BRAZILIAN JOURNAL OF MICROBIOLOGY XXX (2017) XXX–XXX

BRAZILIAN JOURNAL OF MICROBIOLOGY

http://www.bjmicrobiol.com.br/

Food Microbiology

In vitro antimicrobial and antimycobacterial activity and HPLC–DAD screening of phenolics from *Chenopodium ambrosioides* L.

Roberta S. Jesus[a,*], Mariana Piana[a], Robson B. Freitas[a], Thiele F. Brum[a], Camilla F.S. Alves[a], Bianca V. Belke[a], Natália Jank Mossmann[a], Ritiel C. Cruz[a], Roberto C.V. Santos[b], Tanise V. Dalmolin[c], Bianca V. Bianchini[c], Marli M.A. Campos[c], Liliane de Freitas Bauermann[a,*]

[a] Phytochemical Research Laboratory, Department of Industrial Pharmacy, Federal University of Santa Maria, Avenida Roraima 1000, Camobi, Santa Maria, RS, Brazil

[b] Microbiology Research Laboratory, Franciscan University Center, Rua dos Andradas, 1614, Centro, Santa Maria, RS, Brazil

[c] Laboratory of Mycobacterial Research, Department of Clinical Analysis and Toxicological, Federal University of Santa Maria, Avenida Roraima 1000, Camobi, Santa Maria, RS, Brazil

ARTICLE INFO

Article history:
Received 25 January 2016
Accepted 11 February 2017
Available online xxx
Associate Editor: Luis Henrique Guimarães

Keywords:
Amaranthaceae
Antimicrobial potential
Rutin
Quercetin
Chrysin

ABSTRACT

The main objective of this study was to demonstrate the antimicrobial potential of the crude extract and fractions of *Chenopodium ambrosioides* L., popularly known as Santa-Maria herb, against microorganisms of clinical interest by the microdilution technique, and also to show the chromatographic profile of the phenolic compounds in the species. The Phytochemical screening revealed the presence of cardiotonic, anthraquinone, alkaloids, tannins and flavonoids. The analysis by HPLC–DAD revealed the presence of rutin in the crude extract (12.5 ± 0.20 mg/g), ethyl acetate (16.5 ± 0.37 mg/g) and n-butanol (8.85 ± 0.11 mg/g), whereas quercetin and chrysin were quantified in chloroform fraction (1.95 ± 0.04 and 1.04 ± 0.01 mg/g), respectively. The most promising results were obtained with the ethyl acetate fraction, which inhibited a greater number of microorganisms and presented the lowest values of MIC against *Staphylococcus aureus* and *Enterococcus faecalis* (MIC = 0.42 mg/mL), *Pseudomonas aeruginosa* (MIC = 34.37 mg/mL), *Paenibacillus apiarus* (MIC = 4.29 mg/mL) and *Paenibacillus thiaminolyticus* (MIC = 4.29 mg/mL). Considering mycobacterial inhibition, the best results were obtained by chloroform fraction against *M. tuberculosis*, *M. smegmatis*, and *M. avium* (MIC ranging from 156.25 to 625 μg/mL). This study proves, in part, that the popular use of *C. ambrosioides* L. can be an effective and sustainable alternative for the prevention and treatment of diseases caused by various infectious agents.

* *Corresponding authors.*
E-mails: robertasj@hotmail.com (R.S. Jesus), lgfbauermann@gmail.com (L.F. Bauermann).

http://dx.doi.org/10.1016/j.bjm.2017.02.012

Please cite this article in press as: Jesus RS, et al. *In vitro* antimicrobial and antimycobacterial activity and HPLC–DAD screening of phenolics from *Chenopodium ambrosioides* L. *Braz J Microbiol.* (2017), http://dx.doi.org/10.1016/j.bjm.2017.02.012

Introduction

The World Health Organization (WHO) recommends worldwide development of research on medicinal plants for therapeutic purposes, in order to obtain new possibilities for the treatment of diseases, especially in developing countries,[1] considering also, the hope to identify new substances of medicinal character that may serve as raw materials for pharmaceuticals industries. In Brazil the use of plants is very common for the treatment of various diseases, including bacterial and fungal infections, and many studies are conducted to detect secondary metabolites in plants with antimicrobial properties as an attempt to find new antimicrobial or antifungal compounds.[2–4] Indeed, because of the increasing bacterial resistance, there is a need to search for new antimicrobial substances from alternative sources, such as plants used in folk medicine. Other important subjects to study are mycobacterial infections, including those caused by *Mycobacterium tuberculosis*, since tuberculosis caused 8 million new cases and 1.8 million fatalities per annum worldwide.[5,6]

Chenopodium ambrosioides L. (*Dysphania ambrosioides* L.) is to current synonymy of the specie belongs to the Amaranthaceae family and is popularly known as "erva-de-santa-maria". This plant is native from South America and it is widely distributed throughout Brazil, being often used in folk medicine as antirheumatic, anti-inflammatory, antipyretic, antihelmintic, antifungal, anti-ulcer and for the treatment of wounds.[7,8] The ointment is prepared with the essential oil of *C. ambrosioides* inhibited, the growth of dermathophytes and some other fungi.[9,10] In 2009, the specie was added to the list of National Medicinal Plants of Interest in Brazil, (SUS RENISUS-BRAZIL) highlighting the need to increase the understanding of the mechanisms behind its medicinal properties.[11] Therefore, it is of great importance to conduct experiments in such species, to identify their phytochemical compounds with pharmacological potential and to identify possible alternatives for antimicrobial therapy, since studies using crude extracts and fractions of *C. ambrosioides* L. are scarce.

Materials and methods

Reagents

All chemicals were of analytical grade. Solvents for the extractions and analytical procedures and, phytochemical screening were purchased from Merck (Darmstadt, Germany). The quercetin, chrysin and, rutin standards were purchased from Sigma–Aldrich (St. Louis, MO, USA). Microbiological assays were performed using Mueller-Hinton Agar and Mueller-Hinton Broth from Sigma–Aldrich.

Plant collection

The leaves of *C. ambrosioides* L. were collected in the city of Uruguaiana, from the State of Rio Grande do Sul, Brazil (altitude 57 m, 29° latitude, 45′20, 12″S, longitude 57°4′0.28″W) in January of 2013. The exsiccate was archived as specimen in the herbarium of the Departament of Biology at the Federal University of Santa Maria, under the register number of SMDB 137015, for future reference.

Extraction of the plant leaves

Plant material was dried at room temperature and powered in a knife mill. The leaves of the plant (500 g) were macerated at room temperature with ethanol 70% and subjected to a daily shake-up for a week. The extract was collected and filtered and the remaining plant material was subjected to more extraction. After filtration, the hydroalcoholic extract was evaporated under reduced pressure in a rotary evaporator to remove the ethanol. A share of this hydroalcoholic extract was dried in a stove (temperature below 40 °C) yielding the dried crude extract. The remaining of the hydroalcoholic extract was partitioned with solvents of increasing polarity: chloroform, ethyl acetate and n-butanol. Lastly, the fractions obtained were fully dried in a rotary evaporator.

Phytochemical screening

The phytochemical screening followed a series of characterization reactions which used hydroalcoholic extract, according to.[12,13] The identification of alkaloids was performed using Dragendorff reagent, the technique is based on the formation of precipitates after the addition of the reagent, which demonstrates the presence these metabolites. The flavonoids were identified using the reaction with aluminum chloride, filter paper strips were moistened with hydroalcoholic extract, after was added one drop of 5% $AlCl_3$ solution, the presence of flavonoids was revealed by intensification of the yellowish green color on fluorescence. The tannins were identified through of the reaction that used ferric chloride, in which drops of 1% $FeCl_3$ solution in methanol were added to 2 mL of hydroalcoholic extract, the blue color indicated the presence of tannins. The anthraquinone compounds were identified by Bornträger reaction. Briefly, was added small fragment of the drug (about 0.2 g) in a test tube and added 5 mL of diluted NH_4OH solution, the reaction was characterized as positive by pink or reddish coloration. The cardiotonic heterosides were characterized through of the Liebermann-Buchard reaction where acetic anhydride and sulfuric acid were added together to a part of the hydroalcoholic extract. The green coloration characterized the presence of steroidal nucleus, found in cardiotonics compounds.

HPLC–DAD analysis of polyphenols compounds

The analysis were performed with a Prominence Auto-Sampler (SIL-20A) equipped with Shimadzu LC-20AT (Shimadzu, Kyoto, Japan) reciprocating pumps connected to a DGU-20A5 degasser and CBM-20A integrator. UV-VIS detector DAD SPD-M20A and Software LC solution 1.22 SP1 were used. Reverse phase chromatography analyses were carried out with a Phenomenex C-18 column (4.6 mm × 250 mm) packed with 5 μm diameter particles, volume injection was 40 μL, and the gradient elution was conducted with slight modifications in the method.[14] The mobile phase consists of water containing 2.0% acetic acid (phase A) and methanol (phase B), and the elution gradient was 2 min to achieve 5% of B, and changed

Please cite this article in press as: Jesus RS, et al. *In vitro* antimicrobial and antimycobacterial activity and HPLC–DAD screening of phenolics from *Chenopodium ambrosioides* L. *Braz J Microbiol.* (2017), http://dx.doi.org/10.1016/j.bjm.2017.02.012