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Short communication

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Evaluation of the Antimicrobial Activity of *Piper regnellii* (Miq.) C. DC. var. *pallescens* (C. DC.) Yunck

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SUMMARY. The antimicrobial activity of different extracts of *Piper regnellii* was evaluated through the broth microdilution assay. The leaves, roots and stems extracts presented a good activity against *Staphylococcus aureus* and *Bacillus subtilis* with minimal inhibitory concentration (MIC) between 31.25 - 62.5 *µ*g/ml. Although the differences were not significant, the leaves hydroalcoholic extract tended to be more active than the stems and roots extracts. In the anti - yeast assay, all extracts of *P. regnellii* displayed good activity against *Candida tropicalis* (MIC = 62.5 *µ*g/ml) and a moderate response against *Candida albicans* (MIC = 250 *µ*g/ml). The results indicate that the vegetal specie *P. regnellii* demonstrate promising antimicrobial activity and could be used as raw material by pharmaceutical industry.

***RESUMO*. "Avaliação da atividade antimicrobiana de *Piper regnellii* (Miq.) C. DC. var. *pallescens* (C. DC.) Yunck". A atividade antimicrobiana de diferentes extratos de *Piper regnellii* foi avaliada pelo método de microdiluição. Extratos das folhas, raízes e caules apresentaram uma boa atividade em *Staphylococcus aureus* e *Bacillus subtilis* com uma concentração inibitória mínima (CIM) entre 31.25 - 62.5 *µ*g/ml. Diferenças significativas não foram encontradas, o extrato hidroalcoólico das folhas mostrou-se mais ativos do que os extratos do caule e raízes. A atividade antifúngica dos extratos de *P. regnellii* mostrou-se uma boa atividade em *Candida tropicalis* (CIM = 62.5 *µ*g/ml) e moderada em *Candida albicans* (CIM = 250 *µ*g/ml). Estes resultados indicam que a espécie vegetal *P. regnellii* demosntrou uma promissora atividade antimicrobiana e poderá ser utilizado como uma matéria prima vegetal pela indústria farmacêutica.**

INTRODUCTION

Piper regnelii (Miq.). C.DC. var. *pallescens* (C. DC.) Yunck., popularly known in Brazil as "pariparoba", is one of the species belonging to Piperaceae family used in folk medicine, being the leaves and roots used in form of crude extracts, infusions or poultices in the treatment of wounds, swellings and skin irritations [1]. From leaves of *Piper regnellii* (Miq.) C. DC. var. *pallescens* (C. DC.) Yunck were identified eupomatenoid-6, eupomatenoid-5, eupomatenoid-3 and conocarpan. (-mirceno (70%) was identified as the main constituent of the essential oil obtained by hydro distillation from fresh leaves [2]. Neolignans isolated from leaves of *P. regnellii* as eupomatenoid-6 and eupomatenoid-5 showed a good activity against *S. aureus* with MIC of 1.56 µg/ml and 3.12 µg/ml, respectively. Both compounds presented MIC of 3.12 µg/ml against *B. subtilis*. Conocarpan was quite active against *S. aureus* and *B. subtilis* with MIC of 6.25 µg/ml^{-1}. The ethyl acetate extract from *Piper regnellii* leaves presented a significant activity against *Candida albicans* with MIC at 125 µg mL^{-1}, and a moderate activity against both *C. krusei* and *C. parapsilosis* with MIC at 500 µg mL^{-1}. The conocarpan was the only active compound on the yeasts at concentrations of 6.3 to 12.5 µg mL^{-1} isolated from ethyl acetate extract [2] .

In the present study we describe the in vitro antimicrobial activity of hydroalcoholic extracts from leaves, steam and roots of *Piper regnellii* Miq.). C.DC. var. *pallescens* (C. DC.) Yunck.

KEY WORDS: Antimicrobial activity, Neolignans, *Piper regnellii*.
PALAVRAS-CHAVE: Atividade antimicrobiana, *Piper regnellii,* Neolignanas.

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MATERIAL AND METHODS

Plant material

Leaves, stems and roots of *P. regnellii* var. *pallescens* were collected in September 2004 in the Medicinal Plants Garden "Profª. Irenice Silva" of the State University of Maringá *campus,* Maringá, PR, Brazil. The plant material was identified by Marília Borgo of the Botanical Departament of the Federal University of the Paraná. A voucher specimen (number HUM 11411) is deposited at the Herbarium of the State University of Maringá. The samples of leaves, stems and roots of *P. regnellii* were dried at 35 °C in an air oven and were ground in a knife mill before extraction.

Extract preparation

Dried leaves, stems and roots of *P. regnellii* (10 g) were extracted with ethanol:water (9:1, v/v, 100 ml) by maceration method at room temperature for 5 days at dark room. The extracts were filtered, evaporated under vaccum at 40 °C and lyophilized.

HPLC analysis

The analyses were carried out using a Shimadzu LC-10 liquid chromatograph equipped with quaternary pump (LC-10 AD), manual injection valve (Rheodyne) with loop of 20 µl, degasser (DEU-14), thermostatted column compartment (CTO-10Avp) and a UV-Vis detector (SPD-10A), controlled by CLASS LC-10 Software. In the chromatographic analysis a Metasil ODS column, 5 µm, 150 x 4.6 mm, maintained at 30°C, was used . The separation was carried out in an isocratic system, using as mobile phase a mixture of acetonitrile-water (60:40, v/v) containing 2% acetic acid, with flow rate of 1.0 ml/min. The detection was carried out at 280 nm and the running time was 25 min. The sample injection volume was 20 µl.

Determination of antimicrobial activity

Microorganisms used and growth conditions

The test organisms included the bacteria *Staphylococcus aureus* ATCC 25923 and *Bacillus subtilis* ATCC 6623, and the yeasts *Candida tropicalis* ATCC 28707 and *Candida albicans* ATCC 10231. The bacteria were grown in nutrient broth (Difco Laboratories, Detroit, MI) at 37 °C and maintained on nutrient agar slants at 4 °C. The yeasts were grown and maintained on Sabouraud-dextrose agar (Merck SA, São Paulo, Brazil).

Antimicrobial susceptibility testing

The minimal inhibitory concentrations (MICs) of all extracts and reference antibiotics (penicilin, vancomycin and nistatin - Sigma Chemical Co., St. Louis, MO) were determined by microdilution techniques in Müeller-Hinton broth (Merck) for bacteria and Sabouraud broth (Sigma Chemical Co.) for yeasts [3]. Each extract (2 mg/ml) was asseptically mixed with inoculum prepared in the same medium at a density adjusted to a 0,5 McFarland turbidity standard [10^8 colony-forming units (CFU)/ml for bacteria and 10^6 CFU/ml for yeasts], and diluted 1:10 for the broth microdilution procedure. Microtiter plates were incubated at 37 °C and the MICs were recorded after 24 h of incubation. Two susceptibility endpoints were recorded for each isolated. The MIC was defined as the lowest concentration of compounds that the microorganism tested did not demonstrate visible growth compared with control. MBC (minimal bactericidal concentration) and MFC (minimal fungicidal concentration) were defined as the lowest concentration yielding negative subcultures or only one colony.

RESULTS AND DISCUSSION

The evaluation of the activity of hydroalcoholic extracts from leaves, roots and stems of *P. regnellii* against bacteria and yeasts by using the microdilution technique is given in Table 1. The *in vitro* results were classified as follows: if the extracts displayed a MIC less than 100 µg/ml, the antimicrobial activity was considered good; from 100 to 500 µg/ml the antimicrobial activity was moderate [4-6]. The hydroalcoholic extracts from leaves, roots and stems of *P. regnellii* presented a good activity against the gram-positive bacteria *S. aureus* and *B. subtilis* with MIC between 31.25 - 62.5 µg/ml. Although the differences were not significant, the leaves extract tended to be more active (i.e. have a lower MIC) than the stems and roots extracts. In the anti - yeast assay, all extracts of *P. regnellii* displayed good activity against *C. tropicalis* (MIC = 62.5 µg/ml) and a moderate response against *C. albicans* (MIC = 250 µg/ml). The MBCs and MFCs were within two-fold dilutions of the MIC for these organisms. The MICs of the reference antibiotics used in this study were similar to those presented by the literature [7,8].

Analysis by HPLC of *Piper regnellii* (Miq.) C. DC. var. *pallescens* (C. DC.) Yunck compounds demonstrate the presence of neolignans in the

Extracts/ reference antibiotics	Antibacterial activity (µg/ml)				Antifungical activity (µg/ml)			
	S. aureus		*B. subtilis*		*C. tropicalis*		*C. albicans*	
	MIC	MBC	MIC	MBC	MIC	MFC	MIC	MFC
Leaves	31.2	62.5	31.2	31.2	62.5	125	250	250
Roots	31.2	31.2	62.5	125	62.5	125	250	250
-Stems	62.5	62.5	62.5	62.5	62.5	125	250	500
Nistatin		n.d.		n.d.	8.0	n.d.	1.0	n.d.
Penicilin	0.0097	n.d.		n.d.		n.d.		n.d.
Vancomycin		n.d.	0.19	n.d.		n.d.		n.d.

Table 1. Minimal inhibitory concentrations (MICs), minimal bactericidal concentrations (MBCs) and minimal fungicidal concentrations (MFCs) of hydroalcoholic extracts from leaves, roots and stems of *P. regnellii* and of the reference antibiotics. n.d., not determinated.

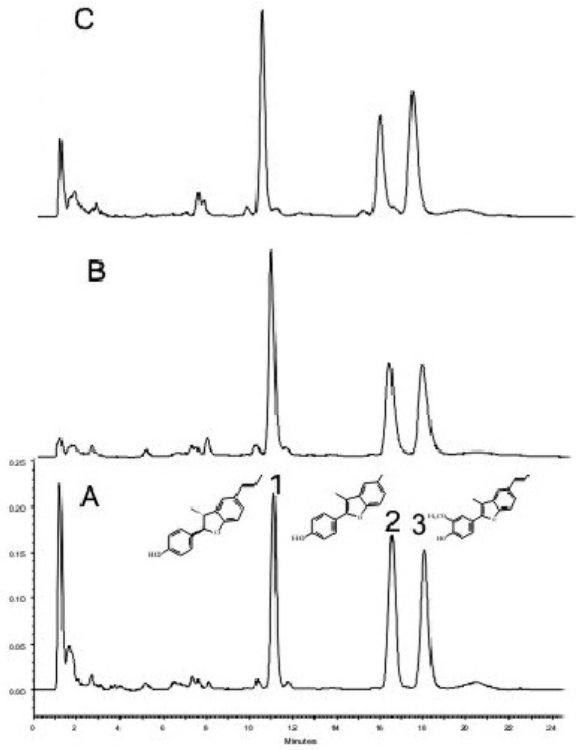

Figure 1. Chromatograms of the *P. regnellii* extracts, (**a**) leaves; (**b**) stems; (**c**) roots, conocapan (**1**), eupomatenoid-6 (**2**) and eupomatenoide-5 (**3**). Chromatographic conditions: Metasil ODS column; mobile phase: acetonitrile/water (60:40, v/v) with 2% acetic acid; flow-rate: 1.0 ml/min; temperature: 30 °C; detection: 280 nm.

different parts of the plant. As can be seen (Fig. 1), roots presented a higher concentration of conocarpan than the leaves and stems, but the difference was significant ($p < 0.05$) in relation to leaves. Leaves and roots presented higher content of eupomatenoid-5 and eupomatenoid-6, respectively, and both showed significant difference ($p < 0.05$) in relation to stems [9].

In spite of the leaves, roots and stems extracts exhibited significant differences in neolignan concentrations; the inhibitory activities against bacteria and yeasts displayed by the extracts were similar.

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