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Short Communication

Antibacterial activity of *Baccharis trimera* (Less.) DC. (carqueja) against bacteria of medical interest¹

Álan Alex Aleixo², Karina Marjorie Silva Herrera³, Rosy Iara Maciel de Azambuja Ribeiro⁴,
Luciana Alves Rodrigues dos Santos Lima⁵, Jaqueline Maria Siqueira Ferreira⁶

ABSTRACT

Baccharis trimera (Less.) (Asteraceae), popularly known as “carqueja”, is a species commonly used in folk medicine for the treatment or prevention of diseases. In this context, the purpose of this work was to study the antibacterial activity of crude hydroalcoholic extract from *Baccharis trimera* against Gram-positive bacterial strains (*Staphylococcus aureus* ATCC 29213, *Staphylococcus saprophyticus* ATCC 15305, *Staphylococcus epidermidis* ATCC 12228, *Enterococcus faecalis* ATCC 19433) and Gram-negative bacteria (*Escherichia coli* EHEC ATCC 43895, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 27736, *Salmonella typhi* ATCC 19430) of clinical interest. Antibacterial susceptibility was evaluated by broth microdilution assay following the CLSI (formerly the NCCLS) guidelines. The extract from *B. trimera* showed antibacterial activity against Gram-positive bacteria and the most interesting result was obtained against *S. epidermidis* that presented Minimal Inhibitory Concentration of 250 µg/mL. These results indicate that *B. trimera* have bacteriostatic potential against Gram-positive bacterial strains of medical interest and could serve as a base for further studies on the use of isolated compounds from this species as future antimicrobials.

Key words: antimicrobial agents, *Baccharis trimera*, *Staphylococcus epidermidis*, minimal inhibitory concentration.

RESUMO

Avaliação antibacteriana de *Baccharis trimera* (Less.) DC. (carqueja) em bactérias de interesse médico

Baccharis trimera (Less.) (Asteraceae), popularmente conhecida como “carqueja”, é uma espécie comumente empregada na medicina tradicional no tratamento ou prevenção de doenças. Nesse contexto, o objetivo deste trabalho foi estudar a atividade antibacteriana do extrato bruto hidroalcoólico de *B. trimera* frente às bactérias Gram-positivas (*Staphylococcus aureus* ATCC 29213, *Staphylococcus saprophyticus* ATCC 15305, *Staphylococcus epidermidis* ATCC 12228, *Enterococcus faecalis* ATCC 19433) e bactérias Gram-negativas (*Escherichia coli* EHEC ATCC 43895, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 27736 e *Salmonella typhi* ATCC 19430) de interesse clínico. A susceptibilidade antibacteriana foi avaliada pelo método de microdiluição em caldo conforme estabelecido pelo CLSI (antigo NCCLS). O extrato de *B. trimera* mostrou atividade contra bactérias Gram-positivas, sendo o resultado mais interessante o obtido por *S. epidermidis*, que apresentou Concentração Inibitória Mínima de

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² Undergraduate of Biochemistry. Laboratório de Microbiologia, Universidade Federal de São João Del-Rei, Campus Centro Oeste Dona Lindu, Rua Sebastião Gonçalves Coelho, 400, Bairro Chanadour, 35501-296, Divinópolis, Minas Gerais, Brazil. aleixocn@hotmail.com

³ Biologist, Master of Science. Laboratório de Microbiologia, Universidade Federal de São João Del-Rei, Campus Centro Oeste Dona Lindu, Rua Sebastião Gonçalves Coelho, 400, Bairro Chanadour, 35501-296, Divinópolis, Minas Gerais, Brazil. kmsherrera@gmail.com

⁴ Biologist, Doctor of Science. Laboratório Biologia Celular e Cultura de Células, Universidade Federal de São João Del-Rei, Campus Centro Oeste Dona Lindu, Rua Sebastião Gonçalves Coelho, 400, Bairro Chanadour, 35501-296, Divinópolis, Minas Gerais, Brazil. rosyiara@gmail.com

⁵ Pharmacist, Doctor of Science. Laboratório de Farmacognosia, Universidade Federal de São João Del-Rei, Campus Centro Oeste Dona Lindu, Rua Sebastião Gonçalves Coelho, 400, Bairro Chanadour, 35501-296, Divinópolis, Minas Gerais, Brazil. lucianaarsantos@yahoo.com.br

⁶ Biologist, Doctor of Science. Laboratório de Microbiologia, Universidade Federal de São João Del-Rei, Campus Centro Oeste Dona Lindu, Rua Sebastião Gonçalves Coelho, 400, Bairro Chanadour, 35501-296, Divinópolis, Minas Gerais, Brazil. jaque@ufs.edu.br (corresponding author).

250 µg/mL. Esses resultados indicam que *B. trimera* possui potencial atividade bacteriostática contra bactérias Gram-positivas de interesse médico e podem apresentar subsídios para estudos posteriores do uso de compostos isolados dessa espécie como futuros agentes antimicrobianos.

Palavras-chave: agentes antimicrobianos, *Baccharis trimera*, *Staphylococcus epidermidis*, concentração inibitória mínima.

INTRODUCTION

Baccharis trimera (Less.) (Asteraceae) is a common species in the tropical regions of South America. Decoction of the aerial parts of this plant are traditionally used for the treatment or control of various diseases (de Oliveira Jr *et al.*, 2012). *In vitro* assays on the antimicrobial activity of the decoct of aerial parts of *B. trimera* showed activity against Gram-positive samples of *Staphylococcus aureus* and *Streptococcus uberis* (Avancini & Mundstock, 2000). Moreover, the synergism between the combination of methanol extract from the plant and some antibiotics inhibitors of bacterial protein synthesis has been reported against *Staphylococcus aureus* (Betoni *et al.*, 2006).

The increase in resistant and multi-resistant strains to the antimicrobials available in the market along with the sustainable production and the search for low-side effect drugs have been contributed to the search for alternative treatments against diseases caused by microorganisms (Clardy *et al.* 2006; OMS, 2010; Jorgetto *et al.*, 2011). The increased resistance occurs as a response of human activity, especially the overuse of antibiotics and also by the natural adaptation that these microorganisms acquire in the environment (Josephson, 2006). Nosocomial infections caused by strains resistant to available antibiotics by the pharmaceutical industry are a serious public health problem whose impact is worldwide, which makes the search for new antimicrobial agents extremely important (Pereira *et al.*, 2013).

Thus, the present study aimed at studying the *in vitro* antibacterial activity of crude hydroalcoholic extract from *B. trimera* against Gram-positive and Gram-negative bacteria causative of nosocomial infections.

MATERIALS AND METHODS

Plant material

Aerial parts of *B. trimera* were collected in a periphery neighborhood of São Sebastião do Oeste, Minas Gerais, located in the coordinates -20° 14' 38.96"S and -45° 2' 14.38"W, with altitude of 712 meters, in August 2011. The voucher specimen was deposited at the Herbarium of the Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil (BHCB 159398).

Aerial parts of *B. trimera* (172.44g) were used for extraction by cold maceration (20% p/v) in ethanol P.A

(Vetec, Brazil). After incubation, the extract was filtered and concentrated in a rotary evaporator at 40°C under reduced pressure to yield the ethanol extract. The dried extract (4.79g) was obtained after lyophilization (Liobras equipment, model K 105) and stored at -70°C.

Determination of the Minimal Inhibitory Concentration and Minimum Lethal Concentration (MLC)

The Minimal Inhibitory Concentration (MIC) was determined using the broth microdilution method performed in accordance with the guidelines of the *Clinical and Laboratory Standards Institute* (CLSI M7-A6 document, 2003). Four Gram-negative bacteria (*Escherichia coli* EHEC American Type Culture Collection (ATCC) 43895, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 27736 and *Salmonella typhi* ATCC 19430) and four Gram-positive bacteria (*Staphylococcus aureus* ATCC 29213, *Staphylococcus saprophyticus* ATCC 15305, *Staphylococcus epidermidis* ATCC 12228 and *Enterococcus faecalis* ATCC 19433) were used in the biological assays. The bacterial strains were kindly provided by the Reference Microorganisms Laboratory of the Oswaldo Cruz Foundation, FIOCRUZ/Brazil.

The extract was diluted in sterile dimethylsulfoxide (DMSO) 20% to the concentrations 1.25; 1.0; 0.50; 0.500; 0.250 and 0.125 mg/mL for antimicrobial tests. Streptomycin (Sigma-Aldrich, USA) and DMSO (Sigma, USA) were included as positive and negative controls, respectively. The MIC was assessed based on the lowest concentration of sample required to inhibit microbial growth (detected as the lack of visible turbidity). The experiments were performed in triplicate and repeated three times.

For assays to determine the Minimum Lethal Concentration (MLC), aliquots of 25 µL were removed from wells without visible turbidity and placed on Agar Plate-count using the Spread-plate Method. After incubation at 37 °C for 24h, colonies were counted. The concentration of sample that resulted in a growth 0.1% of initial inoculum (1.5×10^6 UFC/mL) was determined as the MLC.

Statistical analyses

Analyses were performed by median values of absorbance using the variance test followed by the Tukey test ($p < 0.05$).

RESULTS AND DISCUSSION

The crude hydroalcoholic extract from *B. trimera* showed antibacterial activity against four bacterial strains with 100% of inhibition of Gram-positive bacteria. *S. epidermidis* was the specie that presented the best sensibility, MIC 250 µg/mL. The bacterial strains *E. faecalis* and *S. aureus* showed MIC 500 µg/mL and *S. saprophyticus* was the more resistant species, with MIC of 1250 µg/mL (Table 1). On the other hand, Gram-negative strains did not show sensibility to the *B. trimera* extract in the concentrations tested (MIC >1250 µg/mL). Streptomycin, used as positive control, presented MIC between 3.9 and 62.5 µg/mL. No activity was observed for the negative control (DMSO 2%) (Table 1). The extract showed no bactericidal activity (data not show).

The results of this work demonstrate that the crude hydroalcoholic extract from *B. trimera* has selective action against the biochemical structure of the bacterial cell wall, being mainly active against Gram-positive bacteria. In this respect, similar results were described in which Gram-positive bacteria were more sensitive when tested with the decoct of *B. trimera* after Galenic extraction, confirming the selective character of the extract or its compounds (Avancini & Mundstock, 2000).

Although there is no literature explaining the relationship between the microbial sensitivity to extracts

and structure of bacterial cells, some authors suggest that the effectiveness of inhibitory action of the extracts on bacteria, both Gram-positive and Gram-negative bacteria, may be associated to the peculiarities of the extract composition on the cellular constitution of microorganisms. The activity of certain substances of the plant may be related to the efficacy observed in Gram-positive bacteria, since they exhibit chemically less complex cell wall, less lipid content and lack of outer cell membrane compared to the Gram-negative bacteria (Deans & Ritchie, 1987; Srinivasan et al., 2001).

The presence of diterpenes and flavonoids in plants of the genus *Baccharis* may explain, at least in part, the antibacterial activities observed. Several compounds isolated from these two classes of secondary metabolites have been reported with activity against Gram-positive *Staphylococcus* sp (Tsukiyama et al., 2002; Ulubelen, 2003).

Our results show that bacteria belonging to the genera *Staphylococcus* and *Enterococcus* are more sensitive to the extract of *B. trimera* or its components. In view of the clinical importance of the microorganisms belonging to these genera, the present results encourage further studies in order to isolate and characterize compounds and antimicrobial properties of this species, considering that MIC below 1000 µg/mL for plant crude extract represents the possibility of isolates with better activity and higher antimicrobial specificity.

Table 1. Minimal inhibitory concentration (MIC) of hidroalcoholic extracts of *Baccharis trimera* (Less) against Gram-positive and Gram-negative bacteria of clinical interest

Bacteria (ATCC)	MIC (µg/mL)	
	Crude extract of <i>B. trimera</i>	Streptomycin
Gram-positive		
<i>Staphylococcus epidermidis</i> (12228)	250	3,9
<i>Staphylococcus aureus</i> (29213)	500	3,9
<i>Enterococcus faecalis</i> (19433)	500	62,5
<i>Staphylococcus saprophyticus</i> (15305)	1250	1,95
Gram-Negative		
<i>Klebsiella pneumoniae</i> (27736)	>1250	3,9
<i>Salmonella typhi</i> (19430)	>1250	7,81
<i>Escherichia coli</i> EHEC (43895)	>1250	3,9
<i>Pseudomonas aeruginosa</i> (27853)	>1250	7,81

CONCLUSIONS

The minimal inhibitory concentrations from hidroalcoholic extracts of *B. trimera* ranged between 250 to 1250 µg/mL in Gram-positive bacteria. However, no activity was observed in Gram-negative strains. The results showed that *B. trimera* have an antimicrobial potential against Gram-

positive strains, becoming an important alternative for prospection of new molecules with antibiotic properties.

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