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## Metabolite Profiling (GC-MS), Antioxidant and Antimicrobial Activities of *Baccharis dracunculifolia* Hydroalcoholic Extract

### Perfil Metabólico (GC-MS), Atividade Antioxidante e Antimicrobiana do Extrato Hidroalcoólico de *Baccharis dracunculifolia*

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Alexsandra Iarlen Cabral Cruz: Universidade Federal do Recôncavo da Bahia, Bahia, Brazil. 


Milena da Cruz Costa: Universidade Federal do Recôncavo da Bahia, Bahia, Brazil. 


Jessica Ferreira Maфра: Universidade Federal do Recôncavo da Bahia, Bahia, Brazil. 

Mariza Alves Ferreira: Universidade Federal do Recôncavo da Bahia, Bahia, Brazil. 

Alana Kercia Marques Miranda: Universidade Federal da Bahia, Bahia, Brazil. 

Paulo Roberto Ribeiro: Universidade Federal da Bahia, Bahia, Brazil. 

Floricea Magalhães Araújo: Universidade Federal da Bahia, Bahia, Brazil. 

Norma Suely Evangelista-Barreto: Universidade Federal do Recôncavo da Bahia, Bahia, Brazil. E-mail. [nsevangalista@ufrb.edu.br](mailto:nsevangalista@ufrb.edu.br) 

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#### Abstract

*Baccharis dracunculifolia*, popularly known as ‘field rosemary’, is a shrub native to South America with reported anti-inflammatory, antimicrobial, anticancer, and antioxidant properties. This study aimed to evaluate the metabolite profile of the hydroalcoholic extract of *B. dracunculifolia* using gas chromatography–mass spectrometry (GC–MS) and to determine its antioxidant and antibacterial activities. Sixteen metabolites were identified, predominantly terpenoids (46%), along with ketones, aldehydes, hydrocarbons, and acids. The extract exhibited antioxidant activity with an  $IC_{50}$  of  $17.7 \pm 0.02$  mg/mL in the DPPH assay and  $361 \pm 0.03$   $\mu$ mol Trolox/g in the ABTS assay. Total flavonoid content was  $6.0 \pm 0.14$  mg rutin/100 g extract (w/w), while total phenolic content reached  $17.10 \pm 0.27$  mg GAE/g. Regarding antibacterial activity, the extract displayed bacteriostatic effects (MIC) ranging from 370  $\mu$ g/mL for *L. monocytogenes* to 7,500  $\mu$ g/mL for *P. aeruginosa*, and bactericidal effects (MBC) from 2,500  $\mu$ g/mL for *S. aureus* to 30,000  $\mu$ g/mL for *E. coli*. The bioactive potential observed can be largely attributed to the presence of triterpenes. *B. dracunculifolia* hydroalcoholic extract is a promising source for pharmacological, phytochemical, and microbiological investigations.

**Keywords:** Antibacterial activity. ABTS. DPPH. Flavonoids. Phenols.

## Resumo

*Baccharis dracunculifolia*, popularmente conhecida como "alecrim do campo", é um arbusto nativo da América do Sul que apresenta propriedades anti-inflamatórias, antimicrobianas, anticancerígenas e antioxidantes. Este estudo avaliou o perfil metabólico do extrato hidroalcoólico de *B. dracunculifolia* por cromatografia gasosa acoplada à espectrometria de massas (CG-EM) e determinou sua atividade antioxidante e antibacteriana. Foram identificados 16 metabólitos, predominantemente terpenoides (46%), juntamente com cetonas, aldeídos, hidrocarbonetos e ácidos. O extrato apresentou atividade antioxidante com  $CI_{50}$  de  $17,7 \pm 0,02$  mg/mL (ensaio DPPH) e  $361 \pm 0,03$   $\mu$ mol Trolox/g (ensaio ABTS). O teor total de flavonoides foi de  $6,0 \pm 0,14$  mg de rutina/100 g de extrato (p/p) e o de fenóis totais de  $17,10 \pm 0,27$  mg GAE/g. Em relação à atividade antimicrobiana, o extrato apresentou efeito bacteriostático (CIM) variando de 370  $\mu$ g/mL para *L. monocytogenes* a 7500  $\mu$ g/mL para *P. aeruginosa*, e efeito bactericida (CBM) entre 2500  $\mu$ g/mL para *S. aureus* e 30000  $\mu$ g/mL para *E. coli*. O potencial bioativo observado pode ser atribuído, em grande parte, à presença de triterpenos. O extrato hidroalcoólico de *B. dracunculifolia* é uma fonte relevante para investigações farmacológicas, fitoquímicas e microbiológicas.

**Palavras-chave:** Atividade antibacteriana. ABTS. DPPH. Flavonoides. Fenóis.

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## 1 Introduction

*Baccharis dracunculifolia* is a medicinal plant native to Brazil, primarily found in the Cerrado, Atlantic Forest, and Pampas biomes. This plant species stands out for its numerous biological and pharmacological activities (Costa *et al.*, 2018; Cazella *et al.*, 2019; Zuccolotto *et al.*, 2019). Within the *Baccharis* genus, *B. dracunculifolia* is among the most extensively studied species from a phytochemical and pharmacological perspective. Previous studies have reported the presence of phenolic acids, diterpenes, flavonoids, triterpenes, and various types of glycosides in this species (Gazim *et al.*, 2022).

The occurrence of complex terpenoid structures has also been confirmed in *B. dracunculifolia* (Cazella *et al.*, 2019). According to Iurckevicz *et al.* (2021), the antioxidant capacity of phenolic compounds from *B. dracunculifolia* is closely associated with their redox potential, as well as their ability to neutralize and scavenge free radicals, decompose peroxides, and chelate reactive oxygen species (Bonin *et al.*, 2020).

The antimicrobial and antioxidant activity of the oil and extract of *B. dracunculifolia* is attributed to the presence of various bioactive compounds, including prenylated p-coumaric acid derivatives, such as artepelin C and baccharin, along with flavonoids, diterpenes, and triterpenes isolated from different areas of the plant (Salazar *et al.*, 2018; Silva *et al.*, 2024). However, it is important to highlight that the composition of *B. dracunculifolia* extracts can vary greatly depending on where the plant was grown, climatic and soil conditions, environmental factors, and the parts of

the plant used (Cazella *et al.*, 2019; Bonin *et al.*, 2020), thus influencing the biological activity of the extracts.

Additionally, *B. dracunculifolia* extracts are promising sources of material for the manufacture of medicines, cosmetics, food additives, and supplements. Therefore, this research aimed to evaluate the metabolite profile and antioxidant and antibacterial activity of the hydroalcoholic extract of *B. dracunculifolia*.

## 2 Material and Methods

### 2.1 Obtaining of the extract

The hydroalcoholic extract of *Baccharis dracunculifolia* (3% w/v) was obtained commercially from a certified producer (Abmel, Cruz das Almas, Bahia, Brazil). According to the supplier, the plant material used for extract preparation was collected in the municipality of Goiânia, Goiás, Brazil (16°40'48" S, 49°15'18" W), and the extraction was performed using cereal alcohol (93.5% v/v) as solvent, yielding a final concentration of 3% (w/v).

### 2.2 Metabolite profile via gas chromatography-mass spectrometry (GC-MS)

The metabolite profile of *B. dracunculifolia* hydroalcoholic extract was evaluated by gas chromatography-mass spectrometry (GC-MS) on a Shimadzu gas chromatograph (GC2010) coupled to a mass spectrometer (QP2020) using a DB-5MS capillary column (30 m × 0.25 mm and 0.25 μm). The flow rate of the mobile phase (He) was set at 1.8 mL/minute, the injector temperature was set at 280 °C, and the oven was programmed from 60 °C (for 1.0 minute) to 280 °C with a heating rate of 10 °C per minute, remaining at 280 °C for up to 35 minutes. The injection was carried out using a pulsed mode splitless (at 111.5 kPa), and the temperatures of the source and interface were maintained at 280 °C. Full scan spectra were recorded from 37 to 660 m/z (mass/load) with two scans per second. The compounds were identified by comparing their mass spectra with those available in the NIST08, Mainlib, and Wiley7 databases, as well as with the literature (Bohlmann *et al.*, 1982; Bonin *et al.*, 2020; Gazim *et al.*, 2022; Jaramillo-García *et al.*, 2018; Martinez *et al.*, 2012). The Kóvats Index was calculated by comparing the retention time of the compounds with the retention time of the homologous series of hydrocarbons C<sub>9</sub>H<sub>20</sub>-C<sub>40</sub>H<sub>82</sub>.

### 2.3 Determination of the content of total phenols and total flavonoids

For the quantification of total phenols, an aliquot of 4160 μL of the extract (3% w/v) was diluted

with methanol to reach a final concentration of 0.5% w/v. Then, a 100 µL aliquot of the diluted extract (0.5% w/v) was mixed with 500 µL of Folin-Ciocalteu reagent, 6 mL of distilled water, and 2 mL of Na<sub>2</sub>CO<sub>3</sub> (3% w/v). The reaction mixture was kept in the dark for 2 hours, and the absorbance was measured using a spectrophotometer (Tecnal UV 5100) at 750 nm. Gallic acid (10-60 µg/mL) was used to construct a calibration curve:  $y = 0.0109x + 0.0143$  ( $R^2 = 0.9945$ ). The results were expressed as milligram equivalents of gallic acid per gram of extract (mg GAE/g of extract) (Singleton; Orthofer; Lamuela-Raventós, 1999).

For the quantification of total flavonoids, an aliquot of 5 mL of the extract (0.5% w/v) was mixed with 5 mL of AlCl<sub>3</sub> (20 mg/mL). The reaction mixture was then kept in the dark for 30 minutes, and the absorbance was recorded on a spectrophotometer (Tecnal UV 5100) at 415 nm. Rutin (0-100 µg/mL) was used to construct the calibration curve:  $y = 14.025x + 0.0722$  ( $R^2 = 0.9999$ ). The results were expressed as grams of rutin per 100 g of extract of *B. dracunculifolia* (w/w) (Singleton; Orthofer; Lamuela-Raventós, 1999).

#### **2.4 DPPH and ABTS radical scavenging test**

The antioxidant activity was assessed by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS<sup>+</sup>) radical scavenging assays.

For the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) radical scavenging assay, 300 µL of the extract at different concentrations (0.1-2.0 mg/L) was homogenized in 2.7 mL of DPPH (0.06 mM) and kept under constant stirring for 1 min.

The reaction mixture was kept in the dark for 30 min, and the absorbance was recorded on a spectrophotometer (Tecnal UV 5100) at 515 nm. The results were expressed as IC<sub>50</sub> (mg/mL) (Brand-Williams; Cuvelier; Berset, 1995).

For the 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid radical (ABTS<sup>+</sup>) radical scavenging assay, 30 µL of the extract in different concentrations (1000-6000 mg/L) was homogenized in 3 mL of the ABTS radical<sup>+</sup> and kept under constant stirring for 1 minute. The reaction mixture was kept in the dark for 6 minutes, and the absorbance was recorded on a spectrophotometer (Tecnal UV 5100) at 734 nm.

Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (10-60 µg/mL) was used to construct the calibration curve:  $y = -0.0003x + 0.6582$  ( $R^2 = 0.9901$ ). The results were expressed in µmol Trolox equivalent per gram of extract (Re *et al.*, 1999).

## 2.5 Antibacterial activity of *Baccharis dracunculifolia* extract

The antimicrobial activity of the extract was assessed against Gram-negative (*Escherichia coli* ATCC25922 and *Pseudomonas aeruginosa* ATCC25923) and Gram-positive (*Staphylococcus aureus* ATCC43300 and *Listeria monocytogenes* CERELA) bacteria according to the methodology of Wiegand, Hilpert and Hancock (2008). Initially, 100 µL of Muller-Hinton broth was placed in each of the 96-well ELISA plate wells. Then, 100 µL of the extract (30 mg/mL) was added to the wells of the first line, and after homogenization, microdilution was carried out to obtain final concentrations ranging from 180 to 2500 µg/mL. An aliquot of 10 µL of the inoculum ( $1 \times 10^8$  CFU/mL) of each bacterium was then added to the wells. Chloramphenicol was used as a positive control (1.0 to 30 µg/mL) and the plates were incubated at 37 °C for 24 hours. After this period, 20 µL of resazurin sodium dye (0.01%) was added to the wells, and the plates were reincubated for 3 hours. The minimum inhibitory concentration (MIC) was defined as the lowest concentration capable of inhibiting visible bacterial growth, whereas the minimum bactericidal concentration (MBC) was defined as the lowest concentration capable of causing bacterial cell death.

## 2.6 Statistical analysis

Total phenol and flavonoid content, as well as, antioxidant activity results were submitted to descriptive analysis. Results from the microbiological analysis were submitted to correlation and linear regression analysis, in which the student t-test was used to assess the relationship between the linear regression coefficients. Statistical analyses were performed on SPSS- Statistics for Windows, version 25.0.

## 3 Results and Discussion

### 3.1 Characterization of *Baccharis dracunculifolia* extract by GC-MS

*Baccharis dracunculifolia*, popularly known as "rosemary of the field" or "broom", is a native Brazilian plant that presents a great chemical diversity of compounds that show anti-inflammatory, antibacterial, antioxidant, immunomodulatory, antigenotoxic, and antimutagenic properties (Olivares-Pinto; Barbosa; Fernandes, 2022; Rodrigues *et al.*, 2020; Vilas-Boas *et al.*, 2023).

Sixteen metabolites were identified by GC-MS, including hydrocarbons, aldehydes, alcohols, acids and terpenoids (Table 1). Of these, terpenoids were the most representative metabolites, accounting for approximately 46% of the total relative area of the chromatogram.

**Table 1** - Metabolites present in the *Baccharis dracunculifolia* hydroalcoholic extract

| N° | RT    | %Area | Compound   | IR   | Class                |
|----|-------|-------|--|------|----------------------|
| 1  | 2.97  | 1.41  | 3,3-Dimethoxy-2-butanone                         | 843  | Ketone               |
| 2  | 5.00  | 0.44  | Octane, 2-methyl                                 | 986  | Hydrocarbon          |
| 3  | 8.10  | 0.28  | Decanal  | 1215 | Aldehyde             |
| 4  | 8.34  | 0.20  | 2-Furanmethanol, tetrahydro-                     | 1233 | Alcohol              |
| 5  | 9.99  | 1.71  | Benzenepropanoic acid                            | 1347 | Acid                 |
| 6  | 13.16 | 0.27  | (Z, E) - $\alpha$ -Farnesene                     | 1588 | Monoterpene          |
| 7  | 15.55 | 4.06  | Farnesol   | 1779 | Monoterpene          |
| 8  | 15.92 | 0.85  | 4-(2,2-Dimethyl-6-Methylidenecyclohexyl) butanal | 1870 | Aldehyde             |
| 9  | 17.20 | 1.40  | Palmitic acid                                    | 1925 | Saturated fatty acid |
| 10 | 19.83 | 0.61  | $\gamma$ -cis-sesquicyclogeraniol                | 2279 | Monoterpene          |
| 11 | 20.35 | 1.33  | Linalool   | 2333 | Monoterpene          |
| 12 | 22.55 | 4.43  | $\beta$ -Amyrin                                  | 2563 | Triterpene           |
| 13 | 25.11 | 14.62 | NI   | 2817 | -                    |
| 14 | 25.22 | 33.37 | NI   | 2828 | -                    |
| 15 | 25.32 | 25.05 | 28-Norolean-17-en-3-one                          | 2838 | Triterpene           |
| 16 | 25.95 | 9.95  | Sclareol   | 2848 | Diterpene            |

RT: retention time. IR: retention index.

Source: research data.

Terpenes are naturally occurring compounds commonly found in *B. dracunculifolia* extracts (Brandenburg *et al.*, 2020; Timbe *et al.*, 2020). According to Tomazzoli *et al.* (2021), terpene production levels can vary according to the plant's need for defense. These compounds play a crucial role in providing the plant with natural protection against bacteria, fungi, and insects. The presence of monoterpenes, sesquiterpenes, and their oxygenated derivatives may explain the bacteriostatic and bactericidal action found in the extract. Thus, the diverse collection of terpenoid structures and their functions has aroused growing interest among researchers, and their use has been explored as immunomodulatory, anti-inflammatory, cardiovascular protective, anticancer, antiviral, antibacterial, and antioxidant (Siddiqui *et al.*, 2024).

The metabolites 28-norolean-17-en-3-one and sclareol are reported for the first time in *B. dracunculifolia* (Table 1). The compound 28-Norolean-17-en-3-one was reported by Ostovan *et al.* (2020) in *Pistacia lentiscus*. According to these authors, this metabolite has been employed in traditional Persian and Greek medicine due to its pronounced antibacterial activity, attributed to the induction of cellular apoptosis, as well as exhibiting wound-healing, anticancer, antiarthritic, and antiatherogenic properties (Naouar *et al.*, 2016). Sclareol is a highly valuable diterpene because it has anti-inflammatory and anti-cancer properties. This metabolite is also capable of inducing cell cycle arrest and apoptosis in epithelial cancer cells (Marrelli *et al.*, 2020; Pal *et al.*, 2016).

Additionally, 28-norolean-17-en-3-one has relevant anti-inflammatory efficacy (Peivastegan *et al.*, 2020). *Pistacia lentiscus* is a plant that can grow in calcareous soil that commonly occurs in arid and semi-arid regions, mostly latosols (Cristiano *et al.*, 2016; Bolan *et al.*, 2023; Yildirim, 2012), which may be related to the development of *B. dracunculifolia*. The composition of alcoholic extracts

from plant species can vary according to the species of plant, the part of the plant used in extraction, cultivation, climatic conditions, soil conditions, harvest time, and even storage of the raw material (Apolônio *et al.*, 2023). In this sense, it can be inferred that the presence of 28-norolean-17-en-3-one in the *B. dracunculifolia* extract may be related to the soil conditions in which this species was grown and developed. In this context, the detection of 28-Norolean-17-en-3-one in the extract of *B. dracunculifolia* may be associated with the environmental conditions under which this species was cultivated, considering that this triterpene has not previously been reported for *B. dracunculifolia*.

The triterpene  $\beta$ -Amyrin exhibits gastroprotective effects (Negri; Salatino; Salatino, 2015) and possesses anti-inflammatory, antibacterial, antiulcerogenic, antioxidant, and antiviral properties (Silva; Sahal; Panda, 2023).

Farnesol and palmitic acid are involved in natural antimicrobial defense, which is associated with their importance in pollination and in the foraging behavior of bees, since this compound is used to seal cracks in the walls, reinforce wax combs and act against bacteria and fungi (Silva *et al.*, 2024).

### 3.2 Antioxidant activity and presence of phenols and flavonoids of *Baccharis dracunculifolia* extract

The total phenolic and flavonoid contents observed in the present study (Table 2) differed from those reported in previous studies, which may be attributed to variations in extraction method, plant origin, and environmental conditions. Although lower values were observed when compared to some reports (Rezende *et al.*, 2014; Casagrande *et al.*, 2018), the extract still exhibited significant antioxidant activity. It is important to note that GC-MS analysis is limited in the detection of highly polar phenolic compounds, which may explain their absence in the chromatographic profile (Table 1) despite their quantification by spectrophotometric methods.

**Table 2** - Total phenol content, flavonoids and antioxidant activity of *B. dracunculifolia* extract

| Extract                   | Phenols<br>(mg GAE/g) | Flavonoids<br>(mg RE/g) | DPPH<br>EC50 (mg/mL) | ABTS<br>( $\mu$ mol/trolox g) |
|---------------------------|-----------------------|-------------------------|----------------------|-------------------------------|
| <i>B. dracunculifolia</i> | 17.09 $\pm$ 0.27      | 6.0 $\pm$ 0.14          | 17.7 $\pm$ 0.02      | 361 $\pm$ 0.03                |

GAE: Gallic acid equivalent. RE: Rutin equivalent.

Source: research data.

Extracts of *B. dracunculifolia* with a high flavonoid content act to prevent lipid oxidation by reacting with free radicals involved in the oxidative process and forming relatively stable final radicals (Corrêa *et al.*, 2020). However, phenolic compounds are weak acids that act as hydrogen donors capable of reacting with  $O_2^-$  by different mechanisms, depending on the nature and number of substituents on the phenolic ring (Berretta *et al.*, 2020). Therefore, the significant amount of

phenolic compounds including flavonoids, in the *B. dracunculifolia* extract (Table 2), gives the extract a strong antioxidant potential as observed in Table 2 with the DPPH and ABTS methods.

In Table 3, *B. dracunculifolia* extract was able to neutralize both radicals, DPPH (1,1-diphenyl-2-picrylhydrazyl) and ABTS 2,2'-azinobis (3-ethylbenzenethiazoline-6-sulfonic acid). These results (Table 3) were higher than those reported by Martinez-Correa *et al.* (2012), when they found only 0.036 to 0.2 mg/mL in the capture of the DPPH radical in *B. dracunculifolia* extracts, while Casagrande *et al.* (2018) already reported values closer to those of our study (140.26 to 399.67  $\mu\text{mol TEAC/g}$ ) for the elimination of the ABTS radical (Table 3). These differences found in the antioxidant potential of *B. dracunculifolia* extract are normal, as the metabolites present in the extract vary due to external factors such as the seasonality of the plant and the solvents used in the extraction (Martinez-Correa *et al.*, 2012; Salazar *et al.*, 2018; Bonin *et al.*, 2020).

**Table 3** - Pearson's correlation coefficients (r) of total phenolic compounds, flavonoids and antioxidant activity (DPPH, ABTS) in *B. dracunculifolia* extract

|            | Phenols | Flavonoids | DPPH     | ABTS     |
|------------|---------|------------|----------|----------|
| Phenols    | 1       | 0.3113     | *0.7708  | *0.9933  |
| Flavonoids |         | 1          | *-0.8453 | *-0.9933 |
| DPPH       |         |            | 1        | *0.7777  |
| ABTS       |         |            |          | 1        |

\*Significant at 5% probability ( $p < 0.05$ ).

Source: research data.

The relationship between metabolite content and antioxidant activity was obtained using Pearson's correlation coefficients (r) (Table 3). A strong positive correlation can be seen between phenols and the DPPH (0.7708) and ABTS (0.9933) methods, which suggests a high relationship between the extract's components and its reducing power. However, the flavonoid class showed a strong negative correlation with the DPPH (-0.8453) and ABTS (-0.9933) methods, showing that this class is not directly responsible for the reducing power observed in the *B. dracunculifolia* extract, which corroborates the data presented in (Table 1), in which the extract showed a more significant relative area of terpenes.

With regard to the DPPH and ABTS methods, a strong positive correlation was observed between the two (0.7777), suggesting that the two methods obtained similar responses in relation to the antioxidant capacity of the extract's constituents and can be commonly used to assess the antioxidant potential of plant extracts.

### 3.3 Antibacterial activity of *Baccharis dracunculifolia* extract

The *B. dracunculifolia* extract showed antibacterial activity against Gram-positive bacteria (*S. aureus* and *L. monocytogenes*) and Gram-negative bacteria (*P. aeruginosa* and *E. coli*) (Table 4).

Palmitic acid is a saturated fatty acid that shows antioxidant activity and antimicrobial activity against Gram-positive and Gram-negative bacteria (Al-juhaimi *et al.*, 2021).

Gram-positive bacteria (*S. aureus* and *L. monocytogenes*) showed greater sensitivity to *B. dracunculifolia* extract than Gram-negative bacteria (*P. aeruginosa* and *E. coli*) (Table 4). This result can be attributed to the cell wall of Gram-positive bacteria, which is composed of only a thick peptidoglycan wall that is not dense enough to resist antimicrobial molecules present in the extract, facilitating access to the cell membrane, while Gram-negative bacteria have a more complex outer membrane rich in lipopolysaccharide that limits the diffusion of hydrophobic compounds (Hyldgaard; Mygind; Meyer, 2012). Another important characteristic of Gram-positive bacteria is that they facilitate the infiltration of hydrophobic compounds present in the extract due to the lipophilic ends of lipoteichoic acid present in the cell membrane (Chouhan, Sharma; Guleria, 2017). For Crespo *et al.* (2024), the presence of terpenes in *B. dracunculifolia* is responsible for the antimicrobial action of the extract, as they can cause the rupture of lipophilic compounds in microbial membranes.

**Table 4** - Antibacterial activity of *Baccharis dracunculifolia* extract against Gram-positive and Gram-negative bacteria

| Bacteria                | <i>B. dracunculifolia</i> extract |             | Chloramphenicol |             |
|-------------------------|-----------------------------------|-------------|-----------------|-------------|
|                         | MIC (µg/mL)                       | MBC (µg/mL) | MIC (µg/mL)     | MBC (µg/mL) |
| <i>S. aureus</i>        | 620                               | 2500        | 1.0             | 10          |
| <i>L. monocytogenes</i> | 370                               | 30000       | 1.0             | 10          |
| <i>P. aeruginosa</i>    | 7500                              | >30000      | 3.0             | 30          |
| <i>E. coli</i>          | 1870                              | 30000       | 3.0             | 30          |

MIC: Minimum inhibitory concentration. MBC: Minimum bactericidal concentration.

Source: research data.

*B. dracunculifolia* showed the most pronounced inhibitory activity against *L. monocytogenes*, as evidenced by the lowest MIC value (Table 4). *L. monocytogenes* is a food-borne pathogen that can cause febrile gastroenteritis in healthy people or more serious illnesses such as meningitis, encephalitis, and septicemia in pregnant women, newborns, and immunocompromised people, with mortality ranging from 20 to 40% (Maung *et al.*, 2019; Obaidat, 2020).

*P. aeruginosa* was the most resistant to the *B. dracunculifolia* extract as it had the highest MIC. It was also not possible to observe a CBM value among the concentrations tested (Table 4). *P. aeruginosa* is highly versatile as it has one of the most complex regulatory networks in bacteria. In addition, it is highly resistant to a variety of antibiotics, including aminoglycosides, quinolones, and  $\beta$ -lactams (Pang *et al.*, 2019), and retains innate abilities to find new ways to resist antimicrobials (Bonin *et al.*, 2020).

The antimicrobial action of *B. dracunculifolia* may be attributed to the presence of phenols and terpenes, which exhibit a toxic effect on the structure and function of cell membranes (Salazar *et al.*,

2018). The lipophilic characteristics of terpenes can facilitate their movement from the aqueous phase to membrane structures, resulting in membrane expansion and increased fluidity and permeability, as well as disorder of the proteins incorporated into the membrane. This can lead to inhibition of respiration and alterations in the ion transport process (Trombetta *et al.*, 2005). In addition, studies have shown effective antibacterial action, including against multidrug-resistant bacteria, attributing to plants of the Asteraceae family powerful medicinal action due to the presence of a wide range of bioactive metabolites, such as alkaloids, flavonoids, phenolic acids, coumarins, terpenoids, saponins (Gou *et al.*, 2023; Siddiqui *et al.*, 2024; Vieira *et al.*, 2023).

Casagrande *et al.* (2018), when analyzing the ethanolic extract of *B. dracunculifolia*, found higher MIC values for *S. aureus* (12750 µg/mL), *E. coli* (6370 µg/mL), and *P. aeruginosa* (51000 µg/mL), demonstrating a difference in the biological properties of *B. dracunculifolia* extracts. Furthermore, previous studies have reported the inability to determine the bactericidal concentration of the *B. dracunculifolia* extract against *P. aeruginosa*, which is consistent with the results observed in the study (Table 4). Thus, the extract may be considered a promising source of bioactive compounds with antioxidant and antimicrobial potential.

#### 4 Conclusion

The hydroalcoholic extract of *B. dracunculifolia* exhibited a metabolic profile characterized by the predominance of terpenoids, including mono-, di-, and triterpenes, with particular emphasis on the first-time identification, for this species, of 28-norolean-17-en-3-one and sclareol.

The antioxidant activity of the extract was directly associated with its phenolic compound content, as evidenced by the positive correlations observed, reinforcing the role of these metabolites in the neutralization of reactive species. In addition, the extract demonstrated antibacterial activity against both Gram-positive and Gram-negative bacteria, with greater sensitivity observed for *L. monocytogenes* and a predominantly bacteriostatic effect on Gram-negative strains.

Overall, these findings indicate that the chemical composition of the extract, particularly its terpenoid content, is closely related to its bioactive potential, highlighting *B. dracunculifolia* as a promising source of natural metabolites with antioxidant and antimicrobial applications, with potential relevance for pharmaceutical, food, and biopreservation purposes.

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