SHORT COMMUNICATION

Antimicrobial activity of dimeric acylphloroglucinols isolated from southern Brazilian Hypericum species against to resistant bacterial

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ABSTRACT

The hexanic extracts of Hypericum austrobrasiliense, H. caprifoliatum, H. denudatum, H. pedersenii and H. polyanthemum, and three isolated dimeric acylphloroglucinols (uliginosin B, japonicine A and hyperbrasilol B) were assayed for their antimicrobial activity against some Gram-positive and Gram-negative bacteria (including resistant strains). These extracts were assayed using the disc diffusion test, and the results indicated that the tested species did not exhibit activity on the Gram-negative strains. Subsequently, the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) were measured using the broth dilution technique adopted to macrodilution. The most susceptible strains were the MRSA and the S. aureus MLS\textsuperscript{b}. Regarding these pathogens, the better MIC values were obtained with the extracts from H. austrobrasiliense, H. caprifoliatum and H. pedersenii. The acylphloroglucinols uliginosin B and hyperbrasilol B presented the lowest MIC values against Enterococcus faecalis, Staphylococcus aureus, MRSA and S. aureus MLS\textsuperscript{b} resistance.

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1. Introduction

The antimicrobial resistance is an important public health problem that until now, continues to grow up (Mattar et al. 2020). The plants can be considered an effective source of antimicrobial agents, developed along their co-evolution with the pathogens (Hemaiswarya et al. 2008; Mundy et al. 2016).

The Hypericum genus is represented by ca. 500 species, distributed all over the world (Robson 2012). The native species that occur in South America are allocated in the sections, Brathys and Trigynobrathys. The species of the first section present their biodiversity center in the Andean cordilleras and the latter are encountered principally in the South Brazil (Robson 2012). These plants accumulate several classes of bioactive compounds, principally the phloroglucinols (Bridi et al. 2018). Previous phytochemical investigations with the south Brazilian Hypericum species demonstrated the occurrence of dimeric and prenylated acylphloroglucinols (Ccana-Ccapatinta et al. 2015; Bridi et al. 2018).

Extracts of several species from this genus have been demonstrated antimicrobial activity (Rabanal et al. 2002; Gibbons et al. 2002; Dall’Agnol et al. 2003; Dulger et al. 2005; Radulović et al. 2007; Nogueira et al. 2013; Boga et al. 2016) and the most promising antimicrobial results were obtained with isolated dimeric acylphloroglucinols (Jayasuriya et al. 1989; Jayasuriya et al., 1991) and prenylated phloroglucinols (Shiu et al. 2013). The aim of this study is to determine the antimicrobial potential of lipophilic extracts and dimeric acylphloroglucinols from Brazilian Hypericum species.

2. Results and discussion

2.1. Phytochemical analysis of the lipophilic extracts

The HPLC analysis was performed to determine the content of the major constituents of Hypericum lipophilic extracts. Dimeric acylphloroglucinols were detected in all tested species with contents ranging from 0.11 to 17.42% (Table S1). The benzopyrans
were identified only in the *H. polyanthemum* extract being the main phloroglucinols present in this species.

### 2.2. Antimicrobial assay

The antimicrobial activity of five lipophilic extracts from *Hypericum* was measured with a disc diffusion assay. The extracts from *H. austrobrasiliense*, *H. caprifoliatum* and *H. pedersenii* were active against a panel of Gram-positive bacteria (*Enterococcus faecalis* ATCC 29212, vancomycin-intermediate *E. faecalis*, *Staphylococcus aureus* ATCC 25923, methicillin resistant *S. aureus*, *S. aureus* MLSb). On the other hand, none of the extracts exhibited activity on the Gram-negative strains, *E. coli* ATCC 25922, carbapenemase-producing *E. coli* (bla*NDM*), extended-spectrum beta-lactamase (ESBL)–producing *E. coli*, carbapenemase-producing *K. pneumoniae* (bla*KPC*) and *Acinetobacter baumanii* multidrug resistant (Table S2).

Considering the results obtained by the screening assay, the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) were determined for the same lipophilic extracts that were active against a Gram-positive strains. The results can be observed in the Table 1. The most susceptible strains were the MRSA and the *S. aureus* MLSb resistance. Regarding these pathogens, the lowest MIC values were obtained with the extracts from *H. austrobrasiliense* ($\leq 31.3 \mu g/mL$ and $\leq 15.6 \mu g/mL$), *H. caprifoliatum* ($\leq 31.3 \mu g/mL$ and $\leq 7.8 \mu g/mL$) and *H. pedersenii* ($\leq 62.5 \mu g/mL$ and $\leq 125 \mu g/mL$). Similarly to the observed in the disc diffusion assay, the extracts from *H. denudatum* and *H. polyanthemum* did not exhibit antimicrobial activity.

Three dimeric acylphloroglucinols (uliginosin B, japonicine A and hyperbrasilol B) were tested against four strains of Gram-positive bacteria (Table 2). Uliginosin B and hyperbrasilol B presented the lowest MIC values (31.3 μg/mL) against the bacteria

| Gram-positive bacteria | MIC (H. austrobrasiliense) | MBC (H. austrobrasiliense) | MIC (H. caprifoliatum) | MBC (H. caprifoliatum) | MIC (H. denudatum) | MBC (H. denudatum) | MIC (H. pedersenii) | MBC (H. pedersenii) | MIC (H. polyanthemum) | MBC (H. polyanthemum) |
|------------------------|---------------------------|---------------------------|------------------------|------------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| *Enterococcus faecalis* | 31.3                      | 250                       | > 500                  | 500                    | 500               | > 500             | 250               | 250               | 500               | 500               |
| *Staphylococcus aureus*| 500                       | 500                       | 500                    | > 500                  | 500               | > 500             | 500               | > 500             |
| Methicillin resistant *S. aureus* | 31.3                  | 62.5                      | 31.3                   | 250                    | 500               | > 500             | 62.5              | 125               | 500               | > 500             |
| *S. aureus* (MLSb +) | 15.6                      | 125                       | 7.8                    | 31.3                   | 500               | > 500             | 125               | 125               | 500               | > 500             |

*a* Bactericidal susceptibility in μg/mL.

| Gram-positive bacteria | MIC (Uliginosin B) | MBC (Uliginosin B) | MIC (Japonicine A) | MBC (Japonicine A) | MIC (Hyperbrasilol B) | MBC (Hyperbrasilol B) |
|------------------------|--------------------|--------------------|---------------------|--------------------|-----------------------|------------------------|
| *Enterococcus faecalis* | 31.3               | > 500              | 125                 | > 500              | 31.3                  | > 500                  |
| *Staphylococcus aureus*| 31.3               | 250                | 250                 | > 500              | 31.3                  | 500                    |
| Methicillin resistant *S. aureus* | 31.3          | > 500              | 250                 | > 500              | 31.3                  | 500                    |
| *S. aureus* (MLSb +) | 31.3               | > 500              | 250                 | > 500              | 31.3                  | 500                    |

*a* Bactericidal susceptibility in μg/mL.
Enterococcus faecalis (ATCC 29212), Staphylococcus aureus (ATCC 25923), methicillin resistant S. aureus (MRSA) and S. aureus MLSb resistance.

The antimicrobial potential of Hypericum species was determined several decades ago. The first studies with these genus, reported the bactericidal activity of aqueous extracts and isolated phloroglucinol derivatives (Osborn 1943; Parker and Johnson 1968; Bystrov et al. 1975). Regarding the Brazilian Hypericum species, some studies demonstrated the antibacterial activity of extracts (Dall’Agnol et al. 2003; Dall’agnol et al. 2005; França et al. 2009) and isolated dimeric acylphloroglucinols (França et al. 2009). These plants also exhibited antifungal and antiviral activities (Fritz et al. 2007; Barros et al. 2013; Meirelles et al. 2017).

Comparing the antimicrobial activity with the chemical composition of the Hypericum species, some considerations could be made. The most active species (H. austrobrasiliense, H. caprifoliatum and H. pedersenii) were precisely those that presented the major quantities of dimeric acylphloroglucinols (Table 1). On the other hand, the inactive H. polyanthemum possesses great amounts of benzopyrans and relatively low levels of dimeric acylphloroglucinols in its lipophilic extracts. The presence of dimeric acylphloroglucinols seems to be crucial for the antibacterial activity. This statement was previously demonstrated for drummondin C. This dimeric acylphloroglucinol was active against Staphylococcus aureus, Bacillus subtilis and Mycobacterium smegmatis, while the monomers that make up its structure were not (Jayasuriya et al., 1991).

The obtained data against to resistant bacterial strains indicated that uliginosin B and hyperbrasiliol B are promising molecules to further studies to identify their mechanism of action. They also should be further evaluated using in vivo antibacterial assays

Disclosure statement
No potential conflict of interest was reported by the authors.

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