Microbial Pathogenesis 145 (2020) 104223

Contents lists available at ScienceDirect

Microbial Pathogenesis

journal homepage: www.elsevier.com/locate/micpath

Characterization and antibacterial activity of the essential oil obtained from the leaves of *Baccharis coridifolia* DC against multiresistant strains

Priscilla Ramos Freitas[a], Ana Carolina Justino de Araújo[a], Cristina Rodrigues dos Santos Barbosa[b], Debora Feitosa Muniz[a], Janaina Esmeraldo Rocha[a], José Bezerra de Araújo Neto[a], Maria Milene Costa da Silva[a], Raimundo Luiz Silva Pereira[c], Luiz Everson da Silva[d], Wanderlei do Amaral[d], Cicero Deschamps[d], Saulo Relison Tintino[a], Jaime Ribeiro-Filho[e], Henrique Douglas Melo Coutinho[a,*]

[a] Laboratory of Microbiology and Molecular Biology, LMBM, Regional University of Cariri, Brazil
[b] Laboratory of Bioprospecting of Semiarid and Alternative Methods, LABSEMA, Regional University of Cariri, Brazil
[c] Laboratory of Simulations and Molecular Spectroscopy, LASEMOL, Regional University of Cariri, Brazil
[d] Federal University of Parana, Brazil
[e] Gonçalo Moniz Institute, Oswaldo Cruz Foundation (IGM-FIOCRUZ/BA), Rua Waldemar Falcão, 121, Candeal, 40296-710, Salvador, Bahia, Brazil

ARTICLE INFO

Keywords:
Baccharis coridifolia
Escherichia coli
Pseudomonas aeruginosa
Staphylococcus aureus
Antibacterial agents
Essential oils

ABSTRACT

Essential oils are secondary metabolites with immense pharmacological potential.These substances are abundantly produced by plants of the family Asteraceae, such as *Baccharis coridifolia*. Previous studies have demonstrated that this species has pharmacological properties that make it a promising source of new antibacterial agents. Therefore, the present study aimed to evaluate the antibacterial and antibiotic-modulating activity of *Baccharis coridifolia* essential oil against multidrug-resistant (MDR) strains. The phytochemical analysis was carried out by gas chromatography coupled to Mass Spectroscopy (GC/MS), and realized the Minimum Inhibitory Concentation (MIC) and antibiotic-modulation from the microdilution method in 96-well plates. It was revealed the presence of germacrene D (23.7%), bicyclogermacrene (17.1%), and (E)-caryophyllene (8.4%) as major components. The minimum inhibitory concentration of essential oil against strains of *Pseudomonas aeruginosa* (512 μg/mL) and *Staphylococcus aureus* (128 μg/mL) demonstrated clinically relevant antibacterial activity. In addition, the combination of subinhibitory doses of the oil with conventional antibiotics showed synergism, indicating potentiation of the antibacterial effect. In conclusion, the essential oil of *Baccharis coridifolia* (EOBc) presented antibacterial and antibiotic-modulating activities that place this species as a source of molecules useful in the fight against bacterial resistance.

1. Introduction

The use of plants for therapeutic purposes is an ancient practice that has been empirically transmitted from generation to generation [1,2]. The therapeutic properties of medicinal plants are due to the action of secondary metabolites, also called phytochemicals [3,4]. In addition to providing phytotherapeutic characteristics, these compounds protect plants against pests and microbial infections [5].

In this context, Asteraceae species have been recognized for their high production of essential oils, metabolites with a wide variety of biological effects, and relevant applications in the cosmetics and perfume industries. This family is represented by about 27,000 species and 1,700 genera [6,7], among which the genus *Baccharis* stands out for presenting about 500 species widely distributed in the American continent, mainly in South America. In Brazil, around 178 species are found, predominantly in the state of Paraná, and approximately 83 species are found [8,9].

Species of the genus *Baccharis* have been widely investigated for the medicinal properties reported in folk medicine. Studies indicate that the plants of this genus present in its composition higher concentration of sesquiterpenes in which they can present anti-inflammatory, analgesic, and gastroprotective effect, among other pharmacological properties [10]. *Baccharis coridifolia*, also known as romerillo or mio-mio, is found in regions such as Paraguay, Uruguay, Brazil, and northern Argentina

* Corresponding author.
E-mail addresses: priscilla.r.freitas@hotmail.com (P.R. Freitas), hdmcoutinho@gmail.com, hdmcoutinho@urca.br (H.D.M. Coutinho).

https://doi.org/10.1016/j.micpath.2020.104223
Received 27 March 2020; Received in revised form 16 April 2020; Accepted 20 April 2020
Available online 04 May 2020

[11]. It has been shown that this species has anti-inflammatory activity, besides being popularly used for the treatment of parasitic infections and equine distemper, suggesting that *B. coridifolia* may have antibacterial properties [12].

Multiresistant bacterial infections have become a significant public health problem. Multidrug resistance occurs in both Gram-positive and Gram-negative bacteria, which cause infections that cannot be treated with most conventional antibiotics [13]. Among the Gram-positives, *Staphylococcus aureus* stands out for causing infections ranging from simple skin diseases to severe conditions such as bacteremia and endocarditis [14,15]. The species *Escherichia coli*, of the Enterobacteriaceae family has resistance to several drugs, such as carbapenemases, cephalosporins, among others [16]. *Pseudomonas aeruginosa* are Gram-negative bacteria with high virulence. These bacteria act as opportunistic microorganisms that can cause severe infections and even sepsis [17,18].

The emergence of resistant microorganisms has been directly associated with the indiscriminate use of antibiotics, resulting in a selective pressure that favors the survival of bacteria before treatment with conventional medicines [19]. This scenario has driven the development of research aimed at identifying new antimicrobial agents through chemical synthesis or isolation from natural products [20,21]. Accordingly, the present study aimed to investigate the antibacterial properties of the essential oil of *Baccharis coridifolia* leaves against multiresistant strains, considering that this is the first study to report the antibacterial properties of this species.

2. Materials and methods

2.1. Plant material

The essential oil was extracted from the terminal branches and/or inflorescences of specimens collected in the environmental Protection area “Reserva Particular do Patrimônio Natural” (RPPN), a segment of the Atlantic Forest in the state of Paraná, southern Brazil. The dried specimens were prepared and a deposited in the Herbarium of the “Faculdades Integradas Espiritas” college, under registration number HFIE 8.371.

2.2. Extraction and chemical analysis of Baccharis coridifolia essential oil

The essential oil was extracted in a Clevenger type apparatus using 100g of fresh plant material and 1L of distilled water with three repetitions. After extraction, the samples were centrifuged at 5,000 RPM for 2 min to separate the oil, and the total mass of each sample was determined on an analytical balance.

Gas Chromatography, coupled to Mass Spectroscopy, was used to analyze the chemical composition of the oil. The chemical constituents were identified by comparing their mass spectra with the Wiley and NIST library as well as by analyzing the linear retention indices. These indices were calculated from the injection of a homologous series of hydrocarbons (C7–C26) and compared by the data found in the literature. Only peaks higher than 1% were considered for the identification and quantification of chemical components [22].

2.3. Bacterial strains

The following multiresistant strains were used throughout the present study: *Staphylococcus aureus* 10, *Pseudomonas aeruginosa* 24 e *Escherichia coli* 06 is described in the work of Bezerra and collaborates [23] that used the same strains.

2.4. Essential oil preparation

A test tube was added with 10 mg of the essential oil and 1 mL of DMSO. This solution was transferred to another tube and diluted in 8,765 mL of sterile distilled water, resulting in a solution with a final concentration of 1024 μg/mL. This solution was used throughout the tests.

2.5. Determination of the minimum inhibitory concentration (MIC)

Bacterial strains were seeded in Petri dishes containing HIA culture medium and incubated at 37 °C for 24 h. Then, a sample of each culture was dragged and diluted in test tubes containing sterile saline, in triplicate. After this procedure, the turbidity was adjusted according to the 0.5 McFarland scale. An aliquot of 150 μL of each bacterial inoculum (referring to 10% of the total solution) was transferred to a tube containing 1350 μL of a 10% Brain Heart Infusion Broth (BHI) solution. To prepare the BHI, 10% of the material was weighed, in relation to the total volume used. Every well of a 96-well microdilution plate was filled with 100 μL of this solution, and then the essential oil was diluted at concentrations ranging from 512 μg/mL to 8 μg/mL. A well with no essential oil added was used as bacterial growth positive control. After the treatments, the plates were incubated at 37 °C for 24 h. Bacterial growth was analyzed by adding 20 μL of resazurin to each well, previously prepared at a concentration of 0.04 mg/ml. After the resazurin was added, the plates were incubated for 1 h at room temperature and after this period the colorimetric variation was observed, considering that there was no bacterial growth in the wells that remained with the blue color, and positive for the bacterial growth in the wells that had changed the blue to pink coloring [24,25]. All test was performed in triple.

2.6. Evaluation of the antibiotic-modulating activity

For analysis of the antibiotic-modulating activity, the *Baccharis coridifolia* essential oil was used at a sub-inhibitory concentration (MIC/8). The oil was diluted in a variable volume of 10% BHI enough to obtain this concentration and then added to 150 μL of bacterial inoculum (corresponding to a concentration of 10%) in a tube. Controls were prepared by using 1350 μL of 10% BHI medium and 150 μL of the inoculum. Each well of a microdilution plate was filled with 100 μL of the oil or control solution. Following this procedure, serial dilutions with 100 μL of Penicillin, Gentamicin, and Norfloxacin at an initial concentration of 1024 μg/mL. The plates were incubated in an oven at 37 °C for 24 h, and then, the MIC of these antibiotics in the presence of the essential oil was determined by the addition of resazurin [24]. All test was performed in triple.

2.7. Statistical analysis

Data were expressed as mean ± standard deviation and evaluated by analysis of variance (ANOVA) followed by Bonferroni's post hoc test using GraphPad Prism software. Differences with $p < 0.05$ were considered significant.

3. Results

The essential oil of fresh leaves of *Baccharis coridifolia* had a total yield of 0.17%. The phytochemical characterization by Gas chromatography coupled to mass spectrometry identified the presence of 13 compounds, as shown in Fig. 1. As shown in Table 1, the oil is constituted predominantly by sesquiterpenes (67.1%) and monoterpenes (32.9%). An analysis of individual constituents revealed the presence of germacrene D (23.7%), bicyclogermacrene (17.1%), and (E) -caryophyllene (8.4%) as major components.

Given this evidence, we used the microdilution method to determine the EOBc MIC. The results revealed that this substance has antibacterial activity against strains of *Pseudomonas aeruginosa* and *Staphylococcus aureus*, but not against *Escherichia coli* (Table 2), considering a MIC ≤ 1000 μg/mL [26] as clinically relevant.

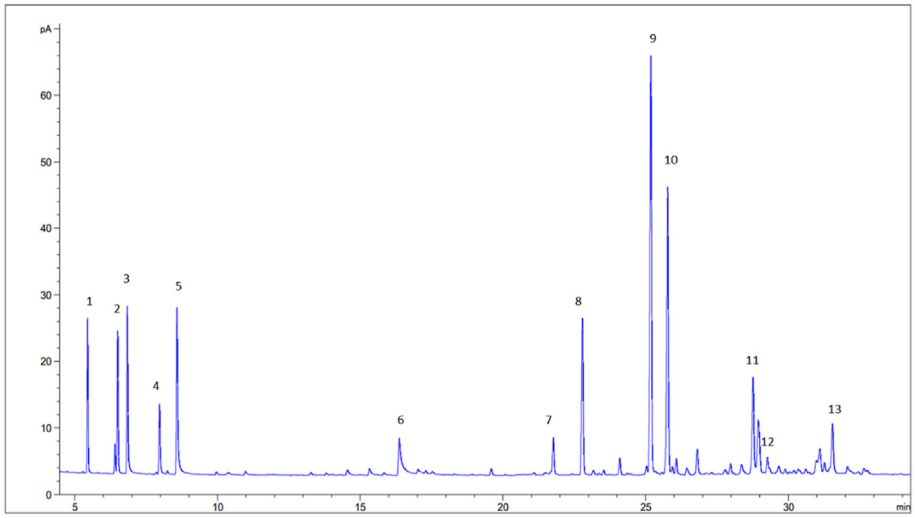

Fig. 1. GC-MS chromatogram of the essential oil of *Baccharis coridifolia*
1 = α-pinene; 2 = β-pinene; 3 = myrcene; 4 = limonene; 5= (E)-β-ocimene; 6 = geraniol; 7 = β-elemene; 8=(E)-caryophyllene; 9 = germacrene D; 10 = bicyclogermacrene; 11 = spathulenol; 12 = caryophyllene óxide; 13 = α-cadinol.

Table 1
Relative composition (%) of *Baccharis coridifolia* Essential Oil.

Retention Index	Compound	%
937	alpha-pinene	4.7
979	beta-pinene	5.8
992	Myrcene	6.7
1031	Limonene	3.2
1051	(*E*)-beta-ocimene	8.2
1258	Geraniol	4.3
1391	beta-elemene	2.8
1417	(*E*)- caryophyllene	8.4
1479	Germacrene D	23.7
1493	Bicyclogermacrene	17.1
1577	Spathulenol	8.1
1581	Caryophyllene oxide	2.8
1651	alpha-cadinol	4.2

Table 2
Minimum inhibitory concentration against multiresistant strains by essencial oil of *Baccharis coridifolia*.

Strain	MIC
Escherichia coli 06	≥1024 μg/Ml
Pseudomonas aeruginosa 24	128 μg/mL
Staphylococcus aureus 10	512 μg/mL

As shown in Fig. 2, besides having a direct antibacterial activity, the essential oil increased the activity of conventional drugs, demonstrating an antibiotic-modulating effect. Tests with *E. coli* revealed that association of the oil with norfloxacin caused a reduction in the MIC from 64 μg/mL to 3.17 μg/mL, and in the case of gentamicin, the MIC was reduced from 32 μg/mL to 20 μg/mL. Combining the oil with the same antibiotics against the *S. aureus* strain also caused a significant reduction in the MIC of these drugs, especially gentamicin, which was reduced from 128 μg/mL to 8 μg/mL. On the other hand, in the tests using *P. aeruginosa*, only the MIC of Norfloxacin was reduced. Interestingly, the essential oil did not reduce the MIC of Penicillin against any of the bacterial strains tested, indicating that the modulating effect varies with the class of the antibiotic.

4. Discussion

The phytochemical composition of the oil analyzed by the present study presented characteristics similar to other species belonging to the same genus. Studies have shown that the essential oils obtained from *Baccharis dracunculifolia* and *Baccharis trimera* are predominantly composed of sesquiterpenes, including germacrene D and α-caryophyllene, respectively as major components [10]. A study by Valarezo et al. [27] found that the essential oil of *Baccharis obtusifolia* has limonene, germacrene D, β-pinene, and bicyclogermacrene as principal constituents. These compounds were also found in *Baccharis coridifolia* essential oil, which had not been previously reported in the literature.

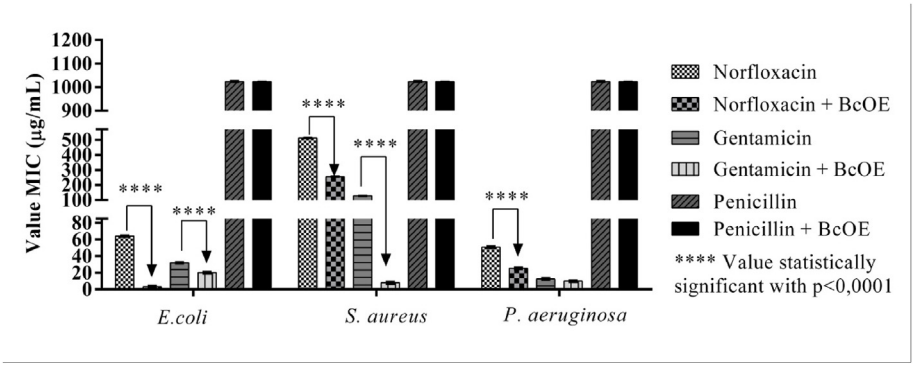

Fig. 2. Antibiotic-modulating activity of *B. coridifolia* essential oil in combination with antibiotics against multiresistant strains of *E. coli 06*, *P. aeruginosa 24* and *S. aureus 10*.

Regarding pharmacological aspects, sesquiterpenes, the leading class of constituents found in the EOBc, were found to present antibacterial activity. Studies have shown that these compounds act on the bacterial membrane, causing an increase in permeability and damage that results in cell autolysis [28].

Literature data demonstrate that the antibacterial spectrum of action of *Baccharis* species is variable. In agreement with the present study, Salazar et al. [29] showed that *Baccharis dracunculifolia* essential oil has antibacterial activity against SA10 and PA24 strains, with MICs of 512 μg/mL and 813 μg/mL, respectively. However, the oil showed no action against the multidrug resistant *E. coli* strain. In addition, the species has a chemical composition similar to that found in the present study, characterized by a high concentration of sesquiterpenes, including Germacrene D (18.4%) as the primary component.

A study with species of the same genus found that *B. dracunculifolia* and *B. uncinella* present an antibacterial activity against *E. coli, P.aeruginosa* and *S. aureus*, indicated by an increase in the inhibition halo in the disc diffusion method [30]. On the other hand, Valarezo et al. [27] demonstrated that *Baccharis latifolia* oil showed no antibacterial activity against *S.aureus, P. aeruginosa*, and *E.coli* strains, considering that the oil presented higher concentrations of sesquiterpenes.

The antibiotic-modulating activity of *B. coridifolia* was previoulsly analyzed through the disc difusion method [31]. The authors demonstrated that the association of the essential oil obtained from this plant with Gentamicin against the *S. aureus* strain had an antagonistic effect. Furthermore, the oil did not modulate the antibacterial effect of gentamicin against *E. coli*, which differs from the results obtained by the present study. On the other hand, a study by Salazar et al. [29], using another species of the same genus (*B. dracunculifolia*), demonstrated synergistic effect when the essential oil was associated with gentamicin and norfloxacin against strains of *E. coli*, *P. aeruginosa* and *S. aureus*.

Essential oils have been shown to have constituents capable of interacting synergistically with antibiotics enhancing their antibacterial effect. Regarding the mechanisms involved in this process, the secondary metabolites present in essential oils seem to facilitate the transport of other oil compounds into the bacteria, enhancing the antibacterial activity of these drugs [32]. In addition, essential oils also have a direct antibacterial activity, whose mechanism involves cell wall lysis, which results in extravasation of cell content, as well as reduction of motor force and coagulation of the cytoplasm. Here, we suggest that these are potential mechanisms involved in the antibacterial and antibiotic-modulating actions of *B. coridifolia* essential oil.

5. Conclusion

The essential oil obtained from the leaves of *B. coridifolia* exerted an antibacterial activity against multidrug resistant strains of *Pseudomonas aeruginosa* and *Staphylococcus aureus*. In addition, the oil potentiated the action of aminoglycosides against multiresistant bacteria, which appears to be dependent on the type of drug and bacterial strain. In that its antibacterial activity possibly occurs due to the present metabolites, which was evidenced as in greater concentration the presence of the germacrene D compound in the essential oil. Thus, further studies with isolated constituents will be crucial to identify the compounds responsible for pharmacological properties of EOBc in this model.

CRediT authorship contribution statement

Priscilla Ramos Freitas: Methodology. **Ana Carolina Justino de Araújo:** Methodology. **Cristina Rodrigues dos Santos Barbosa:** Validation. **Debora Feitosa Muniz:** Methodology. **Janaina Esmeraldo Rocha:** Validation. **José Bezerra de Araújo Neto:** Methodology. **Maria Milene Costa da Silva:** Formal analysis. **Raimundo Luiz Silva Pereira:** Software. **Luiz Everson da Silva:** Conceptualization, Supervision. **Wanderlei do Amaral:** Resources, Supervision. **Cicero Deschamps:** Formal analysis. **Saulo Relison Tintino:** Conceptualization, Supervision. **Jaime Ribeiro-Filho:** Writing - original draft. **Henrique Douglas Melo Coutinho:** Conceptualization, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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