

Extraction and chemical constituents of the essential oils from *Rosmarinus officinalis* L. and *Corymbia citriodora*

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Abstract: The essential oils of *Rosmarinus officinalis* L. and *Corymbia citriodora* (Hook.) K.D.Hill & L.A.S.Johnson leaves have chemical constituents with fungicide potential. The objective of this work was to determine the yield and the main chemical compounds of the essential oils of these species. Plants of the two species were georeferenced and collected in municipalities of Rio Grande do Sul, and identified in the Botany Laboratory of UNIJUÍ. The essential oils were extracted by hydrodistillation and the yield was based on fresh vegetable mass. The analyses of the chemical constituents were determined by CG-MS. A yield of 0.49% was obtained for *R. officinalis* and of 1.54% for *C. citriodora*. Similar to other studies, the major constituents of rosemary essential oil were camphor, 1,8-cineol, canfene and α -pineno, and the ones of eucalyptus essential oil were citronelal, isopulegol and citronelol. It is concluded that the chemical constituents are similar to those reported in the literature and the essential oil yield of the species is low and dependent on external conditions.

Keywords: Volatile oils; Medicinal plants; Chemical Compounds; Camphor; Eucalyptus; Pharmacological.

Abbreviations: VCAM-1_ vascular cell adhesion molecule 1; CG_Gas Chromatography; CG-EM_Gas chromatography-mass spectrometry; CO₂_Carbon dioxide; HURB_ Rogério Bueno University Herbarium; ICAM-1_ intercellular adhesion molecule 1; IL_ Interleukin; IL-1 β _ Interleukin-1 beta; IFN- γ _ Interferon-gama; MCP-1_ Monocyte Chemoattractant Protein-1; NF- κ B_ transcription factor; SISGEN_ National System of Genetic Heritage Management; UNIJUÍ_ Regional University of the Northwest of the State of Rio Grande do Sul.

Introduction

Essential oils, also called volatile or ether oils because of their oily appearance at room temperature, have as main characteristic the volatility, thus differing from fixed oils, which are composed of mixtures of lipid substances (Simões et al., 2007). Essential oils have a complex chemical composition, in which terpenes and phenylpropanoids stand out. These compounds are related to several functions necessary for plant survival, ensuring adaptive advantages in the environment (Oussalah et al., 2007).

Essential oils can be an alternative in the control of diseases caused by fungi (Silva et al., 2021) due to their pharmacological, fungistatic and/or fungicide actions. In the present study, we focus in two plants that have essential oils in their composition with these functions, namely, *Rosmarinus officinalis* L. (rosemary) and *Corymbia citriodora* (Hook.) K.D.Hill & L.A.S.Johnson (eucalyptus) (Farkhondeh et al., 2019). The main constituents of the essential oil of rosemary are camphor, 1,8-cineol, α -pineno, borneol and canfene (Zauli et al., 2023).

Corymbia citriodora, formerly *Eucalyptus citriodora* Hook., is the most widely used eucalyptus species in the commercial exploitation of leaves for the extraction of essential oil. The adult leaves of *C. citriodora* are amphistomatic and glabrous, with stomata restricted to the vicinity of the midrib on both the abaxial and adaxial face of the epidermis, and a

uniseriate epidermis on both faces, as described in *E. globulus* (Moura, Franzener, 2014).

Several studies on the use of different essential oils to control diseases in plants and seeds have been conducted, including with rosemary (Souza et al., 2007; Daronco et al., 2015; Leite et al., 2018; Kesho et al., 2020; Bomfim et al., 2020) and eucalyptus (Caetano, 2018). Therefore, the objective of this work was to determine the yield and the main chemical compounds of the essential oils of *R. officinalis* and *C. citriodora*.

Results

Yield of essential oils

The yields of each essential oil extraction were compiled in Table 2. The highest yield was found for *C. Citriodora* (1.54%).

Chemical composition of essential oils

Regarding the chemical composition, the essential oil of *R. officinalis* presented 21 constituents, according to Table 3, which correspond to 89.24% of the identified components; the predominant constituents were camphor (14.26%), 1,8-cineol (13.90%), canfene (11.46%) and α - pineno (12.46%). The composition of the essential oil of *C. citriodora* is

Table 1. Species of medicinal plants that present essential oils with potential antifungal activity and the parts of the plant used for extraction of essential oil.

Species	Family	Popular name	Part used*	Extraction Time of 200 g of plant
<i>Corymbia citriodora</i>	Myrtaceae	Eucalyptus	Fresh leaves	230 minutes
<i>Rosmarinus officinalis</i>	Lamiaceae	Rosemary	Fresh leaves	180 minutes

*part of the plant used for extraction of essential oil. Source: Adapted from Fenner et al. (2006) and Ehlert et al. (2006).

Table 2. Determination of the yield of *Rosmarinus officinalis* and *Corymbia citriodora* essential oils extracted by hydrodistillation using a modified Clevenger type apparatus. Unijui - Campus Ijuí/RS.

Plant (Species)	Date of Collection and Extraction of the EO	Ambient temperature (°C)	TW (g)	Yield of EO (mL)	% of Yield (v/p)
<i>Rosmarinus officinalis</i>	24/08/2020	±15	2443.56	12	0.49
<i>Corymbia citriodora</i>	22/09/2020	±7	648.96	10	1.54

EO = essential oil; Ambient temperature (°C) = Ambient temperature in °C, at the time of plant collection; TW (g) = Total weight of fresh leaves of the plant for extracting the essential oil. Source: the author, 2021.

described in Table 4. The essential oil of *C. citriodora* presented 9 chemical constituents, and the major constituent was citronellal (62.51%).

Discussion

Yield of essential oils

The yield of *R. officinalis* found in the present study was 0.49%, higher than that found in the study by Verginaci et al. (2024), of approximately 0.10% in relation to the weight of the fresh material, obtained by hydrodistillation of fresh leaves. This difference can be explained by some external factors, such as time of the year and time of collection, type of soil, precipitation and amount of water available to the plant, light irradiation, temperature, and also internal factors such as genetic variability, among others (Chen et al., 2021; Carrubba et al., 2020).

The average yield of *C. citriodora* essential oil was similar to that found in the literature, such as in the study by Vieira (2004) who found a yield of 1.6% and Maffeis et al. (2000) who reported yields varying from 1.0 and 1.6% according to the treatment with macronutrients. Maffeis et al. (2000) explain that the yield of eucalyptus essential oil is tied to external factors, such as climate, planting conditions, soil, plant origin, availability of macronutrients during growth, among others. They also state that the citronellal content in the essential oil correlates with the amount of boron and potassium available in the soil, and the presence of these nutrients also promotes the greater development of leaves and plant growth. In the present study, the plants analyzed did not receive treatment with macronutrients and no soil analysis of the site was performed to ascertain which factors could justify the variability or the similarities observed between the studies.

According to Serafini et al. (2002), regardless of the extraction method used, the content of essential oil extracted from aromatic plants is very low quantitatively, less than 1% in some cases. Differences in the essential oil yield can be expected between species, given the characteristics of each plant. In this study, eucalyptus leaves obtained a higher yield than rosemary leaves, and the difference in yield between these plant species has already been reported in the literature, as described above.

Chemical composition of essential oils

Essential oils have a very complex chemical composition, which can range from tens to hundreds of compounds belonging to different chemical families (De Sousa et al., 2023). Such compounds belong to different chemical groups and their interrelationship results in the biological activities that these oils perform, both in the plant and in other organisms (Maleck et al., 2021).

The main constituents of the essential oil of *R. officinalis* are camphor (5.0-21%), 1,8-cineol (15-55%), α -pineno (9.0-26%), borneol (1.5-5.0%), canfen (2.5-12%), β -pineno (2.0-9.0%) and limonen (1.5-5.0%), in proportions that vary according to vegetative stage of the plant and bioclimatic conditions (Andrade et al., 2018; Satyal et al., 2017).

In the study by Satyal et al. (2017), the chemical composition of rosemary oil was analyzed by gas chromatography-mass spectrometry and chiral gas chromatography, and the predominant constituents were: (+)- α -pineno (13.5%-37.7%), 1,8-cineol (16.1%-29.3%), (+)-verbenone (0.7 8%-16.9%), (-)-borneol (2.1%-6.9%), (-)-camphor (0.7%-7.0%) and racemic limonena (1.6%-4.4%). It is noteworthy that these percentages differ from the ones we found in our study; in our study, we found slightly lower values for α -pineno, 1,8-cineol and borneol; similar values for verbenone; and higher values for camphor (14.26%).

In another research, Kaab et al. (2019) characterized the essential oil of *R. officinalis* by the predominance of the class of monoterpenes, among which 1,8-cineol (54.60%), camphor (12.27%) and α -pineno (7.09%) stood out. Similar values were found by Bomfim et al. (2020) for the constituents camphor (15.2%) and 1,8-cineol (52.2%) and α -pineno (12.4%) in rosemary essential oil extracted by hydrodistillation. These percentages of the major compounds are similar to those found in our study, however, the percentages of 1,8-cineol described are well higher than the value we found in the present study, of 13.90%.

Rosemary plants exhibit different genetic profiles that predispose them to produce essential oils with different chemotypes. Depending on the genes carried by the plant, it can produce higher contents of 1,8-cineol, camphor and verbenone, among others. Essential oils with higher content of cineol are less stimulating than essential oils with higher amounts of camphor, but it is still very useful for memory stimulation because the constituent 1,8-cineol promotes an increase in acetylcholine neurotransmitters, which play a role in the activation of learning and memory in the central nervous system, and also performs other actions such as anti-inflammatory, antinociceptor and myorelaxant actions (Juergens, 2014).

The constituent 1,8-cineol presents great therapeutic potential as an antiviral agent. In the study by Li et al. (2016), it efficiently decreased the level of IL-4, IL-5, IL-10 and MCP-1 in nasal lavage fluids, and the level of IL-1 β , IL-6, TNF- α and IFN- γ in lung tissues of mice infected with influenza virus. It also reduced the expression of NF-kB p65, intercellular adhesion molecule 1 (ICAM)-1 and vascular cell adhesion molecule 1 (VCAM-1 in lung tissues.

Differences in the percentages of major constituents among studies are justified by the natural variability of plants.

Table 3. Chemical composition of the essential oil extracted from leaves of *Rosmarinus officinalis*.

Compounds	Retention Time (min.)	Area (%)
Alpha-Pineno	5.8	12.46
Camphene	6.2	11.46
Beta-Pinene	7.1	3.38
Beta-Myrcene	7.5	1.60
Alta-Phelandrene	8.1	0.74
3-Careno	8.4	0.87
Cymen	8.8	1.90
1,8-Cineole	8.9	13.90
Gamma Terpinolene	9.9	1.66
Alpha-Terpinolene	11	1.49
Linalool	11.5	3.33
Camphor	13.4	14.26
Endo-Borneol	14.2	4.98
Terpinem-4-Ol	14.6	1.24
Alpha-Terpinelol	15.2	2.13
Myrthenol	15.5	0.35
Verbenone	16.1	7.03
Carvone	19.3	3.07
Caryophyllene	24.9	2.81
Humulene	26.2	0.58
n.i.*	31.3	10.76
Total		100%
Total chemical constituents identified		89.24%

*n.i.: compounds not identified. Source: the author, 2021.

Table 4. Chemical composition of the essential oil extracted from leaves of *Corymbia citriodora*.

Compounds	Retention Time (min.)	Area (%)
Alpha-pinene	5.8	0.74
1,8-cineole	8.8	0.98
Linalool	11.4	1.75
Isopulegol	13.3	17.21
Citronellal	14	62.51
Neoisopulegol	14.4	1.33
Citronellol	16.9	9.88
Caryophyllene	24.9	1.44
n.i.*	24.9	4.16
Total		100%
Total chemical constituents identified		95.84%

*n.i.= unidentified compounds. Source: the author, 2021.

Further, according to Bourhia (2019), cultivation in different geographical regions can affect the chemical composition and biological activities of plants, since they are sensitive to climatic variations.

As for the biological activities of the compounds, a molecular docking study evaluated the anti-inflammatory and anti-algic potency of a nanoemulsion containing *R. officinalis* essential oil, with the main compounds limonen, camphor and 1,8-cineol (Borges et al., 2018). The authors stated that camphor presented a higher number of interactions with therapeutic targets related to the inflammatory and pain process, suggesting that camphor was responsible for these therapeutic effects in this experiment with animal models (Borges et al., 2018). Other scientific studies demonstrated that *R. officinalis* essential oil has significant activity against various bacteria (*Staphylococcus epidermidis*, *Staphylococcus aureus*, *Bacillus subtilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Escherichia coli*) and fungi (*Candida albicans* and *Aspergillus niger*), being the major compounds 1,8-cineol (26.54%) and α -pineno (20.14%) considered responsible for such activities (Jiang et al., 2011). These were also the major compounds found in our study.

However, considering the complexity of essential oils, the antimicrobial effect can not be attributed only to the isolated major compounds, since antimicrobial activity can

be rather the result of the synergistic effect of all constituents (Micić et al., 2021). In the studies of Caetano (2018), 88.83% of the chemical constituents in essential oil were found.

Some studies have demonstrated the antibacterial and antifungal activity of different eucalyptus and rosemary species against various microorganisms (Daronco et al., 2015; Leite et al., 2018; Kesho et al., 2020; Bomfim et al., 2020; Aguiar et al., 2014; Caetano, 2018). Thus, new natural actives can be proposed for preclinical and clinical studies against different types of pathogens, and also for field studies in the treatment of phytopathogens (Andrade et al., 2018; Ootani et al., 2016). To advance the understanding on the theme, different concentrations of essential oils and their constituents should be tested in future studies, as they can perform both fungistatic and fungicide actions, depending on the dose used (Brum et al., 2012).

Another important point to highlight is that alterations in the major compounds of essential oils, either by biotic or abiotic factors, can directly influence the results of tests on phytopathogens. Thus, divergences in the results of with the same plant species and pathogens may occur. In order to minimize these effects and prevent non-conclusive data from being published, it is ideal that a chemical analysis of the oil is carried out together with biological activity testing in order to obtain the phytochemical characterization of

the oil. This way it will be possible to ascertain which major compounds are responsible for conferring the detected action on a certain phytopathogen (Morais, 2009).

As Morais (2009) explains, more conclusive results could prevent plants with potential ability to control diseases in relevant crops from being discarded or plants with little or no potential from becoming the target of studies unnecessarily. The association of biological assays with the phytochemical characterization of essential oils can be useful for developing biocompatible products, whether as natural compounds or as models for chemical synthesis or semi-synthesis of products with low cost, ease of application, small spectrum of action, low residual persistence and low toxicity to man, animals and the environment.

In the context of growing interest in the medicinal properties of aromatic plants, Brazil stands out in the world production of essential oils, but the lack of maintenance of quality standards in the country triggers a stationary picture in oil production (Bizzo et al., 2009). On the other hand, the cultivation of aromatic plants in organic family production systems, aiming at the production of essential oil, is a promising commercial alternative. Rodrigues et al. (2021) addressed this fact as a current scenario that requires investments to promote both production and research on the theme, because there is a lack of data on the potential applicability of essential oils in various crops.

Rodrigues et al. (2021) states that the use of essential oils for the control of phytopathogens is somewhat promising, because, in addition to being an alternative method, it does not cause damage to the environment, because they are natural organic molecules. Thus, the use of these products can lead to income generation, especially in the scenario of family farming, because most species with phytochemical potential are easy to grow, requiring no major investments or complex agricultural management.

Materials and methods

Characterization of the raw material

The essential oils of two medicinal plant species, rosemary (*R. officinalis*) and eucalyptus (*C. citriodora*) (Table 1), were extracted from plants cultivated by the researchers in their original sites of occurrence. The plants were collected during winter in the first hour of the morning, before sunrise, on non-rainy days. *R. officinalis* and *C. citriodora* plants were collected, respectively, in the municipalities of Bozano/RS (28°22'04.6 S, 53°46'17.2 W) and Catuípe/RS (28°28'92.5 S, 53°96'90.2 W) and georeferenced. After being identified in the Botany Laboratory of the Regional University of the Northwest of the State of Rio Grande do Sul - UNIJUÍ, the species were cataloged and registered in the Rogério Bueno University Herbarium (HURB) (exsiccates number 8112 - *R. officinalis* and 8055 - *C. citriodora*).

According to Law n. 13123/2015 (Biodiversity Law), all research with Brazilian genetic heritage (plants, animals and microorganisms) or with access to associated traditional knowledge, as well as the development of products with Brazilian biodiversity, should be registered in the National System of Genetic Heritage Management (SisGen) (Brasil, 2015). Therefore, this study was registered in SisGen under the number A734408.

Extraction of essential oils

The essential oils were extracted in the Laboratory of Organic Chemistry of UNIJUÍ through the hydrodistillation technique, using a modified Clevenger type apparatus, according to the technique described by the Brazilian Pharmacopoeia 6th edition (Anvisa, 2019).

For this technique, 300 g of fresh leaves of *R. officinallis* and

C. citriodora were used in each distillation. The leaves were chopped and placed individually in volumetric balloons with a capacity of 2 L, and 1 L of distilled water was added and heating was carried out with the aid of a heating blanket. The distillation time was approximately 3 to 4 hours, computed from the beginning of boiling (± 100 °C), at which time the temperature was reduced to 75 °C. The separation and calculation of yield volume of volatilized and condensed compounds were performed using a graduated Dean Stark apparatus coupled to the hydrodistiller. The essential oils were collected into sterile bottles of amber glass with screw cap and stored under refrigeration (± 8 °C) until the time of their use.

Essential oil yield

The essential oil yield of each plant was calculated as the volume of oil obtained in mL divided by mass in grams of fresh vegetable material used, according to the following formula:

$$\text{Yield (v/p)} = \frac{\text{Volume of essential oil obtained (mL)} \times 100}{\text{Mass of fresh plant material (g)}}$$

Characterization of essential oils

Qualitative analyses of the main chemical constituents of the essential oils of *R. officinalis* and *C. citriodora* were performed using the Gas Chromatography (GC) technique, using an Agilent 7890B triple quadrupole gas chromatograph equipped with a HP-5MS fused silica capillary column (30 m; 0.25 mm; 0.25 μm) coupled to a mass spectrometer. This was obtained with the ionization voltage of 70 eV (source temperature 280 °C). The temperature ramp was programmed from 80-160 °C to 25 °C/min⁻¹ and from 160-270 °C to 8 °C min⁻¹ and maintained an isotherm for 11 min. The drag gas used was helium with a flow of 1.0 cm³ min⁻¹. These analyses were carried out at the Analytical Center - Laboratory of Environmental and Food Analysis of UNIJUÍ. The identification of the compounds was made by comparing their mass spectra with the CG-EM database (Wiley 229, Lib). Quantitative data were obtained from the percentages (%) of the chromatogram areas through standardization.

Conclusion

In this study, *R. officinalis* obtained an average essential oil yield of 0.49%, and *C. citriodora* a yield of 1.54%. The major constituents in the essential oil of rosemary, determined through CG-EM, were camphor, 1,8-cineol, canfene and α -pineno, and the ones of the essential oil of eucalyptus were citronelal, isopulegol and, which is in accordance with what is described in the literature. According to our present findings and previous studies, the essential oil yields of *R. officinalis* and *C. citriodora* are quantitatively low and dependent on external conditions such as climate, planting and soil conditions, plant age, rainfall incidence, as well as plant genetic variability.

Additional assertive research is necessary to search for pharmacological effects of the constituents of the studied aromatic plants, considering the lack of drugs or active drugs for neglected diseases and chronic diseases.

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