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Chemical profile of essential oil in four *Cymbopogon* species grown in southern Brazil

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ABSTRACT. Essential oils (EO) are substances used by pharmaceutical, cosmetic and food industries. These compounds have useful properties for human health and well-being. Among the species cultivated for production of these substances are those of the genus *Cymbopogon*. Several species, subspecies, varieties, and sub-varieties are used worldwide, and studies have been conducted to elucidate the chemical features of their EO, mainly for *C. citratus* and *C. winterianus*. However, there are still species with great potential that have not yet been fully chemically characterized. In addition, EO composition is influenced by genetic and environmental factors. Along this line, we examined the chemical profile of EO of four species of the genus *Cymbopogon* (*C. citratus*, *C. distans*, *C. flexuosus* and *C. winterianus*) grown in southern Brazil. The EO was obtained from fresh leaf tissues by hydrodistillation. Citral was identified as the main component of *C. citratus*, *C. flexuosus*, and *C. distans*, whereas citronellal, citronellol, cis-geraniol and elemol were predominant in *C. winterianus*. The absence of a large range of compounds was noted in *C. distans*, which may be useful for the semi-exclusive synthesis of citral and other compounds of interest.

Key words: Aromatic plants; Citronella; Lemon grass; Metabolomics; Traditional medicine

INTRODUCTION

Essential oils (EO) are mixtures of secondary metabolites that represent the very essence of odor and flavor of a plant species. These compounds are able to exert functions in a plant's self-defense, communication, attraction of pollinators and protection against water losses and extreme temperature fluctuations (Bunse et al., 2022). Essential oils possess numerous biological properties and are a mixture of volatile and non-volatile compounds. Numerous studies have been carried on these oils aiming to better understand their usefulness (Avoseh et al., 2015; Ekpenyong et al., 2017; Zhang et al., 2022).

Essential oils are produced by more than 17,500 aromatic species, including *Cymbopogon* spp. (Bunse et al., 2022). The EO of genus *Cymbopogon* consist mainly of monoterpenes and sesquiterpenes such as citral, citronellol, citronellal, linalool, elemol, limonene, geraniol, β-carofilen, and geranyl acetate (Ganjewala et al., 2009; Omar et al., 2016).

Cymbopogon species EO are used commercially in cosmetics, pharmaceuticals, aromatherapy, food, flavors, fragrances, perfumery, detergents, tobacco products (Mwithiga et al., 2022; Dangol et al., 2023) and insecticidal products (Zhang et al., 2011; Moustafa et al., 2021). The *Cymbopogon* genus is native to Asia, Africa, Australia and tropical islands and comprises 144 species. These species can be found in more than 40 countries, including Brazil (Tibenda et al., 2022). The economically important species are *C. flexuosus*, *C. citratus*, *C. winterianus*, *C. nardus*, *C. pendulus*, *C. khasianus*, *C. martini*, *C. distans*, *C. stracheyi* and *C. jwarancusa* (Verma et al., 2013). Some species of *Cymbopogon* are used locally by native people as tea or in traditional medicine. These species are applied for sore throat, as an analgesic, anti-inflammatory, diuretic and sedative. In addition, they are used in the control of rheumatism and for diabetes and cholera treatment (Shah et al., 2011).

The temporal and spatial variations in total content of secondary metabolites in plants occur due to alterations in gene expression and modifications resulted from the interaction of physiological, biochemical and environmental conditions (Li et al., 2020). Essential oil composition and concentration are influenced by genetic and environmental factors (Aboukhalid et al., 2017; Verma et al., 2020; Kaur et al., 2021; Mwithiga et al., 2022). Therefore, according to species, genotype, cultivation environment (Aboukhalid et al., 2017; Verma et al., 2020; Kaur et al., 2021; Mwithiga et al., 2022) and seasonality (Pinheiro et al., 2016), EO have a specific composition and concentration.

Characterizing the EO profile of *Cymbopogon* genus species cultivated in specific regions is essential for commercial exploitation under local conditions, as well as for the conservation of genetic diversity of the genus. In this sense, the objective of this study was to characterize the chemical composition of EO of four species of genus *Cymbopogon* grown in Rio Grande do Sul/Brazil, highlighting which compounds differentiate them, directing the potential use of each species according to its EO composition.

MATERIAL AND METHODS

Plant Material

The species used in the study were *C. winterianus* (PEL No. 26,973) (citronella), *C. flexuosus* (PEL No. 26,972) (lemon grass), *C. citratus* (PEL No. 26,955) (lemon grass) and *C. distans* (PEL No. 26,956). Each of the species was grown in three replicates in a completely randomized design. The identification of the species was carried out based on the morphological traits contained in the taxonomic key for the genus (Soenarko, 1977). Cultivation was carried out in a raised bed at Federal University of Pelotas, Capão do Leão, Brazil, during the 2016/2017 harvest season.

Essential oil extraction

The EO was obtained from fresh leaf tissues by hydrodistillation using a Clevenger-type apparatus and separated from the water by decantation. The extraction time was 4 hours (Brazil, 2010). The amount of plant material of each species varied between 300 and 400 g. After extraction, the EO was transferred to a graduated cylinder and the yield was determined as % (w/w) with fresh material. Samples were transferred to amber glass vials and stored at -4°C until chemical analysis.

Chromatographic analyses

The identification of the chemical components of the EO (qualitative analysis) was performed by an Agilent 6890 gas chromatograph coupled to an Agilent 5973 mass selective detector (GC/MS). The 2 µL EO aliquot was diluted in 1 mL hexane (HPLC grade). The analyses were performed on an HP5-MS capillary column (Hewlett Packard, 5% phenyl, 95% methylsiloxane, 30 m × 0.25 mm, film thickness: 0.25 µm) at 70 eV. The following conditions were used: flow division 1:100; temperature program: 40°C for 4 min; 40 to 320°C at 4°C min^{-1}; gas carrier was He; flow rate of 1 mL min^{-1}; injector and detector temperature: 250ºC. The EO components were identified by comparing their retention indices, determined by a calibration curve of n-alkanes injected under the same chromatographic conditions as the samples and the mass fragmentation patterns described in the literature (Adams 2007; NIST 2010).

The EO components were quantified by gas chromatography with flame ionization detection (GC/FID) on an Agilent 7890A. The parameters used for the analysis were: non-division mode; temperature program: 40°C for 4 min; 40 to 320°C at 4°C min^{-1}; gas carrier was He; flow rate of 1 mL min^{-1}; Temperature of the injector and detector: 300°C. The percentage of chemical components was based on the normalization of the peak area.

Statistical analysis

Principal Component Analysis (PCA), hierarchical grouping and Partial Least Squares Discriminant Analysis (PLS-DA) were used after the analysis of variance (ANOVA, $P \leq 0.05$). A variable importance score (VIP) > 1.0 was chosen to select the most important discriminant variables and Tukey's honest significant difference test ($P \leq 0.05$) was applied to differentiate the essential oil content and the different classes of compounds (Cruz, 2013; Xia and Wishart, 2016). All analyses were performed with R software.

RESULTS

The *Cymbopogon* species analyzed have differences in leaf-EO content. *C. winterianus* has the highest EO concentration (0.93%), followed by *C. flexuosus* (0.56%). *C. citratus* and *C. distans* had the lowest concentrations, not differing significantly one from each other, with 0.44% and 0.36% respectively (Figure 1A). These species presented different patterns of compounds in their EO (Figure 1B). Most of the compounds found in these species belong to the class of oxygenated monoterpenes (60-90%), and *C. flexuosus* is the one with the greatest variety of chemicals of this class. *C. citratus* has a high concentration of monoterpene hydrocarbons due to the presence of myrcene, while *C. winterianus* presents several compounds belonging to the classes of hydrocarbon sesquiterpenes and oxygenated monoterpenes, with 11 and 13%, respectively (Figure 1C).

Most of the compounds found in EO of *Cymbopogon* species were identified (*C. distans*: 85.6%; *C. winterianus*: 88.1%; *C. flexuosus*: 97.8%; *C. citratus*: 93.1%) as shown in Table 1. *C. winterianus* had the highest number of different compounds (n = 16) and *C. distans* had the lowest (n = 5) (Table 1). In *C. winterianus*, the predominant compounds were citronellal (31.8%), cis-geraniol (12.5%), elemol (10.2%) and citronellol (9.9%), a profile much different from the other species (Table 1).

Table 1. Mean percentage of essential oil compounds identified in each *Cymbopogon* species.

Compounds (%)	***C. distans***	**SD**	***C. flexuous***	**SD**	***C. citratus***	**SD**	***C. winterianus***	**SD**	**RT**	**IK cal**	**IK tab**
Camphene	_	_	0.37	± 0.02	_	_	_	_	11.7	947.1	946
6-Methyl-5-heptene-2-one	_	_	0.56	± 0.05	0.43	± 0.03	_	_	13.2	983.7	986
Myrcene	_	_	_	_	17.47	± 0.17	_	_	13.4	988.6	988
Limonene	_	_	2.93	± 0.26	_	_	2.45	± 0.28	15.0	1027.7	1027
Linalool	2.72	± 0.14	_	_	1.13	± 0.2	_	_	17.8	1098.9	1097
neo-isopulegol	_	_	_	_	_	_	2.81	± 0.66	19.7	1146.7	1148
Citronellal	_	_	3.15	± 0.32	_	_	31.85	± 2.42	19.9	1152.3	1153
cis-chrysanthenol	_	_	4.26	± 0.14	_	_	_	_	20.2	1160.5	1164
cis-Verbenol	_	_	9.20	± 0.53	6.05	± 0.39	_	_	20.9	1179.0	1188
Citronellol	0.94	± 0.14	_	_	_	_	9.90	± 0.26	22.6	1225.6	1226
β-Citral	26.53	± 0.51	28.50	± 0.33	26.02	± 0.26	_	_	23.0	1237.1	1240
cis-Geraniol	_	_	0.27	± 0.05	0.91	± 0.04	12.56	± 0.24	23.4	1248.9	1253
α-Citral	54.47	± 0.08	45.21	± 0.4	40.70	± 0.98	0.70	± 0.05	24.1	1267.1	1268
2-undecanone	_	_	_	_	0.47	± 0.02	_	_	24.9	1291.0	1294
citronellyl acetate	_	_	_	_	_	_	1.74	± 0.18	26.8	1348.2	1353
geranyl acetate	_	_	_	_	_	_	1.21	± 0.08	27.8	1376.4	1381
β-Elemene	_	_	_	_	_	_	4.53	± 0.29	28.2	1387.7	1391
β-Caryophyllene	_	_	1.95	± 0.38	_	_	_	_	29.1	1418.3	1417
Germacrene D	_	_	_	_	_	_	0.61	± 0.10	31.1	1479.8	1485
α-Muurolene	_	_	_	_	_	_	1.48	± 0.10	31.6	1496.5	1497
γ-Cadinene	_	_	0.94	± 0.31	_	_	1.01	± 0.10	32.0	1511.1	1512
δ-Cadinene	_	_	_	_	_	_	3.76	± 0.30	32.2	1516.4	1517
Elemol	_	_	_	_	_	_	10.25	± 1.09	33.1	1546.7	1550
caryophyllene oxide	1.00	0.14	0.52	± 0.26	_	_	_	_	34.1	1580.2	1583
δ-Cadinol	_	_	_	_	_	_	1.21	± 0.13	35.9	1641.6	1646
α-Cadinol	_	_	_	_	_	_	2.06	± 0.32	36.2	1653.6	1654
Total identified	85.65		98.03		93.58		88.15				

RT: retention time; IK cal – calculated Kovats index; IK tab – tabulated Kovats index; SD – Standard deviation.

C. distans, *C. flexuosus* and *C. citratus* presented as major compounds β-Citral and α-Citral, with values ranging between 26.0-28.5% and 40.7-54.4%, respectively. *C. distans* contains a higher concentration of citral, followed by *C. flexuosus* (Table 1).

C. flexuosus has in its composition cis-chrysanthenol and β-caryophyllene, compounds that have not been found in other species. *C. citratus* is characterized by a high concentration of myrcene in its EO (around 17%). The species *C. winterianus* contains elemol and β-elemene in high concentrations (10.2% and 4.5%, respectively), something that is not found in other species (Table 1).

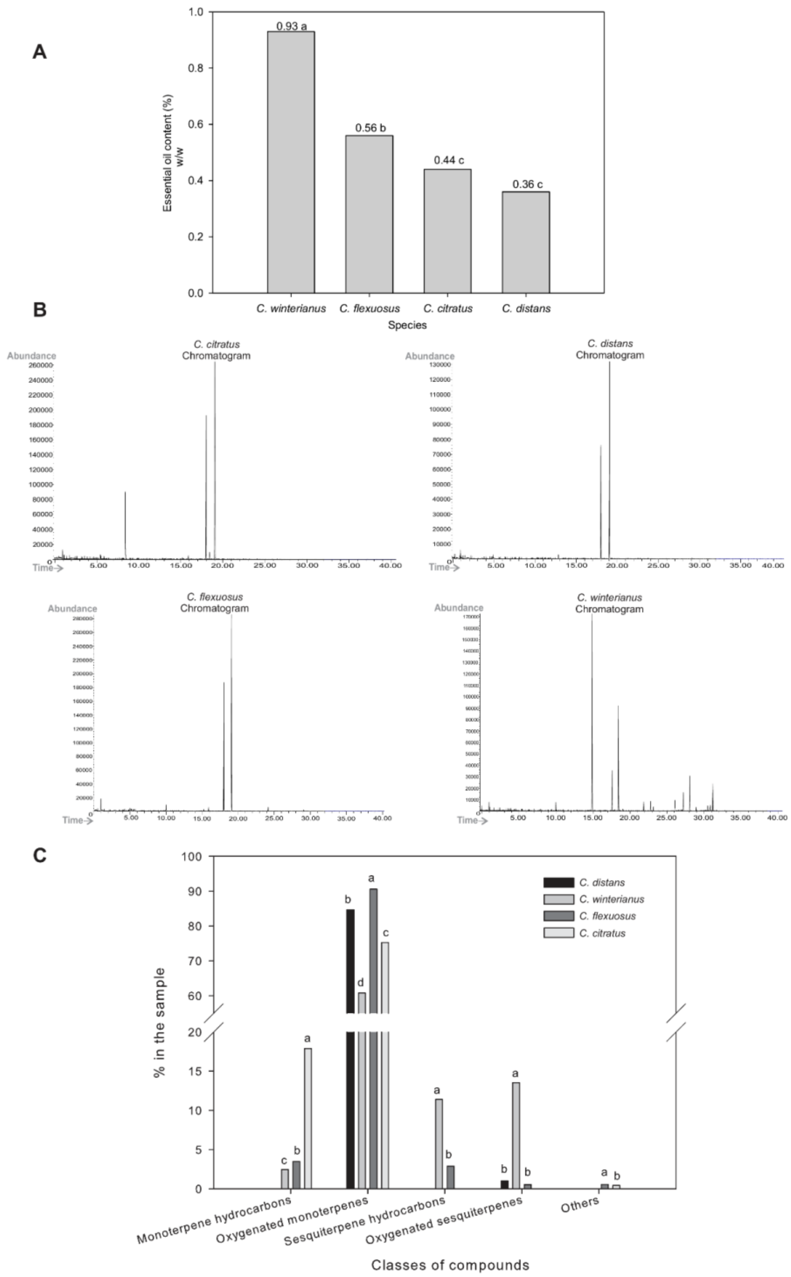

Figure 1. Identification and concentration of the compounds found in plants of the genus *Cymbopogon*. (A) Content of essential oil obtained from different plants of the genus *Cymbopogon*; (B) Typical ion chromatograms obtained by HPLC from *Cymbopogon* oil. (C) Concentration of the main compounds found in the four *Cymbopogon* species.

The PCA analysis highlighted differences among species based on volatile compounds. PC1 presented 65.5% and PC2 presented 21.8% of the total variability of the data set. PC1 separates *C. winterianus* from other species (Figure 2A). VIP scores were used to determine which compounds contributed to cluster separation. Of the 25 evaluated peaks, the one contributing the most for differentiating the species was α-Citral, available in *C. distans*, *C. flexuous* and *C. citratus*. The second compound that most contributed in differentiating the species was citronellal, highly concentrated in *C. winterianus*. β-Citral, available in C. *distans*, *C. flexuous* and *C. citratus*, also contributed to species differentiation (Figures 2B and 2C). Similar to PCA (Figure 2A), the hierarchical clustering separated *C. winterianus* from other species (Figure 2C).

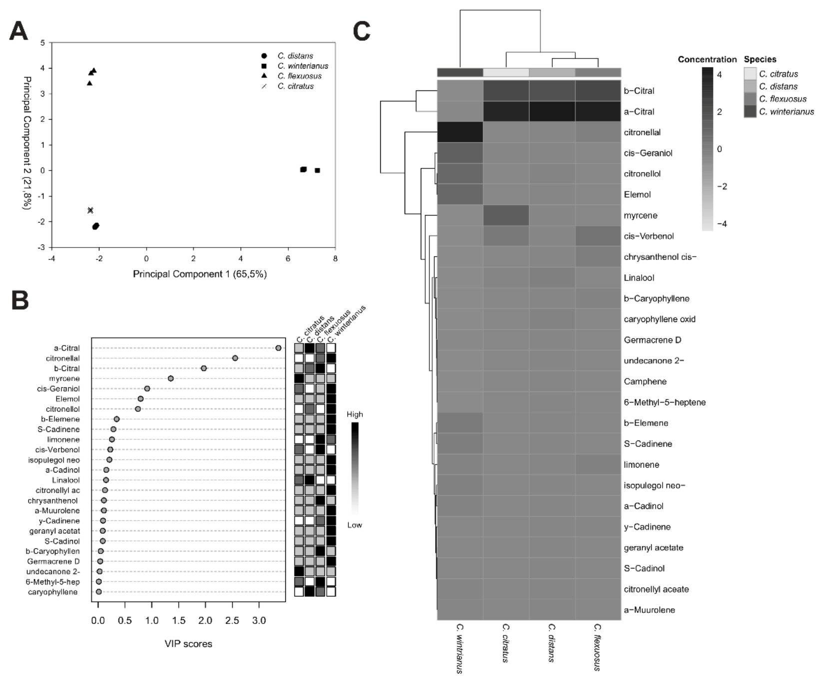

Figure 2. Chemical profile of the oil obtained from *Cymbopogon* spp. (A) Principal component analysis of *Cymbopogon* species based on their constituents. (B) Variable importance in projection (VIP) plot of the initial partial least squares discriminant analysis (PLS-DA) derived from LCMS data. (C) Hierarchical grouping of four *Cymbopogon* species, based on data from non-targeted metabolomics (25 compounds).

DISCUSSION

EO production depends on ecophysiological and environmental factors (Ganjewala, 2009; Aboukhalid et al., 2017). Therefore, in this research, the content and composition of EO in four *Cymbopogon* species grown in southern Brazil was evaluated.

The essential oil content of *C. flexuosus* (0.56%) is within the values found in other countries; however, it could be increased. In this species the EO concentration ranged from 0.17 to 0.24% in Kenya (Mwithiga et al., 2022), 0.65% in Colombia (Montagut et al., 2022) and 1.14% in India (Upadhyay et al., 2022). In *C. citratus* grown in Egypt the EO content ranged from 0.67 to 1.83% according to irrigation interval and harvest season (Mahmoud et al., 2022). These values are higher than those found in southern Brazil (0.44%), demonstrating that improvements can be made for greater EO production.

In India, it was found that EO content of *C. distans* ranged from 0.3 to 0.4% (Chauhan et al., 2016), similar to the value we found (0.36%). These findings suggest that this is the average production of this species. In *C. winterianus* grown in India, researchers found an EO content of approximately 0.8% (Singh and Kumar, 2017), while here this species reached 0.93%. *C. winterianus* had the highest EO content under local conditions, with a difference of 0.37% for *C. flexuosus* and 0.49% and 0.57% for *C. citratus* and *C. distans*, respectively.

The difference in essential oil yield among studies can be associated with environmental factors, evidencing the importance of growth region and management in final EO concentration. In addition, a genotype effect must be considered, and new cultivars can be developed aiming at greater EO content in these species.

Each *Cymbopogon* species may have different chemotypes and presence and concentration of compounds in their EO vary according to their growing environment (Avoseh et al., 2015). The EO composition that we found is similar to that observed for these species cultivated in other environments, and most abundant compounds had concentrations within the ranges described in other publications. In previous studies, it was also observed that *C. citratus* possesses high levels of β-Citral and α-Citral (26-50% and 33-50%, respectively), as well as a high concentration of myrcenene (9-27%) (Akhila, 2010; Mohamed et al., 2012; Avoseh et al., 2015). Similarly, *C. flexuosus* is characterized by presenting β-Citral and α-Citral in high concentrations (29-35% and 30-45%, respectively) while the other compounds are present in lower amounts, and does not contain myrcenene (Akhila, 2010; Avoseh et al., 2015). The same occurs with *C. distans*, which presents β-Citral and α-Citral in high concentrations (15-20% and 20-30%, respectively). However, this species also presents a high concentration of geranyl acetate (Chauhan et al., 2016), a compound that was not found in present study. In addition, here *C. distans* showed higher values for β-Citral and α-Citral, with 26.5% and 54.4%, respectively. Future studies should further explore the use of these compounds, since their concentration is high in these species. The differential metabolic profile present in *C. winterianus* was also reported elsewhere, in which the authors found citronellal (26-36%), citronellol (7-15%), geraniol (16-84%) and elemol (2-10%) (Akhila, 2010; Mohamed et al., 2012; Kakaraparthi et al., 2014; Avoseh et al., 2015; Chauhan et al., 2016; Singh and Kumar, 2017).

The EO quality of lemongrass and many other herbs with lemon fragrance is due to its citral content, which is a mixture of two different monoterpene isomers: the E isomer is known as geranial or α-Citral and has a double bond conjugated to a carbonyl; the Z isomer is known as neral or β-Citral and has a double bond conjugated to an aldehyde carbonyl. The citral is widely used as a flavoring agent in cosmetics, perfumery, colonies, deodorants, and soaps. However, its main use has been in the pharmaceutical industry, acting as a precursor in the synthesis of ionones, methyl ionones, and vitamin A, with potential use as a fungitoxic agent due to its mycelial inhibition properties (Wany et al., 2013). The species *C. distans* stands out for its citral concentration (81%), presenting a high potential for use by industry, with about 7.3% more citral than *C. flexuosus* and 14.3% more than *C. citratus*, which are the most widely used *Cymbopogon* species for the production of EOs rich in citral.

Not only citral, but also the other biomolecules detected in this study have therapeutic potential and several applications in the industry. Myrcene is a monoterpene aromatic hydrocarbon known for its medicinal properties such as analgesic, antioxidant, anti-inflammatory, antibiotic, antibacterial, antinociceptive, antitumor and antimutagenic activities (Ohtsubo et al., 2015; Hwang et al., 2017). Citronellal is a monoterpene widely used as a repellent and studies show that it can have a fungicidal, anticonvulsant, antinociceptive and antioxidant effect (Ramezani et al., 2002; Kim et al., 2005; Quintans-Júnior et al., 2011). Geraniol is an acyclic monoterpene with antitumoral activity, both *in vitro* and *in vivo*, blocking the S phase of the cell cycle, besides being used in the cosmetics and food industries due to its pleasant aroma of rose. It is also known for its antibacterial, antimicrobial and antifungal activity (Carnesecchi et al., 2004; Li et al., 2017; Salvi et al., 2017). Essential oils of *Cymbopogon* are rich sources of acyclic monoterpenes (Bergman et al., 2023). Citronellol, a monoterpene alcohol, has vasorelaxant, anticonvulsive, analgesic, antibacterial and anti-inflammatory activity (de Sousa et al., 2006; Brito et al., 2012; Brito et al., 2015). Elemol is a sesquiterpene alcohol with a sweet smell of green wood which has been proven to have antifungal, repellent and insecticidal action (Carroll et al., 2010; SeonHong et al., 2016).

This wide variety of compounds produced by the plants under study can be used as defense against pathogens and herbivores, and even attract beneficial organisms such as pollinators. Natural selection plays an important role in the evolution of the metabolic pathways involved in the synthesis of these chemicals that can be harmful or beneficial to other species (Chen et al., 2011; Singh and Sharma, 2015). This can partially explain the great variety of compounds found in these plants, which are not commonly used for animal feed, and are not attacked by pests and pathogens.

In general, *C. distans* was the species with the lowest number of different compounds in its EO; however, it has a high citral concentration. Few studies have been made on this species, even though it shows great potential. Its high citral content combined with low concentrations of other similar chemicals can be useful for the production and purification of this compound. Also, further understanding of environmental influences in the precursors of this metabolite can help to increase even more its concentration.

Therefore, the study of compounds produced by different species of *Cymbopogon* is important to avoid inadequate uses and to specify their possible utility, making it possible to direct research according to the composition of each species and to their productive potential.

CONCLUSION

The species *C. distans*, *C. flexuosus* and *C. citratus* grown in southern Brazil produce a high content of citral, a major compound of commercial interest from the genus *Cymbopogon*. Besides citral, *C. citratus* contains myrcene, an important intermediate used in perfumery. On the other hand, *C. winterianus* is the species with the highest EO content, with elevated concentrations of citronellal, citronellol, cis-geraniol and elemol. This species presents a distinct chemical profile with great potential for the cosmetic and pharmaceutical industries.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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