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INFLUENCE OF THE EQUIPMENT IN THE EXTRACTION OF THE LEAF ESSENTIAL OIL OF *Corymbia citriodora* (Hook.) K. D. HILL AND L. A. S. JOHNSON

Juliana de Araujo[1], Wendel Paulo Silvestre[2], Gabriel Fernandes Pauletti[3], Luis Antonio Rezende Muniz[4]

Abstract: The essential oil of *Corymbia citriodora* (Hook.) has a wide application in industry, mainly due to the high concentration of citronellal present in the extracted oil, which gives the species an intense and pleasant odor. The present work aimed to evaluate the influence of the equipment used to extract the essential oil from *C. citriodora* leaves using steam distillation and the influence of this variation on the extraction efficiency of the compounds. Extractions were carried out for 1 h and 4 h, using two extraction systems, the traditional Clevenger apparatus and a Developed System. The type of system had no significant effect on the yield of extracted essential oil. However, both the type of system and the extraction time influenced the individual contents and extracted masses of some of the main components of the essential oil. This indicates the potential for developing a system in which operational parameters can be modulated, allowing the extraction and purification of essential oil components from *C. citriodora* leaves in a single step.

Keywords: Steam distillation; Citronellal; Clevenger; Essential oil; Terpenes.

1 INTRODUCTION

Essential oils comprise mixtures of natural active ingredients (a substance that chemically characterizes the plant) extracted from medicinal, aromatic, spice, and/or forest plants. They are lipophilic substances, mostly odoriferous, that help to defend the plant against attack by possible microorganisms,

1 Chemical Engineer - University of Caxias do Sul - UCS - jaraujo3@ucs.br

2 PhD in Chemical Engineering - University of Caxias do Sul - UCS - wpsilvestre@ucs.br

3 PhD in Agricultural Engineering - University of Caxias do Sul - UCS - gfpaulet@ucs.br

4 PhD in Chemical Engineering - University of Caxias do Sul - UCS - larmuniz@ucs.br

against the loss of water from the plant, and participate in its conservation and reproduction systems (Amaral, 2015; Antunes et al., 2007; Vitti; Brito, 2003).

The yield of extracted essential oil varies from plant to plant, with its chemical composition containing many components. A plant can synthesize several different active ingredients; these can be isolated or not through extraction techniques (Amaral, 2015; Antunes et al., 2007; Vitti; Brito, 2003).

Among the various technologies used to extract essential oils, there is the steam drag extraction method, where water vapor comes into contact with the plant material, diffusing throughout the plant tissues, helping to extract volatile compounds present inside specific glands spread throughout the plant tissue (Antunes et al., 2007; Handa et al., 2008).

The terpenes present in essential oils are thermolabile; that is, they degrade at high temperatures. Therefore, one of the possibilities to alleviate what happened is to reduce the absolute pressure of the system, which can help in the separation/purification of the essential oil components without thermally degrading them. By reducing the pressure, the temperature of the water vapor in contact with the plant material is modulated, altering the volatilization rates of its constituents (Baser; Buchbauer, 2015; Smith; Van Ness; Abbott, 2007).

In the study by Cusin et al. (2023), a vacuum pump was used in the steam distillation process of *Citrus longa* Tenore leaves. By reducing the absolute pressure of the system, there was a reduction in the yield of essential oil obtained compared to the conventional system without using the vacuum pump. This occurred due to the reduction in vapor temperature, which reduced the volatilization rates of the terpenes that make up the essential oil; on the other hand, it was possible to increase the concentration of the majority compound (methyl N-methyl anthranilate), indicating that low-pressure extraction can be an interesting alternative for obtaining and simultaneously purifying terpenes of interest.

The species *Corymbia citriodora* (Hook.) is popularly known as 'lemon-scented gum'. The extracted essential oil has citronellal as its main compound. Due to its intense and pleasant odor, it is widely used in hygiene products, such as disinfectants, detergents, and air fresheners, among other applications (Ayinde, 2016).

Dogenski *et al.* (2016), in studies analyzing the influence of the extraction process on the chemical composition of *C. citriodora* essential oil, observed that samples of essential oils extracted by conventional methods, such as steam distillation/hydrodistillation at atmospheric pressure, generally present compounds originating from degradation at high temperatures (approx. 100 ºC). However, as essential oils comprise a wide range of substances, it is difficult to identify which compounds may have resulted from this thermal degradation process.

In addition to the extraction temperature, other factors can influence the chemical composition of the essential oil, such as the extraction method used, pressure, and other external factors, such as the plant's place of cultivation, harvest period, drying time, and method, among others (Cusin et al., 2023; Araujo et al., 2023). For Dogenski et al. (2016), increasing the system pressure resulted in a greater number of chromatographic peaks, demonstrating that it is possible to fractionate the extracted essential oil, depending on the conditions used in the process (pressure and/or temperature).

Soares *et al.* (2023) studied the hydrodistillation process of *C. citriodora leaves* up to seven times in series, commenting that it was still possible to extract essential oil and bioactive compounds in subsequent extractions, indicating the diversity of components that the essential oil of this species has. As Goodine and Oelgemöller (2020) reported, the wide range of applications of *C. citriodora* essential oil components makes the search for integrated extraction and separation systems important and necessary, aiming for a faster, more efficient process with lower associated costs.

In this work, the essential oil was extracted from the leaves of *C. citriodora* using the steam distillation process. The extractions were carried out in a Developed System and the conventional Clevenger apparatus to verify the performance of the equipment on the essential oil yield, the content, and the extracted mass of the compounds present.

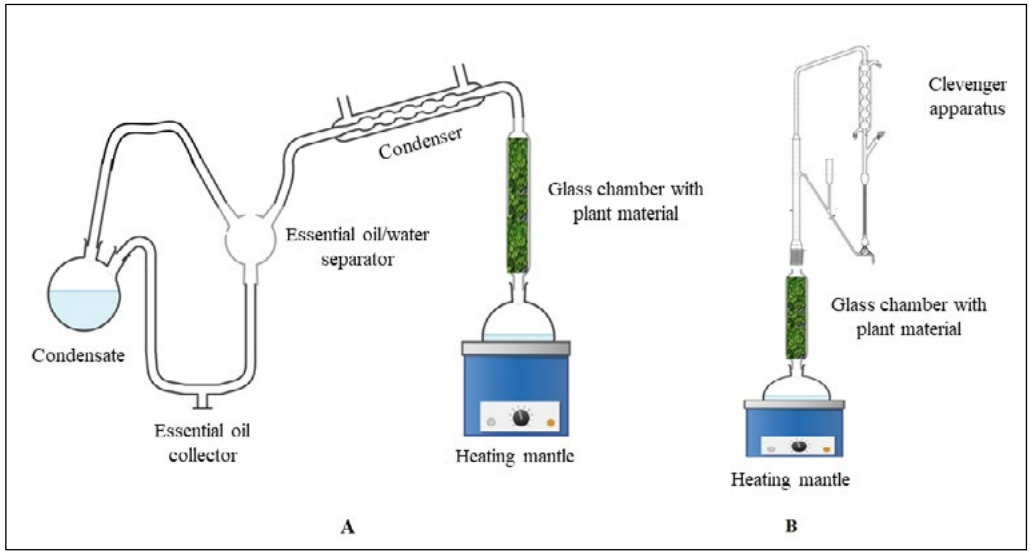
2 DEVELOPMENT

2.1 COLLECTION AND PREPARATION OF PLANT MATERIAL

C. citriodora leaves were collected on a rural property in the municipality of Estrela, RS (29°28'43" S; 51°54'34" W and average altitude of 39 m above sea level) on March 7, 2021. The leaves were dried using a dryer with forced air circulation at room temperature (20±5 °C) for 96 h (Araujo et al., 2023). The extraction was carried out by steam distillation on a laboratory scale, using a Developed System (sized to change the internal pressure to modulate the extraction process) and a conventional Clevenger apparatus manufactured in borosilicate glass, presented in Figure 1.

In both systems, extractions were carried out for 1 h (a period chosen to optimize time, in addition to being the most used industrially) and for 4 h (to extract all the essential oil present in the plant material), both in triplicate, to evaluate reproducibility. Clevenger extraction is a system already known and widely used to extract essential oil; thus, it was compared to the Developed System, checking whether the yield was similar for both, allowing this new apparatus to be applied in future studies.

Figure 1 - Simplified diagram of the Developed System (A) and the Clevenger apparatus (B).



Source: Adapted from Cusin et al. (2023).

Fifty grams of plant material was used in each test; the volume of the extraction chamber was 1.0 L (material density 0.05 g□mL^{-1}). The heating power of the blanket was 415 W, and the condenser temperature was maintained at 10±2 °C. In the Developed System, the volume of extracted essential oil was determined by reading on a graduated scale located in the essential oil collector (Figure 1), whose range was from zero to 5.5 mL, and the resolution was 0.1 mL. In Clevenger, the reading was carried out on the device, which already had a graduated scale (Araujo et al., 2023).

2.2 IDENTIFICATION AND QUANTIFICATION OF ESSENTIAL OIL COMPONENTS

The essential oils obtained were analyzed qualitatively by gas chromatography coupled to mass spectrometry (GC/MS) and quantitatively by gas chromatography with a flame ionization detector (GC-FID). The results were analyzed and interpreted according to the procedures described by Araujo et al. (2023).

The equations used to determine the average yield, average content, extracted mass of the compounds, total mass, and the proportion of the extracted compound mass relative to the total mass are shown in Chart 1.

Chart 1 - Equations used to determine the compounds' average yield, content, and extracted mass.

Equation (1)	Yield	$R = 100x\frac{V}{M}$	Where: R: essential oil yield (% v/w); V: volume of essential oil collected (mL); M: mass of plant material used in extraction (g).
Equation (2)	Essential oil mass	$m = R \times \rho \times \frac{T}{100}$	Where: m: extracted mass ($g \cdot 100\ g^{-1}$ of plant material); R: essential oil yield (% v/w); ρ: specific mass of the compound ($g \cdot mL^{-1}$); T: content of the extracted compound (wt.%).
Equation (3)	Compound quantification	$m_i = \frac{A_i \times m_{PI}}{A_{PI} \times F_{RR}}$	Where: m_i: mass of the compound; A_i: peak chromatogram area of the compound; m_{PI}: internal standard (1-octanol) mass; A_{PI}: internal standard peak area; F_{RR}: relative response factor (calculated based on the response factor of the chemical class of the compound and the response factor of the internal standard).
Equation (4)	Total sample mass	$m_T = \sum_{i=1}^{N} m_i$	The masses of all compounds are summed up, obtaining the total mass (m_T) of the sample.
Equation (5)	Compound mass content (wt.%)	$CMC = 100 \times \frac{m_i}{m_T}$	Where: m_i: mass of the compound; m_t: total sample mass.

Source: From the authors (2023).

2.3. EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS

The study followed a completely randomized experimental design, with factors evaluated such as the type of extraction system (Clevenger and the Developed System) and the extraction time (1 h and 4 h), with each extraction carried out in triplicate. The results obtained were subjected to analysis of variance (ANOVA), and the means were compared using the Tukey test at a 5% probability of error, using the Statistica 12 program (StatSoft, USA).

3 RESULTS AND DISCUSSIONS

The average yield data for the extractions carried out in the two systems and for the two extraction periods are presented in Table 1.

Table 1 – Average essential oil yield for 1 h and 4 h extractions.

Equipment	Average essential oil yield (% v/w)	
	1 h	4 h
Clevenger	6.33±0.30 a	7.99±0.40 a
Developed System	6.53±0.24 a	7.32±0.42 a
p-value	0.4241	0.1174
F-value	0.79 ^{NS}	3.96 ^{NS}
CV (%)	4.21	1.64

Means followed by the same letter in a column do not present a significant statistical difference according to the Tukey test at a 5% probability of error. CV - coefficient of variation. ^{NS} - not significant.
Source: From the authors (2023).

Concerning the average yield obtained, the two equipment did not present significant differences in both extraction times. It is possible to verify that the Developed System presented a similar performance to the conventional Clevenger apparatus. Therefore, it can be noted that the use of the Developed System did not have a negative effect on the oil extraction and collection process after condensation.

C. citriodora (Hook.) leaves used in this study showed an average yield (4 h of extraction) of 7.99 % v/w for Clevenger and 7.32 % v/w for the Developed System. Tolba et al. (2015) observed a yield of 2.26 wt.% (≈ 2.86 % v/w) for the leaves of the same species. However, these authors used plant material from a region with a hot summer Mediterranean climate (Csa). In contrast, the plant material used in this study came from a humid subtropical climate (Cfa). Therefore, it is worth highlighting that the climate, time, and place of harvest influence the yield of extracted essential oil (Silva Júnior et al., 2022).

Dogara (2023) reported an essential oil yield from *C. citriodora* leaves of 1.8 wt.%. However, this author carried out a grinding process before extraction, which may have caused the early evaporation of some of the volatile compounds in the material. In experiments conducted by Silou et al. (2009), extraction occurred by hydrodistillation for 3 h, obtaining essential oil yields in the range of 6.1 – 6.9 wt.% (6.77 – 7.67 % v/w). These values were the closest to the essential oil yields found in the present study. As noted by Silva et al. (2009), the yield of essential oil from *C. citriodora* is variable and susceptible to nutritional and environmental factors, as well as the plant's genetics.

The chemical composition of the oil extracted in both systems, in the two extraction periods, is compiled in Table 2.

Table 2 – Chemical composition of the essential oil of *Corymbia citriodora* (Hook.), obtained after 1 h and 4 h, using Clevenger and the Developed System.

Component	IRL[1]	Chemical Class	Average content[2] (wt.%)			
			Clevenger		Developed System	
			1 h	4 h	1 h	4 h
β-pinene	974	M	0.21 Aa	0.18 Aa	0.09 Bb	0.23 Aa
Eucalyptol	1026	OM	0.39 Aa	0.33 Aa	0.13 Bb	0.33 Aa
Terpinolene	1086	M	-	0.04 a	-	0.08 a
Isopulegol	1145	OM	0.26 Aa	0.60 Aa	0.09Ba	0.44 Ab
Citronellal	1148	OM	87.60 Bb	89.72 Ab	91.51 Aa	90.60 Aa
Citronellol	1223	OM	5.45 Aa	4.75 Aa	4.89 Bb	4.60 Ab
PMD[3]	1301*	OM	0.57 Aa	0.34 Bb	0.42 Ba	0.57 Aa
Caryophyllene	1408	S	1.02 Aa	2.11 Ab	0.70 Ab	2.34 Aa
Total identified			95.50	98.07	97.84	99.19
Not identified			4.50	1.93	2.16	0.81

Means followed by the same letter, uppercase (type of system), and lowercase (extraction time) did not show a statistically significant difference by the Tukey test at a 5 % probability of error (p-value >0.05). [1] IRL – Linear Retention Index reported by Adams (2017) and * - reported by Yadegarinia *et al.* (2006). [2] Mean of triplicates. [3] p-menthane-3,8-diol. M – monoterpene; OM – oxygenated monoterpene; S – sesquiterpene.
Source: From the authors (2023).

According to the data presented in Table 2, it can be observed that the majority compound of the studied *C. citriodora essential oil* was present in high concentration (around 90 wt.%) in both equipment and periods analyzed, that is, practically all the oil extracted is composed of citronellal. This result was compared with the study by Tolba *et al.* (2015), who also performed extraction by steam drag, reporting a peak area relative to the chromatogram of 69.77 % for this compound. Benchaa, Hazzit, and Abdelkrim (2018), Chahomchuen, Insuan, and Insuan (2020), and Souza et al. (2016) performed the extraction by hydrodistillation, and obtained 64.70 %, 68.43 %, and 68.20 % of relative peak areas of the chromatograms, respectively.

Citronellol was the second compound in the highest concentration for the 4 h extraction (4.60 wt.% and 4.75 wt.%) and caryophyllene (2.11 wt.% and 2.34 wt.%). In the literature, the levels of these compounds are reported in the range of 3.39 – 10.63 % for citronellol and 0.62 – 1.34 % for caryophyllene,

considering the relative areas of the chromatograms and not a quantification itself (Caetano, 2018; Chahomchuen; Insuan; Insuan, 2020; Tolba et al., 2015).

According to the statistical analysis presented in Table 2, except for caryophyllene, the type of equipment or extraction time had some influence on the components of the extracted essential oils. Considering the 1 h extraction time, Clevenger extraction promoted higher levels of the most volatile compounds and lower levels of citronellal. For 4 h, the Developed System had a higher citronellal content. However, it was lower than the content obtained in 1 h due to the dilution effect of extracting other components in the plant material.

These results indicate that modulation of the extraction time and/or the controlled change of other parameters, such as system pressure, as discussed by Araujo et al. (2023) and Cusin et al. (2023), can be a strategy for obtaining richer fractions in each compound or classes of compounds.

Still, it is important to highlight that, as it is a plant material, even if the leaves came from the same plant and the sample was adequately homogenized, there may be variability in the levels obtained, either due to experimental effects or uncertainty that is inherent in analysis methods (Maldonado Júnior; Barbosa, 2015).

Data relating to the average masses extracted from the major compounds of the studied *C. citriodora oil* are described in Table 3.

Table 3 – Average extracted masses of the major compounds from *C. citriodora essential oil* for extraction times of 1 h and 4 h, using Clevenger and the Developed System.

Component	Extracted mass* ($g \cdot 100\ g^{-1}$)			
	Clevenger		Developed System	
	1 h	4 h	1 h	4 h
Citronellal	4.74 B	6.13 a	5.11 A	5.67 a
Citronellol	0.29 A	0.32 a	0.27 A	0.29 b
Caryophyllene	0.04 A	0.16 a	0.04 A	0.15 a
PMD	0.06 A	0.04 a	0.03 B	0.03 b

Line averages, followed by the same uppercase (1 h) or lowercase (4 h) letter, do not present a statistically significant difference according to the Tukey test at a 5 % probability of error (p-value > 0.05). * Average of triplicates, in grams of compound per 100 g of plant material.
Source: From the authors (2023).

Regarding the extracted masses of the individual major compounds, in 1 h of extraction, the Developed System extracted a greater amount of citronellal. In comparison, the amount of PMD was lower than Clevenger. On the other hand, after 4 h of extraction, the mass of citronellal showed no significant

difference. In contrast, the extracted masses of citronellol and PMD were lower in the Developed System than in the Clevenger apparatus. Thus, changes in process parameters can enhance the separation of components and/or classes of components, facilitating purification and enabling a joint extraction and fractionation/separation process.

Given the studies reported, extraction efficiency is influenced by different factors depending on the matrix used and possible variations in relation to the extraction method used and/or equipment used (Araujo et al., 2023; Soares et al., 2023). However, as Cusin et al. (2023) noted, developing a system capable of carrying out the extraction and purification steps must consider the need to work with a variable raw material.

4 CONCLUSION

The Developed System employed did not significantly affect the yield of extracted essential oil. However, both the contents and the individual extracted masses of some essential oil components were influenced, indicating the potential for the development and use of a system in which operational parameters can be modulated, allowing the concomitant extraction and purification of volatile components of the leaf essential oil of *C. citriodora*.

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