Chemical composition and antioxidant activity of leaf essential oil from Calyptranthes concinna DC. (Myrtaceae)

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ABSTRACT. The biodiversity found in Brazilian’s ecosystems brings the possibility of discovering new natural products with wide application potentials. However, knowing their availability and chemical composition is crucial. Thus, the aim of this study was to evaluate the extraction yield, chemical composition and antioxidant activity of essential oil from fresh leaves of Calyptranthes concinna DC., a native species of Myrtaceae occurring in Brazilian Atlantic Rain Forest. Plant samples were collected in Southeastern Brazil and the essential oil was extracted by hydrodistillation. The chemical composition was evaluated by Gas Chromatography associated with Mass Spectrometry and antioxidant activity was measured using ABTS, DPPH and FRAP methods. The extraction yield obtained was 0.015% (v), and the chemical composition revealed elemicin, a phenylpropanoid as the major component (36.46%). Still, β-caryophyllene (16.94%), germacrene B (8.28%) and spathulenol (7.33%) proved to be relevant for the same essential oil. Antioxidant activity was obtained for ABTS and DPPH radical scavenge (134.82 ± 2.9 and 93.70 ± 1.7 µM TE mL⁻¹, respectively) and FRAP (11.31 ± 0.2 µM FeSO₄ mL⁻¹ OE), revealing hydrogen-donation as the main antioxidant mechanism. To our knowledge, this is the first report of antioxidant activity of C. concinna essential oil. The product presented compounds of great relevance, with possibilities of application in different areas including food, agriculture and pharmaceutical segments

Keywords: bioeconomy; natural products; volatile compounds; Atlantic Rain Forest; chemical inhibitors.

Introduction

The use of aromatic plants is a practice dating back to ancient times, being used as a source of bioactive compounds, applied as anti-infective, antiseptic, flavoring and seasoning agents, depending on their chemical composition (Cerqueira et al., 2007). The presence of volatile compounds, such as terpenes and phenylpropanoids characterize aromatic plants, which can be obtained as essential oils (EO) from leaves, flowers, stems, barks, roots, fruits and seeds by conventional (solvent extraction, hydrodistillation, steam distillation) and advanced (microwave extraction, supercritical fluid extraction, subcritical liquid extraction) methods (Aziz et al., 2018; Fitsiou & Pappa, 2019; Mohamed et al., 2020).

Within the groups of plants of great importance in Brazil, Myrtaceae family is characterized by presenting species of great medicinal relevance (Carneiro et al., 2017). This group has more than 1,054 species of trees and shrubs distributed in 23 genera (BFG, 2015), with the vast majority of species being a source of essential oils (Silveira, Carvalho, Bünger, & Costa, 2021).

Essential oils are used for different purposes, such as antimicrobial agents and have built great interest as antioxidants (Teixeira et al., 2013). Antioxidants are able to capture free radicals originated in biological and biochemical processes, that can be harmful for living organisms and processed products (Munteanu & Apetrei, 2021). In addition, some essential oils have shown potential to replace synthetic products in the pharmaceutical, chemical, food, cosmetic and agricultural industries (Gatto et al., 2020).

More specifically, in recent studies Myrtaceae essential oils showed in vitro antioxidant activity with Eugenia uniflora L. (Brazilian cherry) and Syzygium aromaticum L. (clove) essential oils providing excellent perspectives of applications (Batiha et al., 2020; Jerônimo et al., 2021).

On the other hand, among the naturally occurring taxa in Brazil, the genus Calyptranthes sp. still remains underexplored, being extremely important to know the chemical composition of its essential oils in order to
propose applications and extractive strategies. Thus, the main objective of this study was to evaluate the extraction yield, the chemical composition and the antioxidant activity of the EO from fresh leaves of *Calyptranthes concinna* DC., popularly known in Brazil as guamirim-facho.

**Material and methods**

**Plant material**

Plant samples containing reproductive structures (Figure 1) were collected in April 2020, from Dois Vizinhos, Paraná, Southeastern Brazil (25°52′38.0″S 53°04′39.6″W) (Figure 2). The species was identified using specialized literature and comparisons with material from Herbaria DVPR of Universidade Tecnológica Federal do Paraná (formerly UTFPR). Later, the voucher specimen was deposited under number 7001.

![Figure 1. (A) Leaves and (B) fruits of *Calyptranthes concinna* DC.](image)

**EO extraction**

The extraction of EO fresh leaves was carried out by hydrodistillation, using Clevenger apparatus, for four hours, using 1:10 plant:distilled water ratio (m v⁻¹). The recovered EO was placed in a falcon tube, in dark and low temperature (4°C) until subsequent analysis.
Yield extraction determination

The yield in terms of EO (%) was calculated according to Girard, Koehler and Neto (2007) equation, with modification (Equation 1).

\[
Y(\%) = \frac{EOV \times d}{B} \times 100
\]  

(1)

Where \( Y \) is the Yield in EO (%), \( EOV \) is the volume of EO obtained, \( d \) is density and \( B \) is the plant biomass used.

CG/MS

Chemical composition was accessed using Gas Chromatography/Mass Spectrometry (Shimadzu CG-MS Chromatograph, QP 2020) with RTX-5ms column (5% polar, dimensions: 30 m x 0.25 mm; 0.25 μm). The equipment had automatic injector split mode set at 280 °C. Helium was used as carrier gas, with a linear flow of 1.8 mL min\(^{-1}\). Column pressure was 107.4 KPa. The mass analyzer was a Quadrupole type. The scan data were collected from 37 – 600 m z\(^{-1}\). All chemical compounds were identified according to the data collection, using specialized literature.

Antioxidant activity

Antioxidant potential of EO \( C. \) concinna was measured using three different methodologies, including the ability to scavenge ABTS (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) and DPPH (2,2-diphenyl-1-picrylhydrazyl) radicals and evaluating Ferric Reducing Antioxidant Power (FRAP). All analyses were performed in triplicates.

ABTS

Stock solutions of ABTS (7 mM) and potassium persulfate (140 mM) were prepared in aqueous medium and reacted overnight to radical activation. The radical was diluted in ethanol 95% and absorbance was adjusted to 0.700 in spectrophotometer with wave length of 734 nanometers (Rufino et al., 2007). ABTS solution was reacted with EO solution during six minutes under dark and room temperature conditions and read in spectrophotometer.

This method is based in ABTS radical scavenge capacity of antioxidants through hydrogen-donating mechanism (Jerônimo et al., 2021), producing a colorless compound that decrease absorbance in a short-term reaction (Figure 3).

![Figure 3. Oxireduction of ABTS radical (Rufino et al., 2007).](image)

All calculations of antioxidant activity were based in a standard curve and all results were expressed as μM TE (Trolox Equivalent) mL\(^{-1}\) OE.

DPPH

A stock solution of DPPH (0.1 mM) was prepared in methanol and reacted with EO solution (in methanol) for thirty minutes under dark and room temperature conditions. The absorbance measurement was performed in spectrophotometer with wave length of 517 nanometers, using methanol as control (Shirwaikar, Shirwaikar, Rajendran, & Punitha, 2006; Elansary et al., 2012).

This method is based in DPPH radical scavenge of antioxidants, mainly through hydrogen-donating, but also trough electron-donating mechanisms (Pisoschi, Cheregi, & Danet, 2009) (Figure 4). All calculations of antioxidant activity were based in a standard curve and all results were expressed as μM TE (Trolox Equivalent) mL\(^{-1}\) OE.

FRAP reagent was prepared using TPTZ (2,4,6-Tri-(2-Pyridyl)-1,3,5-Triazine) (10 mM, solubilized with acidification using concentrated HCl) and Iron (III) chloride (20 mM) in acetate buffer (\( \nu/\nu/\nu \), 1:1:10) (Rufino et al., 2006). FRAP reagent was reacted with EO solution (in methanol + 1% of Tween 80) in dark and hot water-bath at 37°C.
FRAP

This method is based on the reduction ability of antioxidants, mainly due to electron-donation mechanism. When \([\text{Fe(III)(TPTZ)}_2]^3+\) is reduced to \([\text{Fe(II)(TPTZ)}_2]^2+\), a dark blue compound is formed and quantified by spectrophotometry (Figure 5).

![Figure 5. Mechanism of action of FRAP method (Rufino, 2006).](image)

The calculation of reducing power was based on a standard curve plotted with Iron (II) sulfate. All results were expressed as \(\mu\text{M Fe(II)}\text{SO}_4 \text{ mL}^{-1} \text{ OE}\).

Results and discussion

The yield of EO extraction from \(C. \text{ concinna}\) was 0.015% (v) and 20 chemical compounds were identified (Table 1).

| Nº  | Compound                  | Molecular formula | R.T.  | \(C. \text{ concinna} \text{ EO}\) |
|-----|---------------------------|-------------------|-------|-----------------------------------|
| 1   | cis-β-Ocimene             | \(\text{C}_{10}\text{H}_{16}\) | 11.506| 1.69                              |
| 2   | Elixene                   | \(\text{C}_{10}\text{H}_{14}\) | 24.145| 2.47                              |
| 3   | Copaene                   | \(\text{C}_{10}\text{H}_{14}\) | 25.867| 0.88                              |
| 4   | β-Bourbonene              | \(\text{C}_{10}\text{H}_{14}\) | 26.236| 1.40                              |
| 5   | β-Elemene                 | \(\text{C}_{10}\text{H}_{14}\) | 26.600| 4.91                              |
| 6   | β-Gurjunene               | \(\text{C}_{10}\text{H}_{14}\) | 27.284| 0.78                              |
| 7   | β-Caryophyllene           | \(\text{C}_{10}\text{H}_{14}\) | 27.699| 16.94                             |
| 8   | 9-epi-trans-caryophyllene| \(\text{C}_{10}\text{H}_{14}\) | 28.484| 2.50                              |
| 9   | Humulene                  | \(\text{C}_{10}\text{H}_{14}\) | 29.076| 2.39                              |
| 10  | Aromadendrene             | \(\text{C}_{10}\text{H}_{14}\) | 29.566| 0.95                              |
| 11  | Germacrene D              | \(\text{C}_{10}\text{H}_{14}\) | 30.208| 1.19                              |
| 12  | β-Selinene                | \(\text{C}_{10}\text{H}_{14}\) | 30.408| 0.73                              |
| 13  | Germacrene B              | \(\text{C}_{10}\text{H}_{14}\) | 30.845| 8.28                              |
| 14  | α-Amorphene               | \(\text{C}_{10}\text{H}_{14}\) | 31.924| 1.57                              |
| 15  | Elemicin                  | \(\text{C}_{10}\text{H}_{14}\text{O}_{3}\) | 33.408| 36.43                             |
| 16  | Spathulenol               | \(\text{C}_{10}\text{H}_{14}\text{O}\) | 34.032| 7.33                              |
| 17  | Globulol                  | \(\text{C}_{10}\text{H}_{14}\text{O}\) | 34.265| 5.43                              |
| 18  | Ledol                     | \(\text{C}_{10}\text{H}_{14}\text{O}\) | 34.565| 1.56                              |
| 19  | Criptomeridiol            | \(\text{C}_{10}\text{H}_{14}\text{O}_{2}\) | 34.970| 1.22                              |
| 20  | Neointermedeol            | \(\text{C}_{10}\text{H}_{14}\text{O}\) | 36.907| 1.55                              |

For the EO of \(C. \text{ concinna}\) 90% of the identified components were sesquiterpenes, with elemicin, a phenylpropanoid, being the major component (36.46%). Furthermore, β-caryophyllene (16.94%), germacrene B (8.28%) and spathulenol (7.33%) proved to be relevant for the same EO (Figure 6, Table 3).
Elemicin is a compound also identified in the EO of *Daucus carota* L. (wild carrot), which showed an antimicrobial effect against *Campylobacter* spp., being the molecule responsible for the observed biological effect (Rossi et al., 2007). This compound is also found in nutmeg, which may be associated with toxic effects observed with the ingestion of large amounts (Duarte, Mendonça, & Ramos, 2021).

Other compounds similar to those in Figure 6, were found as major compounds in *Kyllinga pumila* Michx (Cyperaceae) EO (Z-caryophyllene, E-caryophyllene and germacrene D with 11.3, 5.6 and 7.1%, respectively) (Jaramillo-Coráloro, Martínez-Cáceres, & Duarte-Restrepo, 2016). Spathulenol was also identified in *Piper sancti-felicis* Trel (Piperaceae) EO (8.2%), with a similar amount to *C. concinna* (7.33%) (Medrano-Ochoa et al., 2021). Both essential oils presented insecticide/repellent activity against *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), an insect considered a pest in stored cereals and other foods.

![Figure 6. Chromatogram of Calyptranthes concinna essential oil and major components.](image)

Studies aimed at evaluating the chemical composition of the EO of *C. concinna* have already been carried out, with elemicin (Menut et al., 1997; Silva et al., 2019) and germacrene derivatives (Limberger et al., 2002) identified as major components.

In the present work, the volume of *C. concinna* EO extracted was considered low. However, when analyzing antimicrobial potential against resistant bacterial strains by Costa et al. (2020), it was found inhibitory activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*, thus showing an antimicrobial effect. It shows the need to focus on studies that can increase the productive capacity of the plant in terms of EO, or identify and select more productive genotypes for use in cultivation.

Although, antioxidant activity was observed, being obtained for ABTS radical scavenging 134.82 ± 2.9 µM TE mL⁻¹ of OE and for DPPH, 93.70 ± 1.7 µM TE mL⁻¹ of OE (Table 2), revealing hydrogen-donation as the main antioxidant mechanism. FRAP result was considered weak, with 11.16 ± 0.2 µM Fe(II)SO₄ mL⁻¹ of OE, confirming low activity of electron-donating compounds. To our knowledge, this is the first report of antioxidant activity of *C. concinna* EO.

| Antioxidant activity | *C. concinna* EO |
|----------------------|------------------|
| ABTS (µM TE mL⁻¹)    | 134.82 ± 2.9     |
| DPPH (µM TE mL⁻¹)    | 93.70 ± 1.7      |
| FRAP (µM Fe(II)SO₄ mL⁻¹) | 11.16 ± 0.6 |

Different results were obtained for *Calyptranthes grandifolia* (O. Berg) (guamirim) and *Calyptranthes tricona* (D. Legrand) (guaburiti), where no antioxidant activity was observed for essential oils (Faleiro et al., 2017). These differences can be explained by the divergence in the chemical composition of essential oils between...
different species, since the antioxidant activity is strongly influenced by the composition and chemical structure of major compounds.

In comparison with other species from Myrtaceae family, to in natura leaves EO from *E. klotzschiana* Berg, 57.81 µM TE g⁻¹ were obtained with ABTS scavenge radical (Carneiro et al., 2017). Still, for *Myrcia sylvatica*, 32.85 µM TE g⁻¹ was calculated (Silva et al., 2018). Both results were lower than obtained for *C. concinna*, revealing remarkable antioxidant activity. However, *E. uniflora* EO presented superior results, from 176.66 to 867.57 µM TE g⁻¹ of EO, showing better antioxidant activity, possibly due to the presence of high concentrations of germacrone (Cipriano, Maia, & Deschamps, 2021).

**Conclusion**

This is the first report of antioxidant activity from *C. concinna* EO. Despite the low yield of extraction obtained by hydrodistillation, the EO presented interesting antioxidant activity against synthetic free radicals.

The EO was characterized by elemicin, a phenylpropanoid, but sesquiterpene compounds such as β-caryophyllene, germacrone B and spathulenol also presented relevance. According to these preliminary results, there is the possibility of applications in food, agriculture and pharmaceutical sectors, however, studies aimed at other biotechnological potentialities and extractive methods with higher yields are still required.

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