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Phytochemical characterization of the *Baccharis dracunculifolia* DC (Asteraceae) essential oil and antibacterial activity evaluation

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ABSTRACT

The present study had as its objective to evaluate the antibacterial and antibiotic modulatory activity of the *Baccharis dracunculifolia* (DC) essential oil (EOBD), a species commonly known as "Alecrim do campo". The essential oil chemical composition analysis and the direct *in vitro* antibiotic activity evaluation, as well as in combination with the antibiotics Ampicillin, Gentamicin, and Norfloxacin against standard *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 9027, and *Staphylococcus aureus* ATCC 25923 strains, as well as *E. coli* 06, *P. aeruginosa* 24, and *S. aureus 10* resistant strains, were performed. Gas chromatography-mass spectrometry (GC–MS) revealed the major terpenoids: Germacrene D (18.4%), (E)-Nerolidol (14.0%), Mustacone (11.2%), Spathulenol (11%), β-pinene (9.5%) and Bicyclogermacrene (8.4%). A direct *B. dracunculifolia* essential oil antibacterial activity against *S. aureus* 25923 with a MIC of 102 μg/mL and one of 512 μg/mL against *S. aureus* 10 were observed. The EOBD synergistically modulated the Norfloxacin antibiotic against *P. aeruginosa* 24, with a MIC reduction from 50.8 to 6.35 μg/mL, a Gentamicin MIC reduction from 20.16 to 4 μg/mL, and an Ampicillin MIC reduction from 645.1 to 512 μg/mL. The essential oil synergistically modified Norfloxacin activity against *E. coli* 06 with a MIC reduction from 40.32 to 3.17 μg/mL, and a Gentamicin MIC reduction from 50.8 to 25.4 μg/mL. The essential oil also showed synergism against *S. aureus* 10 when associated with Norfloxacin, with a MIC reduction from 322.54 to 203.2 μg/mL, whilst when associated with Ampicillin, it demonstrated synergism by reducing the MIC from 80.6 to 20.16 μg/ml. Results from this research revealed that the EOBD is a promising natural product with antimicrobial potential, both directly and in combination with antibiotics, for clinical use against Gram-positive and Gram-negative bacteria.

1. Introduction

Indiscriminate antibiotic use in the fight against common bacteria such as *S. aureus, E. coli* and *P. aeruginosa* has contributed to the selection process of resistant microbial strains (Silveira et al., 2006). Consequently, it is imperative to invest in the discovery of new biologically active natural products which can be used in antibiotic modulation research to more effectively assist the fight against infectious agents, especially multiresistant ones.

The use of medicinal plants to treat diseases is a practice performed since antiquity by humanity, often being the only therapeutic form available for a population (Rakelly de Oliveira et al., 2012). This has stimulated a great scientific interest in chemical and pharmacological research surrounding the biological properties of medicinal plants, making them the source of many medicinal products which are now used in clinical practice (Johann et al., 2010; Coutinho et al., 2009, 2008).

Among various plants, *B. dracunculifolia* DC (Asteraceae), a plant native to Brazil, commonly known as "Alecrim do Campo" stands out (Kumazawa et al., 2003). Teas and decoctions prepared from the flowering plant are widely used in alternative medicine to treat inflammation, liver disorders and stomach ulcers (Lemos et al., 2007; da

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Silva Filho et al., 2009). A variety of pharmacological activities have been attributed to this plant, including anti-ulcerative (De Barros et al., 2007), antibacterial (Park et al., 1998) antifungal and antirheumatic (Pereira et al., 2011) as well as hepatoprotective properties (Seo et al., 2003).

Cristiane et al., 2016 showed the EOBD possessed antibacterial activity against mutant *Streptococci* biofilms, reducing the standard lineage biofilm by 39.3%, indicating its usefulness in the control of microorganisms that cause dental caries. The *B. dracunculifolia* essential oil antimicrobial activity can be attributed to the high presence of prenylated p-coumaric acid, especially artepillin C and baccharin, flavonoids, diterpenes and triterpenes isolated from aerial parts of the plant (Park et al., 2002; Missima et al., 2007). Johann et al. (2012), isolated from the *B. Dracunculifolia* hexanic fraction, ursolic acid, methyl linolenate, caryophyllene oxide and trans-nerolidol, observing that these compounds exhibited antifungal activity against *Paracoccidioides brasiliensis* isolates. This suggests that the use of natural products or their isolated chemical constituents associated with clinically used antimicrobials may result in a synergistic or antagonistic modulating effect of these products (Oliveira et al., 2006; Almeida et al., 2013); in this respect, terpenoid compounds are the components most related to EO bacteriostatic and/or bactericidal activity (Burt, 2004).

Synergistic interactions between antibacterial agents offer improvements in: clinical treatment efficacy; antibacterial agent MIC, and the risk associated with the development of bacterial resistance, reduction; and toxic antibiotic effect reduction for the host. Therefore, the use of associated substances has been widely studied and used as a treatment for infections (Ncube et al., 2012; Silva et al., 2015).

The application plants and drugs known as the “targeting multiple synergistic effects” is an approach which can affect not just a single target, but also multiple targets, in which the different therapeutic compounds act synergistically or antagonistically. This is a viable method for natural product and antibiotic combinations (Coutinho et al., 2010; Wagner and Ulrich-Merzenich, 2009).

Consequently, this study aims to evaluate the *B. dracunculifolia* essential oil antibacterial effect against *S. aureus*, *E. coli*, and *P. aeruginosa* strains by determining the Minimum Inhibitory Concentration (MIC) using direct antibacterial activities, as well as its modulatory effect on standard antibiotics (norfloxacin, gentamicin and ampicillin) in an attempt to supply the need for new antimicrobial agents.

2. Materials and methods

2.1. Plant material

The plant material was obtained from “The Private Reserve of Natural Heritage Butuguara”, a segment of the Atlantic forest, with altitude ranging from 985 to 1145 m and located at 25° 20.884′S and 049° 47.258′ W, Parana State, South Brazil. Collection of plant material in the reserve was made under license of the Environmental Institute of Paraná State by number 284/11. The species *B. dracunculifolia* DC. was collected in the (HFIE nº 8.372). Duplicates of specimens were sent to the “Municipal Botanical Museum Herbarium (MBM)” and the “Herbarium of the Biological Sciences, University of Parana - BRAZIL (UPCB)”.

2.1.1. Essential oil isolation and analysis method

The essential oil sample was obtained from 100 g of fresh or 50 g of dried sample by hydrodistillation for 4 h using a Clevenger-type apparatus. Dried sample were obtained after drying the plant material for 24 h in an electric dryer FANEM (320 SE Mod) with air circulation at 40 °C. The extracted oil were stored in dark vials at-20 °C until analysis. GC–MS analysis was performed using 1.0 L of samples 1% in dichloromethane which was injected with split ratio of 1:20 in an Agilent 6890 gas chromatograph (Palo Alto, CA) coupled to a mass selective detector Agilent 5973 N. The injector was maintained at 250 °C. The separation of the constituents was obtained in an HP-5MS capillary column (5% phenyl - 95% - dimethylpolysiloxane, 30 m × 0.25 mm × 0.25 μm) using helium as carrier gas (1.0 mL min^{-1}). The oven temperature was programmed from 60 to 240 °C at a rate of 3 °C min^{-1}. The mass detector was operated in electronic ionization mode (70 eV) at a rate of 3.15 scan s^{-1} and mass range from 40 to 450 u. The transfer line was maintained at 260 °C, the ion source at 230 °C and the analyzer (quadrupole) at 150 °C.

For quantification, the diluted sample was injected into an Agilent 7890A chromatograph equipped with a flame ionization detector (FID), operated at 280 °C. The same column and the analytical conditions described above were employed, using hydrogen as the carrier gas, at a flow rate of 1.5 mL min^{-1}. The percentage composition was obtained by electronic integration of the FID signal by the division area of each component in the total area. The chemical constituent’s identification was obtained by comparing their mass spectra with the libraries (23–24) and by the linear retention indexes, calculated after the injection of a homologous series of hydrocarbons (C_7–C_{26}) and compared with the literature of data.

Analyses of variance for essential oil yield as well as the Scott-Knott test ($P < 0.05$) of mean comparison procedures were performed using ASSISTAT, release 7.6 Beta.

2.2. Strains

The experiments were carried out with clinical isolates of E. coli 06 (EC06), S. aureus 10 (SA10) and P. aeruginosa 24 (PA24) resistant to the antibiotics gentamicin, norfloxacin and ampicillin. The EC-ATCC2592 strain of E. coli, the SA-ATCC25923 strain of S. aureus, and the PA-ATCC9027 strain of P. aeruginosa were used as positive controls. All bacterial cultures were maintained at 4 °C in Heart Infusion Agar (HIA, Difco). Prior to the assays, all the cells were grown overnight at 37 °C in Brain Heart Infusion (BHI, Difco).

2.3. Drugs

Gentamicin, ampicillin and norfloxacin were obtained from Sigma Chemical Corp., St. Louis, MO, USA. All of the drugs were dissolved in sterile water to obtain the appropriate concentrations and decrease in toxicity.

2.4. Direct antibacterial test (MIC) and modulation of antibiotic activity

MIC (Minimal Inhibitory Concentration) was determined in a microdilution assay according Javadpour et al. (1996). The final concentrations of the oil varied 1024–0.5 μg/mL.

For the evaluation of the oil as modulator of resistance to the antibiotics, MIC of the antibiotics was determined in the presence or absence of (EOBD) at subinhibitory concentrations (ranging from 128 to 64 μg/mL), and the plates were incubated for 24 h at 37 °C. Each antibacterial assay for MIC determination was carried out in triplicate according (Coutinho et al., 2005; Javadpour et al., 1996).

2.5. Statistical analysis of microbiological results

The results of the tests were done in triplicate and expressed as geometric mean. Statistical analysis was applied to two-way ANOVA followed by Bonferroni posttests using GraphPad Prism 5.0 software.

3. Results

3.1. Essential oil yield and composition

Table 1 shows the essential oil yield (%) from fresh and dried *B. dracunculifolia* samples. Table 2 shows that the most abundant compounds are oxygenated sesquiterpenes (45.7%), followed by