; Teixeira et al., 2020).

Piper aduncum (Piperaceae), is commonly named in Brazil as "pimenta-de-macaco" and "pimenta-longa", and it is described in traditional medicine for its diuretic, anti-inflammatory properties, and it is also used for wound treatment (Andrade et al., 2009; Pohlit et al., 2006). This plant is a shrub species, native but not endemic from Brazil, with confirmed occurrences throughout the Brazilian territory. Its phytogeographic domains are Amazon, Cerrado, and Atlantic Forest (Flora do Brasil, 2020). The *P. aduncum* essential oil has been described for its cytotoxic effect (Barros et al., 2016), anti-helminthic (Corral et al., 2018), acaricide (Silva et al., 2009), antifungal (Valadares et al., 2018), trypanocidal (Villamizar et al., 2017), antibacterial (Brazão et al., 2014), repellent (Mamood et al., 2016), and antioxidant (Rodriǵuez et al., 2013) activities. The chemical composition of *P. aduncum* essential oil may vary and some compounds described as its major compounds are (*E*)-caryophyllene (Barros et al., 2016), dillapiole (Corral et al., 2018; de Oliveira et al., 2019; Maia et al., 1998), nerolidol (Villamizar et al., 2017), and piperitone (Potzernheim et al., 2012; Valadares et al., 2018).

It is remarkable the research for environmentally friendly methods, that uses less solvent, energy, and time. Therefore, new methodologies based on the use of microwave energy have been described, such as microwave-assisted extraction, microwave-assisted hydrodistillation, solvent-free microwave extraction, and microwave hydro diffusion and gravity extraction (Asofiei et al., 2017; Ferhat et al., 2006; Megawati et al., 2019).

Microwave consists of a form of non-ionizing electromagnetic radiation with a frequency band of 300MHz to 300 GHz (Fan et al., 2017). Microwave heating involves an energy conversion from electromagnetic energy to thermal energy. Products that contain water have dipolar properties, so the microwave energy passes through the sample and causes the vibration of the dipolar molecules, resulting in internal friction which produces heat (Graham, 2003). Therefore, the microwave energy is delivered directly to the material through molecular interaction with the electromagnetic field (Sun et al., 2016). A typical microwave system consists of the following components: magnetron, a waveguide, a microwave cavity, and a circulator. Microwave energy is produced by the magnetron, propagated through the waveguide and is introduced directly into the microwave cavity, where the circulator distributes the microwave in the forward direction only (Castro and Priego-Capote, 2016).

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Magnetrons convert electrical energy into electromagnetic fields with positive and negative charge centers, which change direction billions of times per second (Graham, 2003). Conventional microwaves ovens operate at a frequency of 2450 MHz which results in oscillations of 4.9×10^9 times per second and as a consequence, molecules subject to electromagnetic radiation are extremely agitated (Lew et al., 2002).

Essential oils have been used in pharmaceutical, cosmetic, and food products. On a laboratory scale, its production has been carried out mainly by hydrodistillation and the steam distillation is commonly applied in industrial applications. The use of microwave energy in different methodologies is a trend that has proved satisfactory, and therefore an alternative to obtaining essential oils. In the present study, the *P. aduncum* essential oil was obtained through hydrodistillation and microwave-assisted extraction, using a prototype extractor. The essential oil obtained was characterized by its yield and dillapiole (1,2-methylenedioxy-5,6-dimethoxy-4-allylbenzene) content, using a factorial design.

2 MATERIALS AND METHODS

2.1 PLANT MATERIAL

Leaves of *P. aduncum* were collected in the municipality of Santo Antônio do Tauá (Pará/Brazil), dried at an oven at 35°C for 7 days and pulverized. Subsequently, it was used a series of Tyler sieves (following decreasing openings: 4,76; 2,00; 1,18; 0,84; 0,425, and 0,05 mm) to determine the granulometric distribution and Sauter diameter, as described by Nascimento et al. (2015). Particles retained in sieves 4,76; 2.00 and 1.18 mm were grouped into a single portion, called as coarse leaves fraction, and the material retained in the following sieves (0.84; 0.425; and 0.05 mm) were grouped into a second portion, called thin leaves fraction (Figure 1). The moisture content was quantified in an oven at 105°C for 24h, as proposed by the Association of Official Analytical Chemists (AOAC, 1997).

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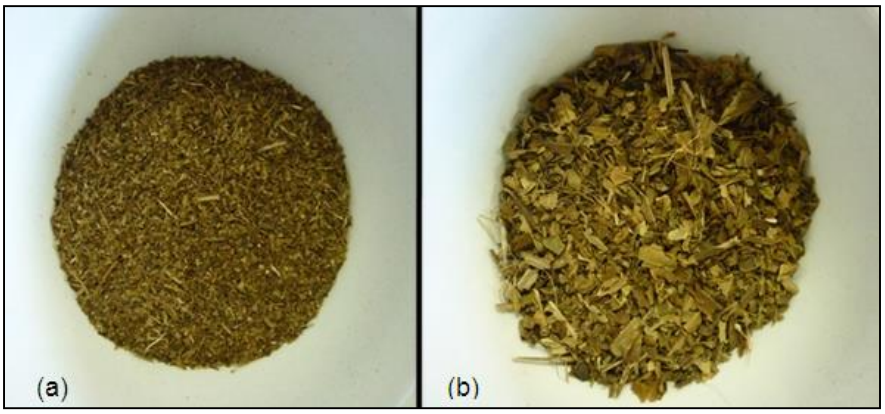
Figure 1 – Fractions of the leaves of *Piper aduncum.*



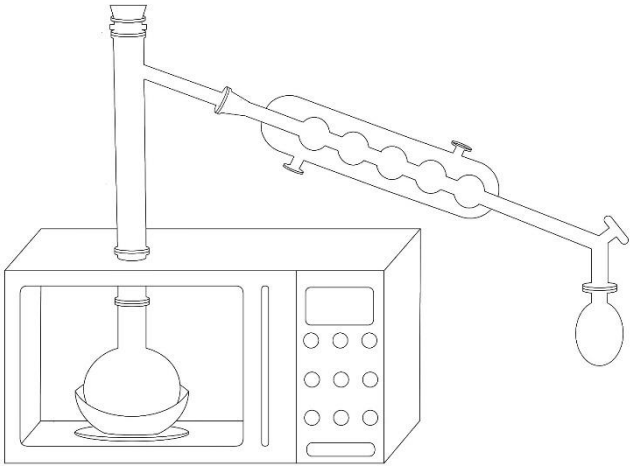
Thin (a) and Coarse (b) leave fractions.

2.2 MICROWAVE-ASSISTED EXTRACTION AND HYDRODISTILLATION PROCESS

Samples of 30 g of each fraction (coarse and thin leave fractions) were put into a boiling flask, which was inserted in the microwave prototype extractor (Figure 2). During the distillation process, the water vapor drags the chemical components present in the sample, then passes through a condenser (10°C - 15°C), and the condensate is deposited in a collecting flask. The process occurred at a fixed power of 800W for 10 min (parameters determined in preliminary tests).

A Clevenger-type apparatus was used in the hydrodistillation process for 180min and it was used 100g of each fraction (coarse and thin). For both methodologies, the essential oil obtained was centrifuged and dried with Na_2SO_4 and stored in a refrigerator at 8°C. The essential oil yield (%) was expressed as the percentage of oil in relation to the dry matter of the leaves.

Figure 2- Adapted microwave oven components.



2.3 CHEMICAL COMPOSITION ANALYSIS

The chemical composition was analyzed by gas-phase chromatography coupled to mass spectrometry (GC-MS) using a THERMO DSQ II GC–MS instrument, equipped with a fused-silica

capillary column DB-5 ms (30 m × 0.25 mm; 0.25 μm film thickness) under the following conditions: programmed temperature: 60–240 °C (3 °C·min^{-1}); injector temperature: 250 °C; carrier gas: helium, adjusted to a linear velocity of 32 cm·s^{-1} (measured at 100 °C); injection type: splitless (2 μL of a 1:1000 hexane solution); split flow was adjusted to yield a 20:1 ratio; septum sweep was a constant 10 mL·min^{-1}; EIMS: electron energy, 70 eV; the temperature of the ion source and connection parts: 200 °C. A FOCUS GC/FID was used to describe the quantitative data of the volatile constituents, using the same conditions employed in the GC–MS, except for the carrier gas, which was nitrogen. The retention index was calculated for all the volatiles constituents using an *n*-alkane (C8–C40, Sigma–Aldrich) homologous series. The chemical constituents were identified as described by Silva et al. (2018), using (Adams, 2007) as literature.

2.4 EXPERIMENTAL DESIGN

Microwave power, irradiation time (extraction time), and moisture content are some important parameters analyzed during the microwave extraction (Singh Chouhan et al., 2019). In the present study, it was used a commercial microwave oven adapted as a prototype extractor. Therefore, a factorial design (4 factorial planning experiments and 4 replicates, totaling 8 experiments) was used to evaluate the influence of the entrance variables fraction diameter (F_R) and load of material plant/water volume (LW) on the response variables essential oil yield (Y_d) and dillapiole content (DC) during the process of obtaining of *P. aduncum* essential oil. The levels assigned to the variables of the entrance are listed in Table 1 and all the experiments were carried out randomly (Box et al., 2005). The statistical analyses were conducted using the software Statistica® version13 (TIBCO Software Inc., USA), considering significance level $\alpha = 0.05$ (confidence of 95%) and pure error. The proposed model and its response surface were obtained to examine the relations between the entrance and response variables.

3 RESULTS AND DISCUSSION

The average diameters of coarse and thin leave fractions were 3.63 ± 0.23 mm and 0.82 ± 0.11 mm, respectively. The moisture content of the samples was 15.69 ± 0.01% on wet basis. The hydrodistillation process was conducted during 180 min and the highest values for essential oil yield and dillapiole content were equal to 2.72% and 87.63%, respectively. Microwave-assisted extraction was performed for 10 min at 800W and presented a maximum dillapiole content (91.07%), and the essential oil yield ranged from 0.23 to 1.27% (Table 1).

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Table 1 - Experimental conditions and results for hydrodistillation and microwave-assisted extraction process.

	Coded variables		Real variables		Hydrodistillation		Microwave	
Experiment	X_1	X_2	F_R (mm)	LW (g/mL)	Y_d	DC	Y_d	DC
1	-1	-1	0.82	1:7	1.26	81.05	0.94	84.29
2	1	-1	3.63	1:7	2.69	83.35	0.23	90.82
3	-1	1	0.82	1:5	2.14	84.12	0.41	89.58
4	1	1	3.63	1:5	1.62	80.29	1.27	89.47
5	-1	-1	0.82	1:7	2.72	87.08	0.94	90.47
6	1	-1	3.63	1:7	2.69	81.65	0.72	91.07
7	-1	1	0.82	1:5	2.27	87.63	0.81	86.59
8	1	1	3.63	1:5	2.34	80.41	1.07	88.63

F_R = average fraction diameter (mm); LW = load of material plant/water volume (g/mL); Y_d = essential oil yield (%); DC = dillapiole content (%).

3.1 EFFECTS ON ESSENTIAL OIL YIELD (Y_D)

The classical methods of obtaining essential oils are the hydrodistillation and steam distillation, which are carried out for 2h to 3h. The microwave-assisted extraction process described in the present study consumed only 10 min at 800W. However, microwave-assisted extraction provided essential oil yields of *P. aduncum* lower than those obtained through hydrodistillation.

For hydrodistillation, the essential oil yields of *P. aduncum* varied between 1.26 to 2.72% (Table 1). The analysis of variance (ANOVA) (Table 2) for the response variable essential oil yields (Yd) displayed that in the hydrodistillation process, the entrance variables X_1 and X_2 and its combination X_1X_2 were not statistically significant for the Y_d response ($F_{1,4} = 7.71$; $p \leq 0.05$) (Box et al., 2005).

Table 2 – Analysis of variance for essential oil yield.

	Responses						
	Hydrodistillation– Y_d				Microwave – Y_d		
Source	df	SS	F-value	*p*-value	SS	F-value	*p*-value
X_1	1	0.1128	0.3384	0.5919	0.0045	0.0820	0.7888
X_2	1	0.1225	0.3675	0.5771	0.0666	1.2109	0.3329

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X_1X_2	1	0.4278	1.2833	0.3206	0.5253	9.5489	0.0366
Pure error	4	1.3334			0.2201		
Total	7	1.9966			0.8165		

$F_{tab} = 7.71$

Andrade et al. (2009) collected specimens of *P. aduncum* from Amazon region and subjected them to hydrodistillation. They reported yields from 1.1 % (Parauapebas, Pará, Brazil) to 4.0% (Manaus, Amazonas, Brazil). Corral et al. (2018) obtained a yield of 3.12% for the leaves of *P. aduncum* from Manaus (Brazil). Valadares et al. (2018) used the hydrodistillation for 2h to evaluate the yields of essential oils from *P. aduncum* (Goiás, Brazil). The inflorescences and leaves resulted in yields of 0.42% and 0.34%, respectively.

For the microwave-assisted extraction process, only the combination X_1X_2 caused significant effects on the Y_d (Table 2). Therefore, a polynomial model was proposed from the regression analysis with the respective coefficients of the entrance variables and their combinations (Equation 1). The proposed model provided $R^2 = 0.73$ and residues in the interval of -0.25 to 0.25 and the absence of systematic pattern, indicating that homoscedasticity condition (equality of variances) was established. The response surface of the model proposed for Y_d (Figure 3), displayed that the highest essential oil yields were obtained when the entrance variables corresponded to level +1. Thus, it was observed that particles with a diameter of 3.63 ± 0.23 mm (coarse leaves fraction) and a load of material plant/water volume in the proportion of 1:5 favored the essential oil yield. The thin leave fraction presented an average diameter of 0.82 ± 0.11 mm, characteristic of a powdered material, which may have caused compaction of the material in the boiling flask, resulting in lower essential oil yields.

Water molecules have dipolar properties, so the moisture in the sample and the water added to the sample, affects the microwave-absorbing ability, which facilitates the heating and improves the extraction efficiency. When microwaves pass through a sample, the water molecules absorb energy which increases the temperature and pressure. At internal pressure limit, the cells can withstand and releases the essential oil, which is evaporated together with the internal water of the plant material and the water that acts as a solvent (Asofiei et al., 2017; de Castro and Castillo-Peinado, 2016; Lucchesi et al., 2004).

$$Y_d = 0{,}80 + 0{,}02X_1 + 0{,}09X_2 + 0{,}26X_1X_2 \quad (1)$$

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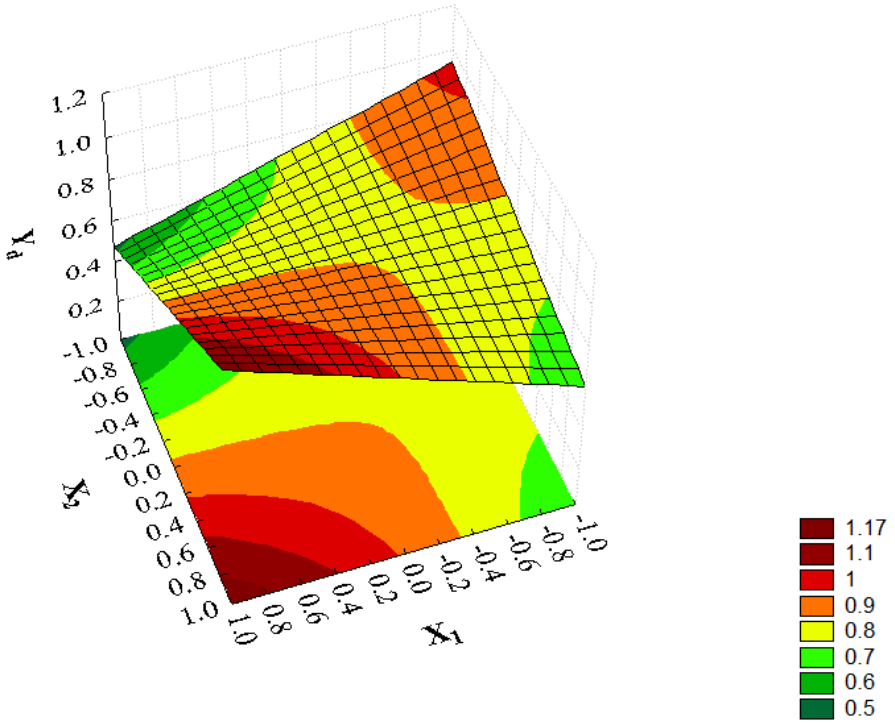
Figure 3 – Response surface for the essential oil yield model.



Moradi et al. (2018) compared the hydrodistillation and microwave-assisted hydro-distillation (MAHD) of essential oil from *Rosmarinus officinalis* (rosemary). For MAHD, it was used a sample of 100*g* of, 300mL of water, and 30 min of operation, which resulted in an essential oil yield of about 1.5%. Kusuma and Mahfud (2018) evaluated the effect of the presence and absence of additional airflow in the microwave hydrodistillation method. In the conventional microwave hydrodistillation, the oil yield of sandalwood obtained was 1.2184 ± 0.1139%. In contrast, for microwave air-hydrodistillation, the increasing airflow (0.1 – 5.0L/min^{-1}) provides higher yields (1.2248 ± 0.1037% – 1.3170 ± 0.0973%). Both methodologies were conducted at 600W.

3.2 DILLAPIOLE CONTENT (DC)

According to Maia et al., (2001), *Piper* species from Amazon, are rich in phenylpropanoids (apiole, dillapiole, myristicin, elemicin, eugenol, methyl eugenol, and safrole), which are responsible for their biological activity.

The *P. aduncum* essential oils obtained using hydrodistillation displayed an average of 83.19 % of dillapiole content. Nevertheless, for microwave-assisted extraction, it was possible to achieve the maximum dillapiole content (91.07%) when the coded variables X_1 was +1 and X_2 was -1, i.e., the

fraction diameter was 3.63 mm (coarse leaves fraction) and a load of material plant/water volume was 1:7 (30g of coarse leaves fraction and 210 mL of water), as showed on Table 1. Dillapiole compound is described by its anti-inflammatory (Parise-Filho et al., 2011), insecticide (Fazolin et al., 2016), and as an antileishmanial agent (Parise-Filho et al., 2012).

Regardless of the distillation method applied, both the individual variables and their combination do not present significance in the response dillapiole content (DC) ($F_{1,4} = 7{,}71$; $p \leq 0{,}05$) (Box et al., 2005). According to the experimental conditions used in the present study, the variables fraction diameter (F_R) and load of material plant/water volume (LW); not influenced the DC (Table 3). Therefore, dillapiole was the major constituent found in the essential oil of *P. aduncum*, for both methods used.

Table 3 – Analysis of variance for dillapiole content.

	Response						
	Hydrodistillation – DC				Microwave – DC		
Source	df	SS	F-value	*p*-value	SS	F-value	*p*-value
X_1	1	25.1340	3.8987	0.1196	10.2604	1.7136	0.2606
X_2	1	0.0578	0.0090	0.9291	0.7080	0.1182	0.7482
X_1 X_2	1	7.8408	1.2160	0.3320	3.3800	0.5645	0.4942
Pure error	4	25.7927			23.9503		
Total	7	58.8253			38.2988		

$F_{tab} = 7.71$

Dillapiole was the major compound identified in different essential oils of *P. aduncum.* Silva et al. (2013) evaluated the dillapiole content as a function of the plant densities (83.98-86.13%) and cutting periods (85,23-85.57%). Corral et al. (2018) reported 92% of dillapiole, while Maia et al. (1998) studied some *P. aduncum* species distributed in the Amazon region and found that dillapiole was the main compound, ranging from 31.5 to 97.3 %. Barros et al. (2016) described a *P. aduncum* essential oil composed by (*E*)- caryophyllene (9.7%), β-cubebene (9.5%), γ-gurjunene (9.3%), and α- farnesene (8.2%). Meanwhile, piperitone (23.4 %), myristicin (12.4 %), and terpinen-4-ol (12.3 %) were the majority compounds identified in the oils from *P. aduncum* collected in Goiás (Brazil) (Valadares et al., 2018). In another study, some *P. aduncum* specimens were collected in different locations of Brasilia (Brazil) and the essential oil chemical composition was characterized by trans-

β-ocimene, bicyclogermacrene, safrol, and sarisan (group 1), while β-phelandrene, trans-β-ocimene, piperitone, γ-terpinene, and 4-terpineol composed the group 2 (Potzernheim et al., 2012).

The main methods for obtaining essential oils are hydrodistillation and steam distillation, but new methodologies have been developed to reduce extraction time, solvent, and energy consumption. Dillapiole was the major component of essential oils obtained through hydrodistillation and microwave-assisted extraction. Nevertheless, the chemical composition of *P. aduncum* may vary depending on location, stage of development, secretory structures, harvest time, climate and other factors (Figueiredo et al., 2008; Gelmini et al., 2015; Gobbo-Neto and Lopes, 2007; Jezler et al., 2013).

4 CONCLUSION

Microwave-assisted extraction was performed in a time interval of 10min and its best results for essential oil yield and dillapiole content were 1.27% and 91.07%, respectively. Hydrodistillation provided higher essential oil yields, but lower levels of the constituent dillapiole. The essential oil yield obtained from microwave-assisted extraction was adequately described using a polynomial model. The highest essential oil yields of *P. aduncum* were obtained using the 30g of coarse leaves fraction and 150 mL of water, using the microwave-assisted methodology.

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