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Content and chemical composition of the essential oils of *Aristolochia cymbifera* Mart. and Zucc. extracted from leaves submitted to different temperatures

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The drying of plant materials ensures the preservation of the active constituents of medicinal plants. This experiment was conducted at the Federal Institute of Education, Science and Technology at the Goiás, Rio Verde Campus. The study sought to evaluate the effects of three drying air temperatures on the content and chemical composition of the essential oils of *Aristolochia cymbifera* (*A. cymbifera*). Drying was performed in a fixed bed dryer with average temperatures of 28.8, 36.4 and 44.8°C with four replicates used for each temperature. By increasing the drying air temperature, the drying time was decreased without altering the essential oil content or chemical composition.

Key words: Content of essential oil, medicinal plants, drying conditions, extraction.

INTRODUCTION

The *Aristolochia cymbifera* Mart. and Zucc., also commonly known in Brazil as *jarrinha*, *milhomem* or *cassaú*, is a species of the genus *Aristolochia* (Aristolochiaceae). This plant is an herbaceous perennial vine and is characteristically vigorous. The species is best adapted to hot environments, is native to Brazil and contains mono/diterpenes and sesquiterpenoids in its leaves, stems and roots (Lorenzi and Matos, 2002).

Plants of this genus have been extensively studied mainly because of their pharmacological properties as well as pesticide, antibiotic and antifungal activities (Barbosa et al., 2006). In folk medicine, *A. cymbifera* Mart. and Zucc. is used to treat various health problems and is considered a diuretic, sedative and antiseptic (Lorenzi and Matos, 2002).

The essential oils of species of the genus *Aristolochia* have been demonstrated to contain monoterpenoid linalool, the sesquiterpene derivatives of farnesane, bisabolene, elemane, germacrane, bicyclogermacrene, humulane, aristolane, caryophyllales, eudesmane, cadinane, guainane, aromadendrane, cubebane, himachalane, santalane, copaane and bourbonane. All of these compounds are found in varying quantities depending on the specific species. The essential oils β-caryophyllene, germacrene A, α-farnesene α-trans-bergamotene and α-cubebene have also been detected in *A. cymbifera* Mart. and Zucc. (Urzúa and Sotes, 2008).

The quality of the essential oils from medicinal plants depends on several factors such as climatic variations, the type of soil, harvest time, genetic characteristics of the plant, drying conditions and storage time (Corrêa et al., 2004; Blank et al., 2007; Gobbo-Neto e Lopes, 2007). The final quality of the essential oil begins with the correct identification of the species as well as the planting,

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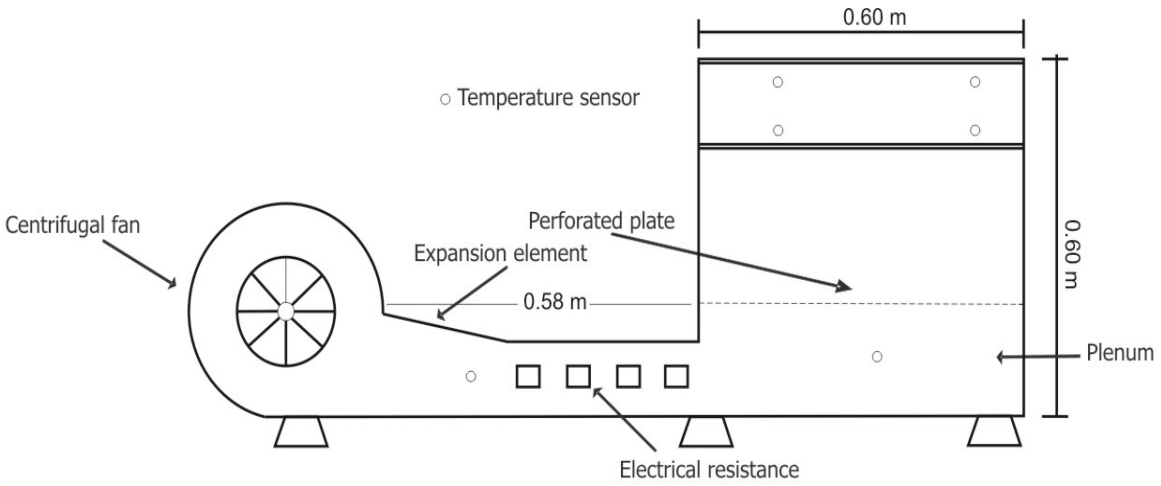

Figure 1. Lateral view of the experimental dryer.

harvesting and processing (Barbosa et al., 2006).

Drying is performed to preserve the plants and maintain their physical and chemical quality for long periods of time. For plants that produce essential oils, drying should be carefully performed due to the high volatility of essential oils. Therefore, the most appropriate drying methodology must be defined for each species to ensure the levels of the active substances (Corrêa et al., 2004).

In addition to the aforementioned features, the quality of an essential oil depends on its processing. The extractive yields of the essential oils of *Ocimum basilicum* L. were obtained when the drying process was conducted with air temperatures equal to 40°C. However, the highest linalool yield was obtained when the drying process was conducted with air temperatures ranging from 50 to 60°C (Soares et al., 2007). Nevertheless, drying of the material is not always appropriate for all samples, e.g., studies have shown that there is a declining trend in the oil content of *Mikania glomerata* Sprengel with increasing temperature when compared with the fresh plant (Rocha et al., 2011).

Based on these previous results, the objective of this present study was to evaluate the effects of three drying air temperatures on the content and chemical composition of the essential oils of *A. cymbifera*.

MATERIALS AND METHODS

Collection and selection of plant

The experiment was conducted at the Laboratories of Postharvest of Plant Products and Plant Tissue Culture of the Federal Institute of Education of Goiano, Science and Technology (Instituto Federal de Educação, Ciência e Tecnologia Goiano - IF Goiano) - Rio Verde Campus, using leaves of *A. cymbifera* Mart. and Zucc. The leaves were obtained in the city of Rio Verde – GO, Brazil.

A. cymbifera plants were collected in the region of Rio Verde at the coordinates S 17°55'56.8", W 50°56'33.2" and an altitude of 682 m between 17:00 and 18:00 h during the month of April, 2012. The exsiccate is recorded in the Jataiense Herbarium under accession number 5,642.

Plants were harvested by cutting the shoots at 5 cm above the ground, packing them in plastic bags and then sending them to the natural products division of the Laboratory of Plant Tissue Culture of the IF Goiano – Rio Verde Campus. After harvest, the plants were subjected to defoliation and selection, and those plants that were in poor health or had been attacked by insects were discarded.

Determination of the moisture content

The moisture content was determined before and after drying according to a methodology for forage and similar materials (plants or leaves) (ASAE, 2000). To determine the moisture content, the leaves were placed in an oven with forced air circulation at a temperature of 103±2°C for 24 h. Four replicate samples were assessed.

The initial moisture content of the leaves was 68.95% wet basis (% wb). During the drying process, the samples were weighed periodically until a moisture content of approximately 10.54 (% wb) was attained.

Drying

Drying was performed in a fixed bed dryer, which was made of 16-gauge sheet metal. The drying chamber had the following dimensions: 0.60 × 0.60 × 0.60 m, with a total volume of 0.216 m^3. A centrifugal fan, driven by an alternate current (AC) motor with a power of 1.5 cv and 1720 rpm rotation, consisted of a rotor, blades, volute and support. The connection between the drying chamber and the fan consisted of an expansion element that transforms a section measuring 0.20 × 0.20 m at the blower outlet to 0.57 m × 0.03 m at the entrance of the drying chamber over a length of 0.64 m (Figure 1).

Each dryer contained six temperature sensors and four 1,500 watts electrical resistors for a total of 6,000 watts. The sensors were positioned before and after the resistors and inside each tray. Four removable trays with perforated bottoms and dimensions of 0.28 × 0.28 × 0.15 m were placed in the drying chamber (Figure 2). The system also included an automatic controller to manage the system and store data.

The leaves of *A. cymbifera* were wrapped in a voile-like fabric

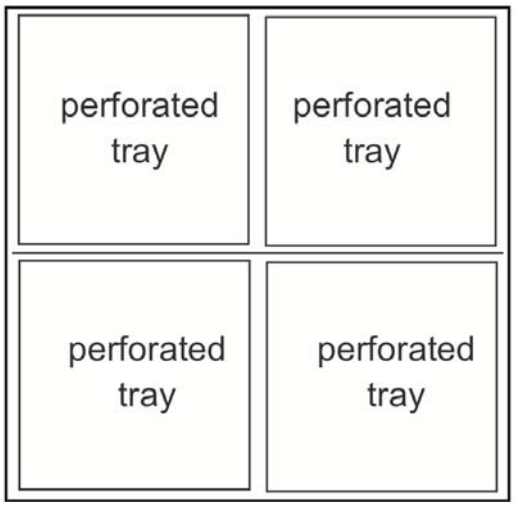

Figure 2. Top view of the experimental dryer - detail of perforated trays.

and spread on trays, forming a layer of approximately 0.06 m thickness. Two treatments were regulated for heating to 36.4±1.32 and 44.8±0.76°C, while another treatment used only ambient air without heating (28.8±1.30°C), with the air velocity controlled at 1.0 m s^{-1} and average relative humidity levels of 40.31, 25.95 and 60.88%, respectively.

The reduction in water weight during the drying process was monitored by the gravimetric method (weight loss), based on the initial water content of the product, until the level reached the desired water content. The monitoring of the mass reduction during drying was performed using a balance with resolution of 0.01 g.

The drying air temperatures were monitored by thermocouples installed inside and outside of the dryer. The ambient temperature and relative humidity were monitored using an integrated digital thermo-hygrometer with a precision of 3%, where the relative humidity inside the dryers was obtained by means of the basic principles of psychrometrics using the software GRAPSI (Melo et al., 2004).

Obtaining the essential oil

Essential oil extraction was performed using a Clevenger apparatus adapted to a 3 L volumetric flask. The sample was placed in the flask together with 2 L of distilled water. Approximately 60 g of dried leaves ground in a Willye TE - 648 micro mill (TECNAL) were used. The extraction time was 150 min counted from the time of boiling.

The essential oils were extracted from the aqueous phase using dichloromethane (3 × 6 ml; 20 min each extraction). The obtained organic fractions were pooled and supplemented with anhydrous sodium sulfate. After 30 min, the sulfate was removed by filtration. The mass of the obtained essential oil was determined by weighing on an analytical balance with a resolution of 0.0001 mg.

Oil samples were transferred to amber glass bottles, capped with aluminum foil containing small holes to allow for evaporation of the solvent and stored in a refrigerator at 4 to 8°C until the analysis.

Chemical analysis by gas chromatography/mass spectrometry

The chemical analyses were performed at the Department of Chemistry of the Federal University of Lavras (Universidade Federal de Lavras), Lavras-MG-Brazil, using gas chromatograph coupled to a quadrupole mass spectrometer (GC-MS). The Shimadzu QP5050A (Kyoto, Japan) instrument was operated under the following conditions: fused silica capillary column, model DB-5 (30 m length × 0.25 mm internal diameter × 0.25 µm film thickness) (Shimadzu, Japan), helium carrier gas with flow rate of 1 ml/mi, and heating according to the temperature program 60°C with a gradient of 3°C min^{-1} to 240°C followed by a gradient of 10°C min^{-1} to 270°C, maintaining an isotherm for 7 min with a total run time of 70 min. The detector ionization energy was 70 eV, and the sample injection volume was 1.0 ml diluted in dichloromethane (ultra-pure grade, Baker, USA) with an injection ratio of 1:20. The injector and detector were maintained at 220 and 240°C, respectively. The analysis was conducted in scanning mode at a rate of 2.0 scans s^{-1} with a mass interval of 45 to 500 *m/z*.

Statistical analysis

The experimental design consisted of randomized blocks with three air drying temperatures. Each treatment consisted of four replicates totaling 12 experimental units. The experimental results were submitted to an analysis of variance ($F < 0.05$), and means were compared by the Tukey test at 5% significance using the program SISVAR (Ferreira, 2011).

RESULTS AND DISCUSSION

The average temperatures inside the dryer containing the leaves for the three drying temperatures, as well as the room temperature and relative humidity data, are as shown in Figure 3. Note that the average temperatures inside the dryer were 44.8±0.76, 36.4±1.32 and 28.8±1.30°C. The average room temperature and relative humidity were 26.1±1.7°C and 71.3±6.87%, respectively. The difference between the ambient air temperatures (26.1°C) and the temperatures of the dryer without heating (28.8°C) was attributed to heating promoted by the centrifugal fan blades.

The leaves of *A. cymbifera* were dried until reaching moisture contents of 10.16, 9.74 and 11.72 (% wb) for the 44.8, 36.4 and 28.8°C treatments, respectively. These values are within the recommended range for different pharmacopoeias, that is, between 8 and 14 (% wb) (Farias, 2003).

The drying curves for the different air temperatures are as shown in Figure 4.

Note that as the temperature of the drying air increased, the water was removed from *A. cymbifera* leaves more quickly, resulting in a reduced drying time of 5.00, 10.58 and 58.13 h for the temperatures of 44.8, 36.4 and 28.8°C, respectively. Heating of the air reduced the drying time relative to the treatment without heating (28.8°C) by 5.49- and 11.63-fold for the temperatures of 36.4 and 44.8°C, respectively.

Studies have demonstrated the influence of the drying air temperature on the content and chemical composition of essential oils from *Lippia alba* (Mill) N. and Brown, wherein increases in temperature reduce the drying time. For example, drying times of 31.42, 3.42, 1.83, 1.17, 0.75 and 0.55 h were observed for drying at room temperature

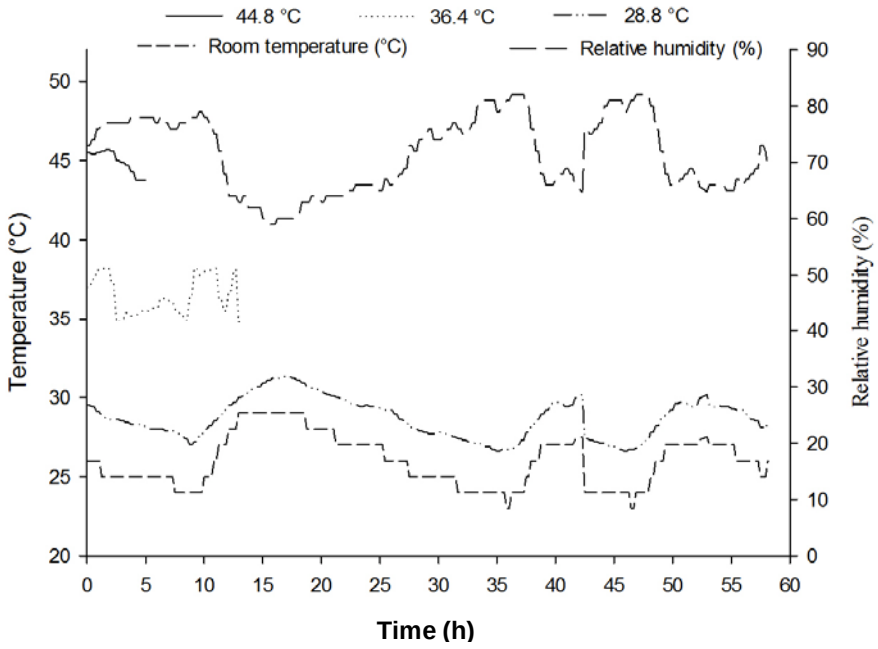

Figure 3. Temperatures inside the trays containing leaves of *Aristolochia cymbifera* Mart. and Zucc. for the three air drying temperatures. The room temperature and relative humidity are also shown.

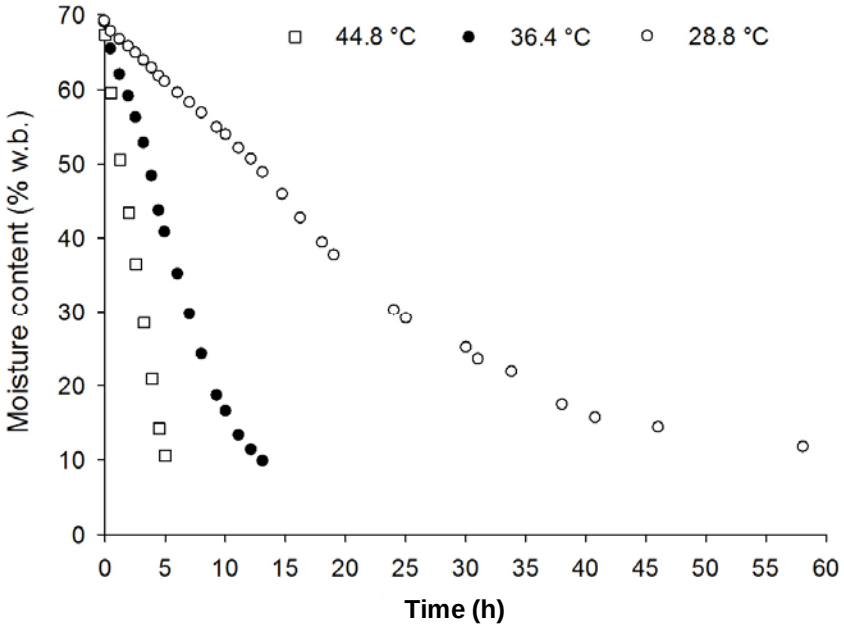

Figure 4. Drying curves for *Aristolochia cymbifera* Mart. and Zucc. with different air drying temperatures.

(25±1°C) and ambient temperatures of 40, 50, 60, 70 and 80°C, respectively (Barbosa et al., 2006).

The measured essential oil contents of *A. cymbifera* leaves obtained using different drying temperatures are as shown in Figure 5.

The essential oil content of *A. cymbifera* was not affected when using drying air temperatures of 28.8, 36.4 and 44.8°C. A similar result was obtained for the extraction yield of essential oils from rosemary pepper (*Lippia sidoides* Cham.) using a fixed-bed experimental

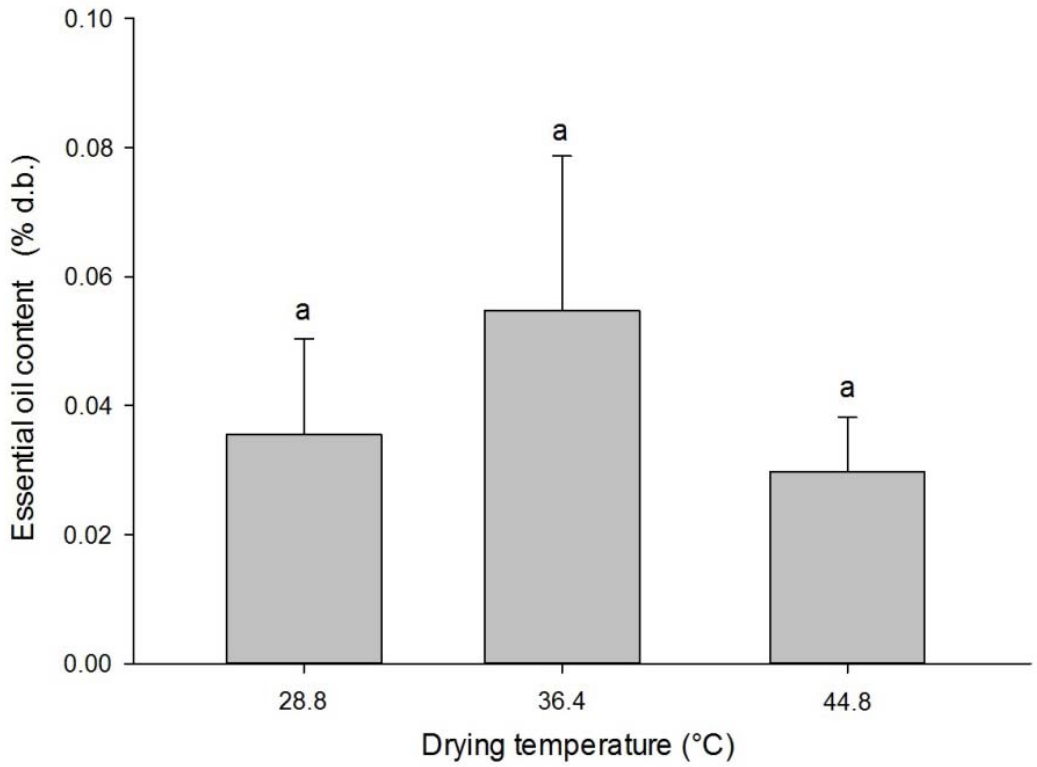

Figure 5. Essential oil contents extracted from leaves of *Aristolochia cymbifera* Mart. and Zucc. submitted to drying at different air temperatures.

dryer at ambient temperature, 40, 50, 60 and 70°C (Radünz et al., 2002).

This trend is different from that was observed for the essential oil contents of *O. basilicum* L. when evaluating the influence of four drying temperatures (40, 50, 60 and 70°C) and two air velocities (0.9 to 1.9 m s^{-1}). In this study, the essential oil content was influenced by both the velocity and the temperature of the drying air, indicating that the oils of *O. basilicum* L. undergo volatilization (Soares et al., 2007).

The chemical composition of the essential oils extracted from the leaves of *A. cymbifera* at different drying temperatures is presented in Table 1.

The chemical analysis results confirmed that all identified components were not influenced by the drying conditions, including the major constituents of the essential oil from *A. cymbifera*, bicyclogermacrene, spathulenol, (E)-nerolidol, δ-cadinene, α-himachalene and viridiflorol, which represent approximately 67.93, 65.52 and 69.93% of the oil obtained from plants dried at 44 8, 36.4 and 28.4°C, respectively. It was also observed that in all treatments the total identified components in the essential oil was more than 94% (Table 1).

These results indicating that the essential oil components were not influenced by the drying air temperature confirming previous studies on the major constituents of oil from *L. alba* (Mill) N. E. BROWN, that is, nerol, neral, genariol and geranial, that did not differ at different drying temperatures (40, 50, 60, 70 and 80°C, and room temperature) (Barbosa et al., 2006).

The major component of *A. cymbifera* was bicyclogermacrene at concentrations ranged from 21.32 to 23.13%. This compound is a sesquiterpenoid with antifungal activity (Silva et al., 2007). The component with the second highest concentration was spathulenol, ranging from 14.09 to 16.72%; this compound has the odor of dry wood and can be used for food flavoring and in sophisticated perfumes. Spathulenol can also be applied in foods, medicines, toothpastes, soaps, detergents, cleaning agents, cosmetics and solutions for skin treatment (Naarden, 1985; citado por Mendes et al., 2008).

Conclusion

The increased air temperature from 28.8 to 44.8°C decreased the drying time of leaves of *A. cymbifera* Mart. and Zucc. from 58.13 to 5 h. With heating, there is a 5.49- and 11.63-fold reduction in the drying time at 36.4 and 44.8°C, respectively.

Temperature of the drying air did not influence the content of the extracted essential oils, and the identified chemical compounds were not affected by the various

Table 1. Chemical composition of the essential oils from leaves of *Aristolochia cymbifera* Mart. and Zucc. submitted to three drying air temperatures.

Compound	IKc	Drying temperatures		
		44.8	36.4	28.8
(E)-Nerolidol	1561	10.84±0.37[a]	11.38±1.48 [a]	10.26±0.59[a]
Alloaromadendrene	1458	1.76±0.11[a]	1.95±0.25[a]	1.94±0.16[a]
Aromadendrene	1439	3.09±1.95[a]	4.50±2.45[a]	5.31±1.92[a]
Bicyclogermacrene	1500	22.50±0.33[a]	21.32±2.11[a]	23.13±1.84[a]
Cedrol	1597	0.32±0.24[a]	0.76±0.18[a]	0.54±0.23[a]
Cyclosativene	1368	2.55±0.10[a]	2.37±0.87[a]	1.67±0.21[a]
Spathulenol	1575	15.14±0.83[a]	14.09±1.27[a]	16.72±1.17[a]
Farnesol (cis, cis)	1715	0.27±0.14[a]	0.27±0.07[a]	0.22±0.17[a]
Germacrene B	1559	3.13±0.22[a]	3.44±0.17[a]	3.38±0.23[a]
Lanceol	1759	0.23±0.20[a]	0.20±0.20[a]	0.33±0.08[a]
Caryophyllene oxide	1582	1.46±0.73[a]	2.36±0.83[a]	1.32±0.61[a]
Valencene	1494	2.44±1.64[a]	0.12±0.21[a]	1.05±1.53[a]
Viridiflorene	1494	0.64±0.18[a]	0.77±0.16[a]	0.80±0.24[a]
Viridiflorol	1589	4.99±0.47[a]	4.55±1.23[a]	4.77±2.40[a]
α-Copaene	1368	1.36±0.15[a]	1.27±0.47[a]	0.98±0.13[a]
α-Curcumene	1480	0.31±0.14[a]	0.50±0.11[a]	0.61±0.06[a]
α-Himachalene	1448	5.86±0.33[a]	6.64±1.16[a]	5.95±0.58[a]
α-Humulene	1452	1.01±0.99[a]	0.48±0.08[a]	0.49±0.06[a]
α-Muurolene	1489	0.93±0.05[a]	0.76±0.16[a]	0.86±0.1[a]
α-Muurolol	1643	0.60±0.11[a]	0.50±0.13[a]	0.50±0.11[a]
β-Bourbonene	1387	1.47±0.13[a]	1.92±0.60[a]	1.76±0.20[a]
β-Chamigrene	1476	1.45±0.18[a]	1.06 ±0.81[a]	0.69±0.70[a]
β-Elemene	1390	2.69±0.36[a]	3.30±1.01[a]	2.78±0.28[a]
β-Selinene	1485	0.82±0.45[a]	0.76±0.69[a]	1.15±0.18[a]
γ-Gurjunene	1473	1.89±0.69[a]	1.76±0.34[a]	1.42±0.31[a]
δ-Cadinene	1522	8.60±0.60[a]	7.54±1.62[a]	9.10±0.48[a]
Total identified	-	94.66±0.73	94.58±1.98	97,72±1.08

drying temperatures that were tested.

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