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Original article

Rupture of glandular trichomes in *Ocimum gratissimum* leaves influences the content of essential oil during the drying method

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ABSTRACT

Medicinal and aromatic plants are commonly sold dried; however, it is necessary to understand the effects different drying methods have on these materials, to ensure their efficiency and quality. *Ocimum gratissimum* L., Lamiaceae, is an aromatic plant whose essential oil is stored in glandular trichomes. This study aimed to confirm the effects of different drying temperatures and methods of *O. gratissimum* leaves on trichome integrity and essential oil content. Leaves dried in a forced ventilation oven at 60°C display damaged trichomes and a reduction in the essential oil content. The different drying methods (oven, dehumidification and air drying) were not identified to elicit changes in the essential oil content or damage to trichomes. All of the drying methods showed a reduction in fungal contamination in a logarithmic cycle.

Introduction

Freshly harvested medicinal plants occupy large volumes of space and pose difficulties for transportation and storage. The reduction of the water content of freshly harvested medicinal plants is imperative for handling and storage purposes, given that by reducing the water content, the material becomes easier to handle and less susceptible to microorganisms (Tanko et al., 2005). The drying method can be performed by either a natural or an artificial method, depending on the source of heat and use of energy. Drying reduces the enzymatic activity inhibiting chemical reactions as hydrolysis, oxidation, and fermentation (Costa et al., 2005). Nevertheless, the drying method can be catastrophic to medicinal plants if not properly conducted, like drying using extensive high-temperature that can cause both physical and chemical changes (Tanko et al., 2005). Desiccation must be carried out immediately after harvest to avoid enzymatic degradation. However, if it is done too quickly it can damage secondary metabolites, and if it is done too slowly, it can facilitate the proliferation of bacteria and fungi. Solar drying is not recommended, as it commonly leads to photodecomposition reactions (Barbosa et al., 2006).

Medicinal and aromatic plants may present high microbial contamination due to the plant's contact with the soil, as well as by the pre and post-harvest handling (Zaroni et al., 2004). The reduction in the quality of the active principle is related not only to enzymatic degradation, but also to the action of the microorganisms that can grow in the harvested plants. The drying method may represent a means through which to contain the microbial growth, thus avoiding changes in metabolite content of the plants caused by microorganisms.

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Volatile aroma compounds are the most sensitive components to the drying methods. The effect of desiccation of various aromatic plants and vegetables has been the subject of numerous studies, which have shown that changes in the content of these compounds depend on several factors, such as the drying method and characteristic parameters of the plant material (Venskutonis, 1997; Costa et al., 2005; Barbosa et al., 2006, Borsato et al., 2008).

Among the *Ocimum* genus, *O. gratissimum* L., Lamiaceae, popularly known as ornamental basil (Prabhu et al., 2009), is of economic and medicinal importance due to the biological activity attributed to its essential oil, of which the eugenol is the most widely known and studied component due to its medicinal and cooking uses (Almeida and Albuquerque, 2002; Matasyoh et al., 2007). The essential oil of *O. gratissimum* is stored in glandular trichomes, which are external and sensitive structures that can undergo changes during desiccation (Gang et al., 2001), affecting the essential oil content and composition.

Although *O. gratissimum* is a species with a well-studied chemical composition (Silva et al., 1999; Keita et al., 2000; Vieira and Simon, 2000; Matasyoh et al., 2007; Singh, 2012), little is known about the influence of the drying method on the quality of the raw material sold on the market.

The present study aims to analyze the effect of the different drying temperatures and drying methods of *O. gratissimum* leaves on trichome integrity, essential oil content and chemical composition, and possible morphological damage and fungal contamination in relation to treatment.

Materials and methods

Plant material

The plants (*Ocimum gratissimum* L., Lamiaceae) were cultivated in the Medicinal Plant Garden of the Universidade Estadual de Santa Cruz. The plants were harvested in the morning during October 2011 (after 180 days of cultivation) for experiments applying different drying temperatures in forced ventilation ovens; and in January 2012 (240 days of cultivation) for experiments related to different types of drying processes. After harvest, the plants' leaves were removed and selected, discarding those considered sick and/or attacked by insects. The specimens were identified by Larissa C. do B. Costa, and a voucher specimen was deposited in the UESC Herbarium of the Universidade Estadual de Santa under voucher number 2494.

Drying of plant material

Two separate experiments were carried out to evaluate the influence of temperatures and drying methods on the content and chemical composition of the essential oil from *O. gratissimum* leaves.

Drying temperatures in a oven with forced air

The fresh leaves (400 g) were dried in an oven with forced air (Marconi MA035) at four drying temperatures; 30, 40, 50, and 60°C, respectively. The drying process was monitored by decreasing moisture content until a constant weight was reached.

Drying methods

The fresh leaves (400 g) were dried by three different drying methods: oven with forced air (Marconi MA035) at 50°C; dehumidifier (Arsec model 160 with a controlled room temperature at 22°C), and at room temperature averaged at 30°C. The methods were monitored according to decreasing mass content, until they reached a constant mass.

Extraction of essential oil

The essential oil from the dry leaves (15 g) was extracted by hydrodistillation, using a Clevenger apparatus for 90 min. The oil was separated using ethyl ether, dried with anhydrous sodium sulfate (Na_2SO_4) and further concentrated. The content was determined by the essential oil mass, expressed in mass/mass percentage (g of essential oil/100 g of dry plant material) for four repetitions. The essential oils were cooled at -10°C until undergoing chromatographic analysis.

Chemical composition of the essential oil

The quantitative analysis of the chemical composition was performed by gas chromatography equipped with flame ionization detector (GC-FID), using the Varian Saturn 3800 gas chromatograph with a fused silica capillary VF5-MS column (30 m × 0.25 mm). The stationary phase was 5% phenyl-95% dimethylpolysiloxane (0.25 µm of film thickness), with helium as a carrier gas at a flow rate of 1.2 $ml.min^{-1}$. The temperatures of the injector and detector were 250°C and 280°C, respectively, and 1.0 µl of 10% methanol solution was added, in triplicate, in the *split* mode (1:10). The temperature of the column started at 70°C and increased $8°C{\cdot}min^{-1}$ until reaching 200°C, and then increased $10°C{\cdot}min^{-1}$ until reaching 260°C. The column was then kept at this temperature for another 5 min. An Agilent 5975C mass spectrometer was used to perform the qualitative analysis of the oil. The column and the programming conditions were the same as those used for the GC-FID analysis. The method used was the 70 eV electronic impact at a scan velocity of 1/s within a range of 40 to 450 *m/z*. The analysis and identification of the oil's components was performed using the fragmentation patterns observed in the mass spectra, compared with NIST 8.0 database. Identification was also supported by the comparison of Retention Indices, obtained by injecting a mixture of C_8-C_{26} alkanes, acquired from Sigma-Aldrich (USA), with data from the literature (Adams, 2007).

Integrity of trichomes

A micromorphological analysis of the leaf's surface was performed using JEOL JSM-6390V scanning electron microscopy (SEM), to evaluate the integrity of glandular trichomes. Samples of the median region of the dried leaves were fixed in glutaraldehyde, dehydrated, submitted to critical point drying (BAL-TEC, model CPD030), and then coated in gold (Sputter Coater, BAL-TEC, model SCD050). The images were recorded

in five repetitions at 160x magnification to determine the quantity of ruptured, deflated, and intact glandular trichomes.

Microbiological contamination

Leaf samples (10 g) were weighed in aseptic conditions and homogenized with 90 ml of saline solution (0.85%). The mixture was left to rest for 10 min. The material was then homogenized and serial dilutions (up to 10^{-4}) in saline solution (0.85%) were made. From each dilution, 0.1 ml was transferred and spread on Sabouraud Agar plates containing chloramphenicol (antibiotic) and incubated at 25ºC for seven days. After this period, the filamentous fungi colonies were counted, and the results were expressed in UFC·g^{-1} of the sample.

Statistical analysis

Both experiments were arranged in a randomized blocks with four repetitions. Four drying temperatures in an oven with forced air were evaluated (30, 40 50 and 60°C) and three drying methods (oven at 50°C, dehumidifier and ambient). For each repetition 15 g of dry mass of the leaves were used. An analysis of variance (ANOVA) was used to compare the averages followed by Tukey's test at 5% significance to evaluate the variables: moisture content, essential oil content and chemical composition of the essential oil. A polynomial regression analysis was applied to determine the moisture reduction variable.

Results and discussion

Drying temperatures in a oven with forced air ventilation

The present study observed the effect of temperature on the drying time regarding the plant material's mass loss. The regression analysis of the loss of mass, compared to the drying time were applied in a quadratic form of the different temperatures tested, in the attempt to reach a final average mass of 22.2%, thus indicating that the experiments held similar parameters. This study required 96 h of drying at 30°C to reach constant mass, while at 50 and 60°C, only 48 h of desiccation are needed (Fig. 1).

Drying under forced air ventilation is considered to be the most efficient method for medicinal and aromatic plants (Simões and Spitzer, 2004); however, the ideal temperature can vary greatly among species. To dry *O. basilicum* and *O. selloi*, the recommended temperature is 40ºC (Carvalho-Filho et al., 2006; David et al., 2006; Soares et al., 2007), for *Cymbopogon winterianus* it is 60°C (Rocha et al., 2000), and for *Lippia alba* the ideal temperature ranges between 40 and 80°C (Barbosa et al., 2006).

No significant difference could be identified in the essential oil contents of *O. gratissimum* extracted from dry leaves at temperatures of 30, 40, and 50°C (Fig. 2). However, there was a reduction of 48% of the content obtained from leaves dried at 60°C, indicating a loss of volatile components (Fig. 2).

The chemical analyses of the essential oils of the *O. gratissimum* leaves submitted to different drying temperatures had similar chemical profiles (Table 1).

Fifteen chemical constituents, among phenylpropanoids, monoterpenes, oxygenated monoterpenes, sesquiterpenes, and oxygenated sesquiterpenes, were found. The main component was eugenol (81.07-83.45%) and showed no significant variation among the tested temperatures. Significantly, (Z)-β-ocimene, a monoterpene, was found to be at its lowest content (8.14-12.19%) at a temperature of 60°C, most likely to be associated with a greater volatility of monoterpenes when compared to the other classes of compounds. In smaller quantities, germacrene D (2.23-3.27%), a sesquiterpene, was identified. No significant variations of phenylpropanoids, monoterpenes, and oxygenated monoterpenes were observed at different temperatures; however, variation in content of sesquiterpenes and oxygenated sesquiterpenes were observed (Table 1). The desiccation of the medicinal plants is necessary for preservation, but the volatile components of the essential oil may be very sensitive to drying procedures affecting the properties of the plant material (Tanko et al., 2005).

The *O. gratissimum* leaves display peltate and capitate trichomes. According to Gang (2001) the volatile oil

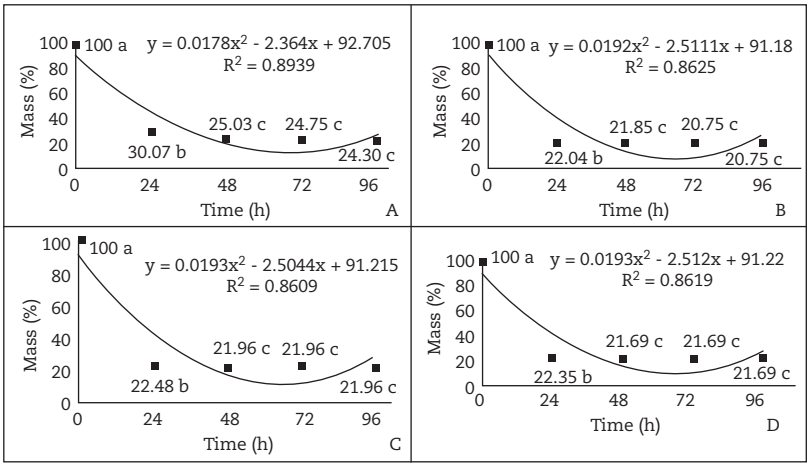

Figure 1 – Mass content of *Ocimum gratissimum* submitted to different drying temperatures in a forced air oven, A, 30°C; B, 40°C; C, 50°C, D, 60°C.

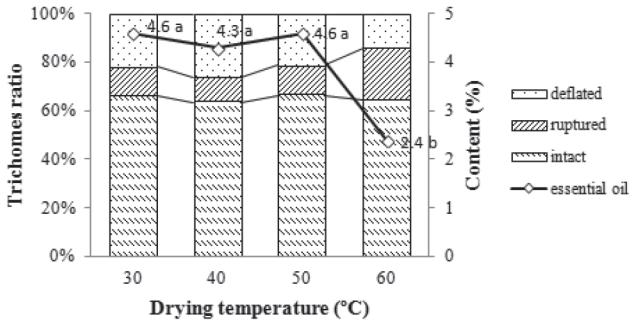

Figure 2 – Proportion of trichomes and essential oil content of *Ocimum gratissimum* submitted to different drying temperatures in a forced air ventilation oven. Mean values followed by the same letter are not significantly different according to Tukey's test ($p > 0.05$).

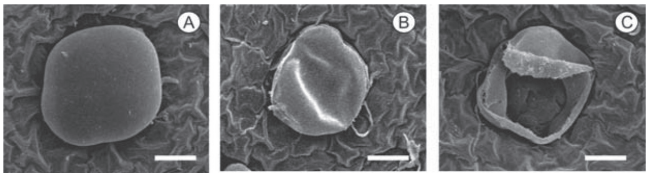

Figure 3 – SEM photomicrographs showing glandular trichomes of *Ocimum gratissimum* leaf submitted to different drying temperatures in a forced air ventilation oven: A, intact; B, deflated; C, ruptured. Scale = 25 µm.

constituents, mainly eugenol, accumulates in the peltate trichomes. Different drying temperatures kept the integrity of the peltates trichomes in both adaxial and abaxial surfaces, but significant difference was observed in the abaxial leaf surface.

This study verified the effect of temperature on the proportion of intact and damaged (ruptured or deflated) trichomes related to *O. gratissimum*'s essential oil content (Fig. 3).

The proportion of intact or damaged trichomes (deflated and ruptured) does not change among treatments, however, at temperatures of 30, 40, and 50°C, a greater number of deflated trichomes was observed; nevertheless, at 60°C this proportion decreased due to the increase in ruptured trichomes. The essential oil content showed a similar behavior, presenting a significant reduction only at 60°C, coinciding with the increased rupture of trichomes (Fig. 2). Since these are external and very fragile structures, trichomes become vulnerable to external conditions, principally those related to the harvesting, beneficiation, and drying methods. Likewise, glandular trichomes on floral structures of *Chamomilla recutita* were progressively affected by the drying method, which creates a predisposition to essential oil loss, in turn progressively reducing the moisture and essential oil content of chamomile during the drying method (Borsato et al., 2008).

Drying methods

A quadratic adjustment of mass loss could be observed in relation to the drying time for the different drying methods and no significant differences were identified. All of the samples

Table 1
Relative percentages of the constituents from the essential oil of *Ocimum gratissimum* submitted to different drying temperatures in a forced ventilation oven.

Compounds	RI	Temperature (°C)			
		30	40	50	60
	930	0.11 ± 0.00	—	—	—
sabinene	978	0.31 ± 0.19	0.36 ± 0.01	—	—
mircene	991	0.30 ± 0.07	0.33 ± 0.01	0.34 ± 0.00	0.33 ± 0.00
(Z)-β-ocimene	1038	11.88 ± 2.16	12.19 ± 0.23	8.38 ± 2.61	8.14 ± 1.52
(E)-β-ocimene	1048	0.77 ± 0.13	0.68 ± 0.01	0.49 ± 0.00	0.37 ± 0.06
trans-4-thujanol	1070	0.44 ± 0.03	0.44 ± 0.08	0.66 ± 0.07	0.68 ± 0.08
terpinen-4-ol	1181	0.44 ± 0.07	0.50 ± 0.01	0.39 ± 0.00	0.70 ± 0.08
eugenol	1367	81.78 ± 2.52	81.07 ± 0.79	83.45 ± 3.27	82.16 ± 0.78
α-copaene	1382	0.29 ± 0.09	0.38 ± 0.03	0.52 ± 0.06	0.59 ± 0.07
β-bourbonene	1392	0.43 ± 0.03	0.50 ± 0.03	0.67 ± 0.03	0.78 ± 0.02
(E)-caryophyllene	1427	0.88 ± 0.06	0.99 ± 0.06	1.57 ± 0.13	1.59 ± 0.25
Germacrene D	1486	2.23 ± 0.17	2.34 ± 0.02	3.27 ± 0.12	3.28 ± 0.47
δ-cadinene	1526	0.24 ± 0.00	—	0.36 ± 0.12	0.35 ± 0.00
caryophyllene oxide	1589	0.21 ± 0.00	0.33 ± 0.00	0.48 ± 0.01	0.65 ± 0.11
cedr-8(15)-en-9-α-ol	1659	—	—	0.37 ± 0.03	0.42 ± 0.11
Total identified (%)		100.31 ± 0.56	100.1 ± 0.04	100.9 ± 0.75	100.1 ± 0.30
Compound class	**C.V**	**Relative percentual**			
Phenylpropanoids	2.60	81.78[a]	81.07[a]	83.45[a]	82.16[a]
Monoterpenes	20.15	13.37[a]	13.56[a]	9.21[a]	8.84[a]
Oxygenated monoterpenes	28.65	0.88[a]	0.94[a]	1.05[a]	1.38[a]
Sesquiterpenes	14.21	4.07[a]	4.21[a]	6.39[b]	6.63[b]
Oxygenated Sesquiterpenes	73.37	0.21[a]	0.33[b]	0.85[b]	1.07[c]

RI, Retention indices calculated against C_8-C_{26} n-alkanes on the VF-5ms column. Percentages obtained by FID-peak normalization. C.V, Variation coefficient ; —, not detected. Mean values followed by the same letter in line are not significantly different, according to Tukey's test ($p > 0.05$).

reached the same final mass content (Fig. 4), indicating that the experiments had reached similar parameters.

The chemical analyses of the essential oils obtained by the different drying methods presented the same chromatographic profile of the essential oils extracted at different drying temperatures in a forced ventilation oven. Fourteen compounds were identified (Table 2), with the main component being eugenol (84.28-85.14%), followed by (Z)-β-ocimene (7.13-9.32%) and germacrene D (2.65-3.36%). A significant variation could be observed in monoterpenes, with the greatest quantity observed when plants were dried at room temperature. No significant variations were observed for other classes of compounds. The eugenol content, the main constituent of the oil, suffered a minor variation by the different drying methods. The reduction of the volatile compounds during the drying method depends on the volatility and chemical structure of the plants' constituents (Barbosa et al., 2006).

The minor changes in the proportion of intact and damaged trichomes observed among the different drying methods was not enough to interfere with the average content and quality of the essential oil (Fig. 5).

Between the different drying methods, fungal contamination was evaluated by counting the filamentous fungal colonies present in the leaf samples of the different treatments compared to those produced by fresh leaves of *O. gratissimum*. This study found contaminations of 2.1×10^4 CFU·g^{-1} at leaves dried under a forced ventilation oven at 50°C; 6.9×10^4 CFU·g^{-1} at samples dried in a dehumidifier; 7.6×10^4 CFU·g^{-1} at samples dried at room temperature; and of 4.16×10^5 CFU·g^{-1} in the fresh leaves sample. A significant difference, determined by Tukey's test, were observed when compared to fungal contamination of the fresh leaves among the different drying methods. However, no difference in fungal contamination degree could be observed within the different drying methods. Therefore, the different drying methods decreased, in a logarithmic cycle, the count of fungal colonies of the dried leaves of *O. gratissimum* when compared to the fresh leaves, in accordance with findings reported by Zaroni et al. (2004). This reduction in the number of fungal colonies observed in the dry samples may be beneficial to the maintenance and quality of essential oils within a given sample.

The leaves of the *O. gratissimum* can be dried in a forced ventilation oven at up to 50°C, with a dehumidifier and at ambient temperature without significantly changing the content and composition of the essential oil. Taking into consideration the greater drying time, the preservation of trichomes, and the content of essential oils, the forced ventilation oven at 50°C is the most highly recommended method.

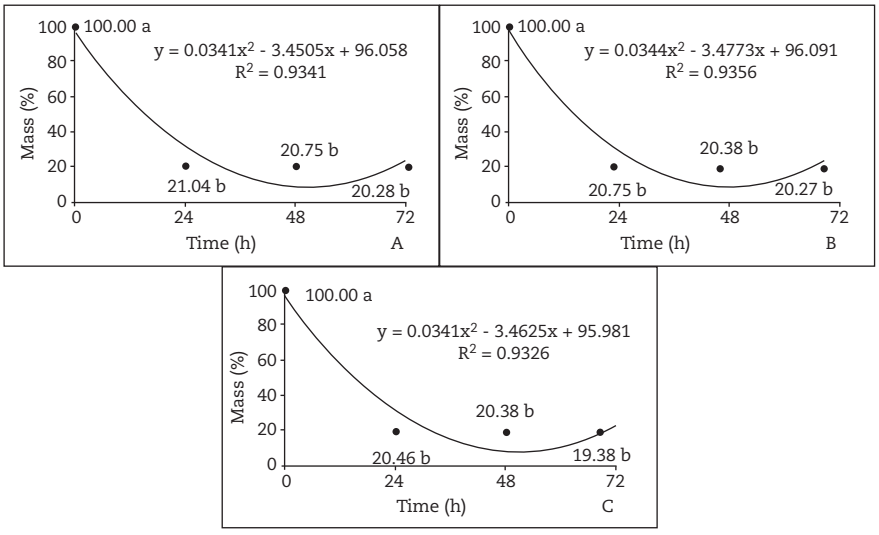

Figure 4 – Mass content of *Ocimum gratissimum* submitted to different drying methods. A, dehumidifier; B, ambient; C, oven at 50°C.

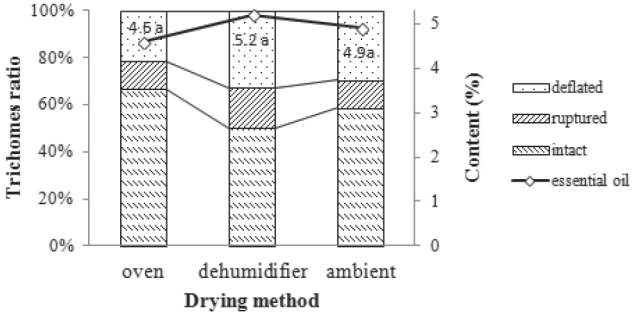

Figure 5 – Proportion of trichomes and essential oil yield of *Ocimum gratissimum* submitted to different drying methods. Mean values followed by the same letter are not significantly different according to Tukey's test ($p > 0.05$).

Table 2
Relative percentages of the constituents from the essential oil of *Ocimum gratissimum* submitted to different drying temperatures in a forced ventilation oven.

Compounds	RI	Drying methods		
		Oven at 50°C	Dehumidifier	Ambient
sabinene	978	0.29 ± 0.03	0.21 ± 0.01	0.25 ± 0.07
mircene	991	—	0.06 ± 0.00	0.09 ± 0.00
(*Z*)-β-ocimene	1038	7.13 ± 0.52	7.82 ± 0.09	9.32 ± 0.24
(*E*)-β-ocimene	1048	0.40 ± 0.03	0.48 ± 0.02	0.46 ± 0.12
trans-4-thujanol	1070	0.45 ± 0.06	0.35 ± 0.04	0.42 ± 0.01
terpinen-4-ol	1181	0.44 ± 0.01	0.28 ± 0.02	0.42 ± 0.06
eugenol	1367	84.28 ± 0.44	85.14 ± 0.15	84.36 ± 0.26
α-copaene	1382	0.39 ± 0.03	0.26 ± 0.02	0.31 ± 0.03
β-bourbonene	1392	0.67 ± 0.01	0.47 ± 0.03	0.54 ± 0.05
(*E*)-caryophyllene	1427	1.63 ± 0.01	1.23 ± 0.05	1.39 ± 0.11
germacrene D	1486	3.36 ± 0.49	3.13 ± 0.21	2.65 ± 0.18
δ-cadinene	1526	0.31 ± 0.02	0.25 ± 0.01	0.27 ± 0.01
caryophyllene oxide	1589	0.38 ± 0.03	0.20 ± 0.02	0.31 ± 0.02
cedr-8(15)-en-9-α-ol	1659	0.28 ± 0.01	0.13 ± 0.01	0.15 ± 0.00
Total identified (%)		100.01 ± 0.16	100.01 ± 0.08	100.94 ± 0.04
Compounds class	**C.V**	**Relative percentual**		
Phenylpropanoids	1.80	84.28[a]	85.11[a]	84.36[a]
Monoterpenes	18.36	7.82[a]	8.57[a]	10.12[b]
Oxygenated monoterpenes	6.49	0.99[a]	0.63[a]	0.84[a]
Sesquiterpenes	8.33	6.36[a]	5.34[a]	5.16[a]
Oxygenated Sesquiterpenes	21.33	0.66[a]	0.33[a]	0.46[a]

RI, Retention indices calculated against C_8-C_{26} n-alkanes on the VF-5ms column. Percentages obtained by FID-peak normalization. C.V, Variation coefficient; —, not detected.
Mean values followed by the same letter in line are not significantly different, according to Tukey's test ($p > 0.05$).

Authors' contributions

ACMS (PhD student) contributed in the harvest, drying and extraction of the essential oil, ran the laboratory work, analyzed the data and drafted the paper. LCBC contributed in cultivation, plant identification, herbarium confection, micro morphological analysis, data analysis and drafted the paper. GSP contributed to chromatographic analysis. APTU and CMB contributed in the microbiological analysis. RAO and LCBC designed the study, supervised the laboratory work and contributed to critical reading of the manuscript. All the authors have read the final manuscript and approved the submission.

Conflicts of interest

The authors declare no conflicts of interest.

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