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ORIGINAL ARTICLE/ARTICLE ORIGINAL

Antifungal activity of *Pterocaulon* species (Asteraceae) against *Sporothrix schenckii*

Activité antifongique de Pterocaulon *sp. contre* Sporothrix schenckii

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KEYWORDS
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Summary Plants of the genus *Pterocaulon* (Asteraceae) are popularly used in the treatment of skin diseases caused by fungi and bacteria. The aim of this work was to investigate the *in vitro* activity of the crude methanolic extracts obtained from the aerial parts of *Pterocaulon polystachyum, P. balansae, P. lorentzii, P. lanatum,* and *P. cordobense* against 24 *Sporothrix schenckii* clinical isolates and determine the minimum inhibitory concentration (MIC) and the minimum fungicidal concentration (MFC). MIC were performed by the broth microdilution method according guidelines recommended by Clinical and Laboratory Standards Institute for filamentous fungi and MFC were determined for transference of aliquots of the well that showed 100% of growth inhibition into tubes with culture medium. The extract from *P. polystachyum* was the most active sample, presenting MIC range of 156 and 312 μg/mL. The popular use of these plants corroborates the importance of ethnopharmacological surveys and opens the possibility for finding new clinically effective antifungal agents.

MOTS CLÉS
Pterocaulon ;
Activité antifongique ;
Asteraceae ;

Résumé Les plantes du genre *Pterocaulon* (Asteraceae) sont couramment utilisées dans le traitement des maladies de peau causées par des champignons et des bactéries. Le but de ce travail était d'étudier l'activité *in vitro* des extraits méthanoliques bruts obtenus à partir des parties aériennes de *Pterocaulon polystachyum*, *P. balansae*, *P. lorentzii*, *P. lanatum*, et *P. cordobense* contre 24 isolats de *Sporothrix schenckii* ainsi que d'en déterminer la

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Sporotrichose ;
Sporothrix schenckii

concentration minimale inhibitrice (CMI) et la concentration minimale fongicide (CMF). Les CMI ont été réalisées par la méthode de microdilution selon les lignes directrices recommandées par Clinical and Laboratory Standards Institute pour les champignons filamenteux et les CMF ont été déterminées pour le transfert des parties aliquotes du puits qui a montré 100% d'inhibition de la croissance dans des tubes de milieu de culture. L'extrait de *P. polystachyum* a été l'échantillon le plus actif avec une gamme CMI de 156 à 312 μg/mL. L'usage populaire de ces plantes corrobore l'importance des enquêtes ethnopharmacologiques et ouvre la possibilité de trouver de nouveaux agents antifongiques cliniquement efficaces.

Introduction

Treatment of invasive fungal infections is not always successful due to the limited number of available antifungal agents and resistance to these antimicrobials among fungal pathogens [9]. Therefore, substantial research studies in the field of antifungal compounds are necessary to develop new prototype antimicrobial agents to avert this situation.

Sporothrix schenckii is the etiological agent of sporotrichosis [6] a mycosis that can affect humans and other animals. This mycosis is acquired by traumatic inoculation of contaminated soil, plants, and organic matter. The lesions are usually limited to the skin, subcutaneous tissue, and surrounding lymph vessels. Sporotrichosis predominates in tropical and temperate zones, and Brazil is one of main endemic area, being the most common subcutaneous mycosis occurring in the state of Rio Grande do Sul, in the south of Brazil [4].

Although most antibiotics in clinical use have been obtained from microorganisms, the interest in plant antimicrobials has emerged in the last few decades. Plants and plant-derived compounds have a long clinically history as sources of potential chemotherapeutic agents [7]. The investigation of plants used by traditional medicine is a strategy for finding alternative antimicrobial agents [17].

An ethnoveterinary study indicated that some *Pterocaulon* species were useful in treating humans and animals skin diseases. In another study, three *Pterocaulon* species native to southern Brazil showed antifungal properties against opportunistic pathogenic yeasts and filamentous fungi, including dermatophytes [14,15]. Besides that, extract of *Pterocaulon alopecuroides* presented a broad spectrum of activity against a panel of chromoblastomycosis agents [5]. In another study, the amebicidal activity of extract of *P. polystachyum* was demonstrated [11].

The broad spectrum of action against some pathogenic fungi shown by extracts of *P. alopecuroides*, *P. balansae*, and *P. polystachyum* [15] makes it important to perform assays aimed at evaluating the antifungal activity against other fungal pathogens such as sporothrichosis agents. Thus, the aim of this work was to test the methanolic extracts of five *Pterocaulon* species against 24 *S. schenckii* clinical isolates determining the minimum inhibitory concentration (MIC) and minimal fungicidal concentration (MFC).

Materials and methods

Plant material

Plant material was collected in the southern region of Brazil in January, 2007 and were identified by N. Matzembacker (PPG-Botânica, Universidade Federal do Rio Grande do Sul, Brazil). Vouchers were deposited in the herbarium of the Universidade Federal do Rio Grande do Sul (ICN) (*Pterocaulon balansae*: ICN 59572; *P. cordobense*: ICN 140001; *P. lanatum* ICN 140002; *P. lorentzii*: ICN 140005; *P. polystachyum*: ICN 140011).

Extract preparation

The crude methanolic extracts of *P. polystachyum*, *P. balansae*, *P. lorentzii*, *P. lanatum*, and *P. cordobense* were prepared with 50 g of the drug, using a drug: solvent ratio = 1:10 (w/v) by maceration. The extract was evaporated to dryness under reduced pressure at 45 °C affording 9—15%.

Microorganisms

For the antifungal evaluation, 24 clinical isolates of sporotrichosis agents from the Pathogenic Fungi Laboratory (Department of Microbiology of the Institute of Basic Health Sciences of Universidade Federal do Rio Grande do Sul, Brazil) were used: *S. schenckii* 20, 339, 424, 441, 478, 611, 794, 13534, 13845, 16498, 1099-18, 1400, s175, SN, STT, UFSM (02, 08, 09, 23, 26), and Santa Casa (I, II, III, F32).

Antifungal susceptibility testing

Antifungal susceptibility assays were performed by the broth microdilution method according to guidelines recommended by Clinical and Laboratory Standards Institute (CLSI) for filamentous fungi — M38-A2 [3]. Strains were subcultured onto potato dextrose agar (PDA/Difco, Becton, Dickinson and Company, USA) at 35 °C for 7 days. The surface was gently scraped with a sterile bent glass after flooding with sterile saline solution. The standard suspensions were adjusted by UV-visible spectrophotometry (Spectrum Instruments Co., Shanghai, China) to show transmittance at 530 nm of 80—82%. Adjusted suspensions were diluted in RPMI-MOPS (1:50) to obtain a final inoculum of 10^4 CFU/mL, and 100 μL of the fungal suspensions were added to each microdilution well containing 100 μL of the extracts