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COMPARISON OF CHEMICAL COMPOSITION VARIATION AND ESSENTIAL OIL YIELD OBTAINED FROM *OCIMUM GRATISSIMUM* LEAVES FROM THE CAATINGA AND ATLANTIC FORESTS

COMPARAÇÃO DA VARIAÇÃO DA COMPOSIÇÃO QUÍMICA E DO RENDIMENTO DE ÓLEOS ESSENCIAIS OBTIDOS DAS FOLHAS DE OCIMUM GRATISSIMUM DA CAATINGA E MATA ATLÂNTICA

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ABSTRACT

Ocimum gratissimum is a medicinal and aromatic species cultivated worldwide. This study compares the variability of essential oil content and the percentage of its components in two biomes in the state of Bahia, Brazil. Leaves of this species were collected from a farm in the Atlantic Forest and urban gardens in the Caatinga biome. The chemical composition of the oils derived from these samples was analyzed by thin-layer chromatography (TLC) and gas chromatography–mass spectrometry (GC-MS). The average oil content was 2.02% ± 0.33% (Caatinga biome) and 2.05% ± 0.47% (Atlantic Forest region). There were no differences between the samples from the two different biomes. The TLC analysis revealed similar qualitative features among the samples. It was possible to identify 12 substances in the oils by GC. The major component identified was eugenol (64.61%

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± 7.45% Caatinga; 37.74% ± 6.39% Atlantic Forest). Eucalyptol was identified only in the Atlantic Forest (15.64% ± 5.59%). Caryophyllene values were 3.44% ± 0.54% (Caatinga) and 8.25% ± 2.73% (Atlantic Forest). For α-pinene, the values were 6.49% ± 3.35 % (Caatinga) and 0.94% ± 0.56% (Atlantic Forest). The essential oil yields and chemical composition were like those described in the literature. Thus, according to the parameters analyzed, samples from Caatinga and the Atlantic Forest (Bahia, Brazil) met the quality requirements; therefore, it is possible to obtain a suitable plant drug to prepare herbal medicines from those regions, considering the parameters analyzed here.

Keywords: chemical variability; quality control; vegetable drug; gas chromatography; thin-layer chromatography

RESUMO

Ocimum gratissimum é uma espécie medicinal e aromática cultivada mundialmente. Este estudo visa comparar a variabilidade do teor de óleo essencial e a porcentagem de seus componentes em dois biomas do estado da Bahia. As folhas dessa espécie foram coletadas em uma fazenda na Mata Atlântica e em jardins urbanos do bioma Caatinga. A composição química dos óleos derivados dessas amostras foi analisada por cromatografia em camada delgada (CCD) e por cromatografia em fase gasosa acoplada à espectrometria de massa (CG-EM). O teor médio de óleo foi de 2,02% ± 0,33% (Caatinga) e 2,05% ± 0,47% (Mata Atlântica). Não foram observadas diferenças entre as amostras dos dois biomas. A análise de CCD revelou características qualitativas semelhantes entre as amostras. Foi possível identificar 12 substâncias nos óleos por CG-EM. O principal componente identificado foi o eugenol (64,61% ± 7,45% Caatinga; 37,74% ± 6,39% Mata Atlântica). O eucaliptol foi identificado apenas na Mata Atlântica (15,64% ± 5,59%). Os valores do cariofileno foram 3,44% ± 0,54% (Caatinga) e 8,25% ± 2,73% (Mata Atlântica). Para α-pineno os valores foram 6,49% ± 3,35% (Caatinga) e 0,94% ± 0,56% (Mata Atlântica). Os rendimentos e a composição dos óleos

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essenciais obtidos foram semelhantes às descritas na literatura. Assim, de acordo com os parâmetros analisados, amostras da Caatinga e da Mata Atlântica atenderam aos requisitos de qualidade. Portanto, conclui-se que é possível obter uma matéria prima vegetal adequada para o preparo de fitoterápicos nessas regiões, considerando os parâmetros aqui analisados.
Palavras-chave: variabilidade química; controle de qualidade; droga vegetal, cromatografia gasosa, cromatografia em camada delgada

INTRODUCTION

Ocimum gratissimum is present in the National List of Medicinal Plants of nterest to Sistema Único de Saúde (SUS) because it is widely used in Brazil and is of therapeutic interest (VILANOVA *et al.*, 2019). *O. gratissimum,* popularly known as “alfavaca,” is used for cooking and in folk medicine (ALBUQUERQUE, DE; ANDRADE, 1998). The volatile oil extracted from *O. gratissimum* has activity against many types of pathogens (CHIMNOI *et al.*, 2018; LEMOS, J. De A. *et al.*, 2005; NGASSOUM *et al.*, 2003). The oil also exhibits anesthetic and analgesic activities (SILVA, L. De L. *et al.*, 2012; RABELO *et al.*, 2003). The essential oil content is influenced by the genetic material, culture conditions, and the environment (GOBBO-NETO; LOPES, 2007; GOMES *et al.*, 2019; GUIMARÃES *et al.*, 2020). Monoterpenoids and sesquiterpenoids are widespread substances present in essential oils that are affected by environmental factors, such as light quality and intensity, temperature, drought, plant pathogens, ozone, seasonality, mineral nutrients, and developmental stage of the plant (REHMAN *et al.*, 2016; REHMAN; ASIF HANIF, 2016). Another factor is that many species produce secondary metabolites with distinct chemical phenotypes called chemotypes. At least seven chemotypes exist for *O. gratissimum,* such as the eugenol, thymol, and linalool chemotypes (KUMAR *et al.*, 2019; OLUGBADE; KOLIPHA-KAMARA; CHRISTIANAH ABIMBOLA ELUSIYAN, GRACE OSARUGUE ONAWUNMI, 2017).

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Water and thermal availability accelerate the growth and development of *O. gratissimum* (COSTA FILHO; ENCARNAÇÃO; OLIVEIRA, 2006). There is also an influence of sunlight on eugenol production in this plant. Eugenol content is higher when the collection is performed between 11 and 13 h (SILVA, M. G. De V. *et al.*, 1999).

By observing the possible variation in terms of *Ocimum gratissimum* leaves essential oil composition, this work aims to compare essential oil variability of content and the percentage of its components in two biomes in the state of Bahia. The results may be relevant for the quality control of herbal medicine produced in the Caatinga and Atlantic Forest (Bahia, Brazil).

MATERIAL AND METHODS

Plant material

Sample of *O. gratissimum* leaves were collected in June in an urban garden (14°50'34.436"S, 40°52'18.260"W) in a transitional region between Caatinga and the Atlantic Forest, Vitória da Conquista city, Bahia, and in a farm (13°22′20″S, 39°22′16″W) in Presidente Tancredo Neves city, Bahia (Atlantic Forest). The samples were dried at 40°C for 10 h and stored in paper bags. Voucher specimens of *O. gratissimum* collected in Vitória da Conquista (# HVC 724) were deposited at the Herbarium Mongoyós in the University of Bahia, campus Vitória da Conquista. Samples from Presidente Tancredo Neves were identified by comparison with the authentic sample. The authors had registered access to the Brazilian System for the Management of Genetic Heritage and Associated Traditional Knowledge–SISGEN (# AB58325) for this study.

Variability in essential oil content

To determine the essential oil yield, samples were subjected to hydro-

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distillation for 4 h, using a Clevenger-type apparatus with 0.5 mL of xylene to dissolve the essential oil extracted, according to the Brazilian Pharmacopoeia procedure (BRASIL, 2010). After extraction, the essential oil was directly measured in the extraction apparatus, and the content (%) was calculated as volume (mL) of essential oil per 100 g of dry plant material. Average, standard deviation, and relative standard deviation (RSD) were calculated for the samples obtained from each region. Comparisons between results obtained for each biome's samples were performed by the analysis of variance (ANOVA), applying Tukey's test ($p < 0.05$) to compare mean values, using the GraphPad Prism software, version 5. The essential oils were stored in a freezer before conducting further chromatographic analyses.

Analysis of the chemical profile of essential oil by thin-layer chromatography

Thin-layer chromatography (TLC) was used to verify the essential oil profile using 250-mm thick silica gel GF254 (BRASIL, 2010). The ratio of mobile phase was hexane/ethyl acetate (8:2). The plates were visualized with an anisaldehyde spray solution. The chemical profiles were compared qualitatively using thymol and eugenol (Sigma-Aldrich) as a reference substance.

Qualitative analysis by gas chromatography–mass spectrometry (GC-MS) and gas chromatography– flame ionization detector (GC-FID)

The essential oils were analyzed using a Shimadzu QP2010 gas chromatography apparatus (Shimadzu, Tokyo, Japan) directly interfaced with MS and FID, equipped with 5% diphenyl, 95% dimethyl polysiloxane capillary column (30 m × 0.25 mm, 0.25 µm of film thickness). Helium was the carrier gas. The mass detector for the GC-MS system was operated in electron-impact mode, with a scan range of 45−500 amu, ionization energy of 70 eV, and a scan rate of 0.30 s per scan. The temperatures of the ionization source and the injector were maintained at

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200°C and 240°C, respectively. Other GC conditions, such as the flow rate, concentration of the sample, and separation temperature program, were optimized to provide better separation of components in a shorter run time. Chemical constituents were identified by comparing their mass spectra with those of known compounds available in the 2008 National Institute Standard and Technology (NIST) database and Flavour and Fragrances of Natural and Synthetic Compounds (FFNSC) 1.3 library (Wiley and Sons, Somerset, NJ, USA). The identification was further supported by calculating the retention index (RI) under identical experimental conditions using the n-alkanes (C_{10}-C_{40}) series (Sigma-Aldrich). The RI was calculated for each main compound and compared to the literature. Reference substances present as major compounds in *O. gratissimum* essential oils (eugenol and thymol) were injected to confirm identification.

The relative percentage of each compound in the leaf oil was calculated by integrating the corresponding peak area, which was automatically performed using a software (GCMSsolution®). The integration of each peak was checked and manually corrected, if necessary. The composition was reported as a relative percentage of total peak area. Average, standard deviation, and RSD (%) of the relative proportion areas were calculated for each major peak identified and their respective retention times.

RESULTS AND DISCUSSION

Variability in essential oil yield

The average percentage of essential oil yield obtained from *O. gratissimum* samples in the Caatinga transitional region was 2.02% ± 0.33% (RSD = 16.30%), and that from the Atlantic Forest biome was 2.05% ± 0.47% (RSD = 23.05%) (range = 1.30%–2.87%. A comparison between biomes revealed no significant difference ($p = 0.8830$). All analyzed *O. gratissimum* samples had much higher oil yields compared to the values found in the literature (0.13%–0.80%), but those

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percentages were calculated on a fresh weight basis (CHIMNOI et al., 2018; CORTEZ, D. A. G. et al., 1998; KATARA et al., 2013; KISHORE DUBEY et al., 2000; KUMAR et al., 2019). Therefore, *O. gratissimum* samples grown in the Brazilian transitional region between Caatinga and the Atlantic Forest and its regions of Bahia state are suitable for use in herbal medicine preparations.

TLC analysis of the essential oil chemical profile

The TLC profiles of the *O. gratissimum* essential oil samples (Fig. 1) demonstrated the presence of eugenol (retention factor (RF) = 0.47) in almost all samples. Thymol (Rf = 0.56) was detected in only one sample from the Caatinga biome.

Figure 1: TLC profiles of the *O. gratissimum* essential oil samples

GC-MS and GC-FID analyses

After testing and evaluating several chromatographic parameters (sample concentration, inlet mode, flow rate, and the temperature separation program), the optimized parameters were a flow rate of 1.5 mL min 1, a concentration of 2.5% of the sample in dichloromethane, and a temperature program column as described in Table 1. Using this method, it was possible to separate all substances in *O. gratissimum* within 20 min as shown in Figure 2. This method was more advantageous than other methods described in the literature for *O. gratissimum* because of its shorter analysis time (KUMAR et al., 2019; OLUGBADE; KOLIPHA-KAMARA; CHRISTIANAH ABIMBOLA ELUSIYAN, GRACE OSARUGUE ONAWUNMI, 2017; SARTORATTO et al., 2004).

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Table 1: Temperature program for the gas chromatographic analysis of the essential oils

Rate (°C/ min)	Final temperature (ºC)	Hold time (min.)
-	80	2
30	130	0
10	150	0
2	156	1
2	170	0
50	240	1

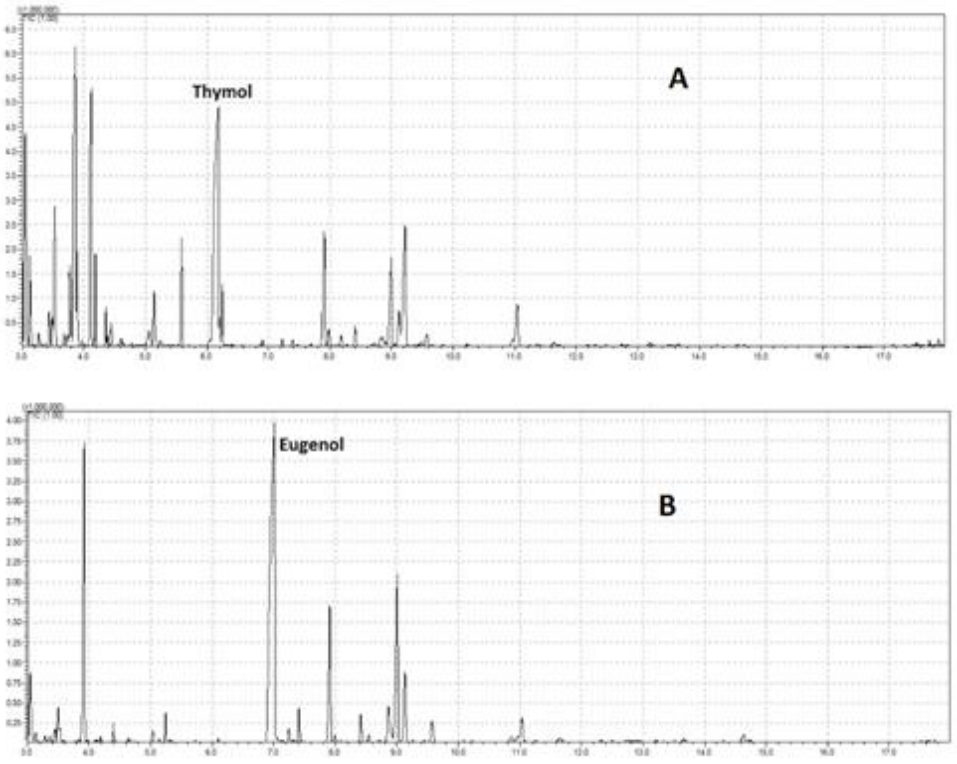

Figure 2: Gas chromatography–mass spectrometry chromatogram of *Ocimum gratissimum* leaf essential oil (A–thymol profile, B–eugenol profile)

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Twelve compounds were identified in the essential oil by comparing the RI (literature and RI taken from NIST 2008 library) and mass spectra of each peak with the NIST and 1.3 FFNSC libraries (similarity >90%). The major compounds identified in *O. gratissimum* were eugenol (**8**), caryophyllene (**10**), and eucalyptol (**5**). Thymol (**7**) was found in only one sample from the transitional biome **(Table 2)**. Some minor components were also identified (**Table 2)**. Analyses of the samples were performed by the peak area normalization. **Table 2** shows the average, standard deviation, and RSD (%) values of the relative proportional area (%).

Table 2: Relative proportional area of *Ocimum gratissimum* essential oil main components

Compound	**Rt[a]**	**RI[b]**	**RI[c]**	**Caatinga biome**	**Atlantic forest biome**
				Average of relative proportion area (%) ± SD[d] (RSD[e])	**Average of relative proportion area (%) ± SD[d] (RSD[e])**
α-Pinene (**1**)	3.20	923	924 (COULADIS *et al.*, 2003)	6.49 ± 3.35 (51.69)	0.94 ± 0.56 (59.89)
Sabinene (**2**)	3.46	987	987 (PINO; MARBOT; VAZQUEZ, 2004)	0.75 ± 0.22 (29.82)	0.91 ± 0.35 (38.02)
β-Pinene (**3**)	3.50	990	987 (HÖGNADÓTTIR; ROUSEFF, 2003)44	-	2.48 ± 1.06 (42.57)

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Mircene (**4**)	3.59	998	994 (HÖGNADÓTTIR; ROUSEFF, 2003)	2.33 ± 2.56 (109.76)	1.07 ± 1.19 (110.49)
Eucalyptol (**5**)	3.93	1054	1053 (EL-GHORAB; FADEL; EL-MASSRY, 2002)	-	15.64 ± 5.59 (35.74)
α- Terpineol (**6**)	5.24	1192	1189 (SENATORE *et al.*, 2006)	-	1.86 ± 0.78 (41.77)
Thymol (**7**)	6.23	1321	1327 (EL-GHORAB; FADEL; EL-MASSRY, 2002)	28.58 ± 1.13 (3.96)	-
Eugenol (**8**)	6.99	1334	1330 (LIU, J. *et al.*, 2006)	64.61 ± 7.45 (11.52)	37.74 ± 6.39 (16.94)
α-Copaene (**9**)	7.26	1341	1349 (LIU, J. *et al.*, 2006)	-	0.77 ± 0.13 (17.19)
Caryophyllene (**10**)	7.93	1415	1415 (MARONGIU *et al.*, 2007)	3.44 ± 0.54 (15.67)	8.25 ± 2.73 (33.14)
Humulene (**11**)	8.43	1455	1455 (MARONGIU *et al.*, 2007)	-	1.76 ± 0.61 (34.65)

[a] RT–Retention time (min.)
[b] RI–Retention indices on Rtx®-5MS (5% diphenyl, 95% dimethyl polysiloxane) capillary, experimentally determined using homologous series of C_{10}–C_{40} n-alkanes
[c] RI–Retention indices from the literature
[d] SD–Standard deviation (%)
[e] RSD–Relative standard deviation (%)

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Table 3 shows the results of a comparison between the relative percentages of *O. gratissimum* essential oils obtained in this study and those reported in the literature. The major compounds reported in the literature were like those observed in this study.

Table 3: Comparison between relative percentages from gas chromatography–mass spectrometry and the *Ocimum gratissimum* literature

Reference	Compound											
	(1)	**(2)**	**(3)**	**(4)**	**(5)**	**(6)**	**(7)**	**(8)**	**(9)**	**(10)**	**(11)**	**(12)**
Current study Caatinga biome	6.49	0.75	-	2.33	-	-	28.58	64.61	-	3.44	-	0.41
Current study Atlantic forest biome	0.94	0.91	2.48	1.07	15.64	1.86	27.39	37.74	0.77	8.25	1.76	1.67
(JOSHI *et al.*, 2013)	0.1	0.3	0.1	tr.	-	0.1	0.5	75.1	0.7	0.9	-	-
(SARTORATTO *et al.*, 2004)	-	-	-	-	-	-	-	93.9	-	-	-	-
(OLUGBADE; KOLIPHA-KAMARA; CHRISTIANAH ABIMBOLA ELUSIYAN, GRACE OSARUGUE ONAWUNMI, 2017)[a]	tr.	-	-	-	-	-	60.5	-	-	-	-	-
(OLUGBADE; KOLIPHA-KAMARA; CHRISTIANAH ABIMBOLA ELUSIYAN,	0.7	0.5	1.9	-	-	-	42.2	-	-	-	-	-

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GRACE OSARUGUE ONAWUNMI, 2017)[b]												
(MOHR *et al.*, 2017)	0.53	0.60	1.25	0.83	-	tr.	-	7.42	tr.	1.68	tr.	-
(BENITEZ; MELÉNDEZ LEÓN; STASHENKO, 2009)[c]	0.95	-	-	0.65	32.71	-	-	43.71	-	-	-	-
(BENITEZ; MELÉNDEZ LEÓN; STASHENKO, 2009)	1.20	-	-	0.9	12.8	1.7	-	43.20	-	-	1.70	-
(CHIMNOI *et al.*, 2018)	-	-	-	0.13	-	-	-	55.55	-	-	-	0.05
(SILVA, M. G. De V. *et al.*, 1999)	-	-	1.3 – 3.70	-	2.50 – 75.50	1.10 – 1.80	-	11.40 – 98.00		1.30-11.60	-	-
(OGENDO *et al.*, 2008)	0.39	0.69	1.21	0.33	1.59	-	-	2.43	0.61	5.14	0.39	-
(SILVA, L. De L. *et al.*, 2012)	-	-	-	-	-	-	-	73.6	-	-	-	4.8

[a] Sierra Leone
[b] Nigeria
[c] Brazil
[d] Colombia
tr. = traces

CONCLUSIONS

Essential oils yield from *O. gratissimum* leaves were much higher compared to values found in the literature. Chemical composition was like those described in the literature; thus, according to the parameters analyzed, samples from Caatinga and the Atlantic Forest (Bahia, Brazil) met the quality requirements. We conclude

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that it is possible to obtain a suitable plant drug to prepare herbal medicines from these regions. Furthermore, this work obtained a GC-MS analysis method with a much shorter analysis time than others found in the literature for this analysis.

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