Revista Brasileira de Farmacognosia
Brazilian Journal of Pharmacognosy
19(1A): 20-25, Jan./Mar. 2009
Received 1 January 2009; Accepted 1 March 2009

Artigo

Initial antimicrobial activity studies of plants of the riverside forests of the southern Uruguay River

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RESUMO: "Estudos antimicrobianos preliminares de plantas da floresta ribeirinha do sul do rio Uruguai". As doenças infecciosas ainda são uma das principais causas de morte no mundo, sendo de significativa importância o desenvolvimento de novos compostos antimicrobianos contra diferentes microrganismos. As plantas podem ser uma boa fonte para direcionar a busca destes compostos. Neste estudo, 66 extratos de 25 plantas da floresta ribeirinha do sul do Rio Uruguai foram estudados para a atividade antimicrobiana contra o *Staphylococcus aureus*, *Listeria inocua*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Mycobacterium tuberculosis*, *Aspergillus niger* e *Candida albicans*. Cinqüenta e três destes extratos apresentaram algum tipo de atividade antimicrobiana. Seis (*Eugenia mansoni*, *Eugenia repanda, Myrcianthes cisplatensis, Paullinia ellegans, Petunia* sp e *Ruprechtia laxiflora*) apresentaram atividade contra o *Mycobacterium tub*erculosis com CIM de 50 µg/mL.

Unitermos: Atividade antimicrobiana, bioprospecção, *Mycobacterium, Candida*.

ABSTRACT: Development of new antimicrobial compounds against different microorganisms is becoming critically important, as infectious diseases are still one of the leading causes of death in the world. Plants can be a useful source of these lead compounds. In this study, 66 extracts of 25 plants of the riverside forest of southern Uruguay River were studied for antimicrobial activity against *Staphylococcus aureus*, *Listeria inocua*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Mycobacterium tuberculosis*, *Aspergillus niger* and *Candida albicans*. Fifty-three of these extracts showed some kind of antimicrobial activity. Six of these (*Eugenia mansoni, Eugenia repanda*, *Myrcianthes cisplatensis*, *Paullinia ellegans, Petunia* sp and *Ruprechtia laxiflora*) presented activity against *Mycobacterium tuberculosis* with MIC values as low as 50 µg/mL.

Keywords: Antimicrobial activity, bioprospection, *Mycobacterium*, *Candida*.

INTRODUCTION

In spite of the great advance in chemotherapeutics, infectious diseases are still one of the leading causes of death in the world. The World Health Organisation (WHO, 2004) states that infectious and parasitic diseases account for nearly 11 million among the 57 million total deaths in 2003.

Although there seems to be a great array of antibacterial and antifungal drugs in clinical use, the appearance of resistant organisms sometimes makes them ineffective or leads to recurrence.

Higher plants have shown to be an important source of new bioactive compounds including antihypertensive, analgesics, cytotoxic compounds, amongst others (Cassady et al., 1990; Lewis & Elvin-Lewis, 1995; Clark, 1996; Amaral et al., 2006; Barbosa-Filho et al., 2006a,b,c; Barbosa-Filho et al., 2007; Rocha et al., 2007; Saúde-Guimarães & Faria, 2007; Araújo et al., 2008; Barbosa-Filho et al., 2008; Corrêa et al., 2008; Sousa et al., 2008). Though no plant derived compound has been found to compete with clinically used antibiotics to date, the great structural variety found in plants makes them attractive as a source of novel lead compounds (Cowan, 1999). In fact, higher plants frequently exhibit significant potency against human bacterial and fungal pathogens (Alonso et al., 1995).

Although Uruguay is usually considered a grassland country it has more than 30 woody families and a considerable amount of native forests, especially along river banks (gallery forests) and "quebradas"

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ISSN 0102-695X

(gulch forests), with a subtropical and tropical vegetation intrusion (Morrone, 2001; Bertucci et al., 2008). These forests comprise more than 810.000 has with a varied and distinctive flora, around 250 comprising woody and arbustive species (Escudero, 2004). The riverside forest along the Uruguay River banks is especially interesting as a great number of tropical species originary from the Chaco and Espinal floristic provinces are also present (Grela & Brussa, 2003).

As part of an ongoing project for the native forest bioprospection we identified 54 species belonging to 25 families. Herein we present the results of the antimicrobial screening of 25 of the plant species collected at this site and selected using chemical, pharmacological and ethnopharmacological criteria.

MATERIAL AND METHODS

Plant material

Plants were collected during the 2006-2007 summer season in different locations along the Uruguay River shore between the Chapicuy stream confluence and river Guaviyu confluence, near Paysandù. Plants were identified by Lic. F. Haretche, Museo y Jardin Botanico "Atilio Lombardo", Montevideo. Voucher specimens are kept in the MVJB Herbarium, Jardin Botanico, Montevideo.

Extraction

The plant material was air dried in the dark and milled to a coarse powder. Samples (20 g) were separately twice extracted by maceration with EtOH/H_2O 70:30, acetone and $CHCl_3$ (100 mL) for 48 h. Combined extracts were evaporated under vacuum and lyophilised when necessary.

Microbiological assay

The antimicrobial activity of extracts to *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 6538p), *Listeria inocua* (CCM-FQ 56), *Candida albicans* (ATCC 10231) and *Aspergillus niger* (ATCC 2601) was determined by an agar-diffusion method (Barry & Thornsberry, 1985).

For *Mycobacterium tuberculosis* H37Rv (ATCC 27294) the antimicrobial activity was performed by the Resazurin Method developed by Palomino (Montoro et al., 2002) at 200 mg/mL extract concentration. For those extracts that showed activity at this concentration, a MIC by the aforementioned method was performed.

The microorganisms, except *M. tuberculosis* were cultured overnight at 35 ºC in blood agar base. Colonies were suspended directly into a small volume of 0.9% saline and further diluted until the turbidity matched the MacFarland tube nº 1 and 2.5 mL of this suspension added to 100 mL of molten Mueller-Hinton agar (Difco) for bacteria, and Sabouraud agar (Difco) for yeasts.

The inoculated medium (20 ml) was poured into Petri dishes. Four stainless steel cylinders (ID 1cm) were placed on the surfaces of the medium and 200 µl of each extract solution (10 mg/mL^{-1}) pipetted into each of three of them. Two hundred microlitres of gentamicin (20 $\mu g/mL^{-1}$) or nystatin (50 $\mu g/mL^{-1}$) were placed into the fourth in order to perform a positive control.

The Petri dishes were incubated at 35 ºC for 24 h for bacteria and 25 ºC for 48 h for yeasts. A positive result was recorded if an inhibition zone bigger than the cylinder's diameter was observed. Nystatin and gentamicin were used as controls.

M. tuberculosis was maintained in Ogawa-Kudoh medium for about 14 days. The bacterial suspensions were prepared in sterile water containing glass beads of 3 mm. The suspension was homogenized by vortex agitation and the turbidity was adjusted in agreement with tube nº 1 of the scale of McFarland (3.2 x 10^6 cfu/mL). The inocula was prepared diluting the bacterial suspension 1:25 in Middlebrook 7H9 OADC medium (4.7 g Middlebrook 7H9 base (Difco-Becton Dickinson) enrich with 10% Oleic acid, Dextrose Albumin, Catalase (OADC-BBL).

RESULTS AND DISCUSSION

The antimicrobial activity of different plant extracts measured by the agar diffusion method is depicted in Table 1. The MICs determined for plants with a positive assay for *M. tuberculosis* are show in Table 2.

The plants collected for this study were selected using an ethnopharmacological criteria (Cox, 1990), and this accounts for the high levels of positive results obtained (Olano et al., 1996), of sixty-six extracts tested, fifty-three presented some kind of antimicrobial activity.

Myrcianthes cisplatensis has a broad spectrum of antimicrobial activity for all its extracts, even including fungi and *Mycobacterium*. On the other hand, some others such as *Pouteria salicifolia* show activity only against a certain group, in this case *Mycobacterium* and fungi. *Paulinia ellegans* results one of the most interesting plants. All its extracts show activity against *M. tuberculosis* and the ethanol/water leaf extract shows activity against Gram negative bacteria as well.

The three species of the *Eugenia* genus, known locally as pitanga or ñangaripé and used as edible, are especially interesting, showing activity against *Mycobacterium*, *Candida* and *Aspergillu*s.

The *Petunia* extracts showed MIC of 50 µg/mL for *M. tuberculosis* and despite the fact that it is a high value considering classical antituberculous drugs we believe that it is a good source for bio-guided bioassay.

The MIC of a crude natural extract may or may

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Table 1. Antimicrobial activity of plant extracts.

Species	Used part	Extract	*S. aureus*	*L. inocua*	*E. coli*	*P. aeruginosa*	*M. tuberculosis*	*C. albicans*	*A. niger*
Combretum fruticosum	L	1	+	-	-	+	-	-	+
		2	+	-	+	+	-	-	+
		3	-	-	+	-	-	-	-
Croton tenuissimus fem	L	1	-	-	-	-	-	-	-
		2	-	-	-	-	-	+	-
		3	-	-	-	-	-	-	-
masc		1	-	-	-	-	-	-	-
		2	+	+	-	-	-	-	-
		3	-	+	-	-	-	-	-
Eugenia mansoni	L	1	+	+	-	-	-	-	-
		2	+	+	-	-	+	-	+
		3	+	+	-	-	+	+/-	+
Eugenia repanda	L	1	-	+	-	-	-	-	-
		2	-	+	-	-	+	-	-
		3	-	-	-	-	+	+	+
Eugenia uniflora	L	1	+	-	+	-	-	-	+
		2	+	-	-	-	-	-	+
		3	-	-	-	-	-	-	-
Galianthe brasiliensis	L	2	-	-	-	-	-	-	-
Guettarda uruguensis	L	1		+	+	-	-	-	-
		2	+/-	-	-	-	-	-	-
		3	-	-	-	-	-	-	+
Hexachlamys edulis	L	1	+	+	+	-	-	-	-
		2	+	+	+	-	-	-	-
		3	-	-	-	-	-	+	-
Inga uruguensis	L	1	-	-	-	-	-	+	-
		2	-	-	-	-	-	-	-
		3	-	-	-	-	-	-	-
Luehea divaricata	L	1	-	+	-	-	-	-	+
		2	-	+/-	-	-	-	-	-
		3	-	+	-	-	-	-	-
Mitracarpus megapotamicus	AP	2	-	-	-	-	-	-	-
Myrcianthes cisplatensis	L	1	+	+	+	+	-	+	+
		2	+	+	+	-	+	+	+
		3	+	+	-	-	-	+	-

Species	Used part	Extract	*S. aureus*	*L. inocua*	*E. coli*	*P. aeruginosa*	*M. tuberculosis*	*C. albicans*	*A. niger*
Nectandra angustifolia	L	1	-	+	-	-	+	+	-
		2	-	+	-	-	+	-	-
		3	-	+	-	-	-	+	-
Paullinia elegans	L	1	-	+	+	+	+	-	-
		2	-	+	-	-	+	-	-
		3	-	+	-	-	+	-	+/-
	F	1	-	-	+	-	+	-	-
Pavonia sepium	AP	2	-	-	-	-	-	+	-
Petunia sp	AP	1	-	-	-	-	-	-	-
		2	-	-	-	-	+	-	-
		3	-	-	-	-	+	-	-
Phyllanthus sellowianus	L	1	+	-	+	-	-	-	+
		2	+	+	-	-	-	-	-
		3	-	-	-	-	-	-	-
Pouteria salicifolia	L	1	-	-	-	-	-	-	+
		2	+	-	-	-	+	+	-
		3	-	-	-	-	+	+	-
Psychotria carthagenensis	L	1	+	+	+	-	-	-	-
		2	+	+	+	-	-	-	+
		3	-	-	-	-	-	-	-
Ruprechtia laxiflora	L	1	-	+	+	-	+	-	-
		2	+/-	-	-	-	-	-	-
		3	-	-	-	-	-	-	-
Rupretchia salicifolia	L	1	-	-	-	-	-	+	-
		2	-	-	-	-	-	+	-
		3	-	-	-	-	-	-	-
Scutia buxifolia	L	1	+	-	-	-	-	-	-
		2	+	-	-	-	-	-	-
		3	-	-	-	-	-	+	-
Sebastiana commersoniana	L	2	+	+	-	-	-	-	-
Terminalia australis	L	1	+	+	-	-	-	-	-
		2	+	+	+	-	-	-	-
		3	-	-	-	-	-	+/-	-
Teucrium vesicarium	L	2	+	-	-	-	-	-	-

Used part: L-leaves, AP-aerial parts, F-fruits Extraction solvent: 1-EtOH-H_2O 70:30, 2-CH_3COCH_3, 3-$CHCl_3$

Table 2. Minimum inhibitory concentration of selected extracts against *M. tuberculosis* H37Rv.

Species	Used part	Extract	MIC µg/mL	Species	Used part	Extract	MIC µg/mL
				Paullinia elegans	L	1	>200
Eugenia mansoni	L	2	200			2	200
		3	200			3	200
Eugenia repanda	L	2	100		F	1	200
		3	100	*Petunia* sp	AP	2	50
Myrcianthes cisplatensis	L	2	200			3	50
		3	100	*Ruprechtia laxiflora*	L	1	200

Used part: L-leaves, AP-aerial parts, F-fruits Extraction solvent: 1-EtOH-H_2O 70:30, 2-CH_3COCH_3, 3-$CHCl_3$

not be a reliable indicator of the chances for success in isolating a potent antimycobacterial agent from that extract. The possibility exists that (i) an extract with a relatively low MIC (high activity) may contain large quantities of only very few moderately active major constituents, or (ii) moderately active crude materials could lead to minor compounds with high activity (Pauli et al., 2005). The extracts with MIC values of 100 and 200 mg/L will be further studied by bioassay-guided fractionation to search for the active (s) molecule (s).

ACKNOWLEDGEMENTS

The authors greatly acknowledge the PDT programme (Uruguay) for funding through the grant 32-34. The support of CNPq (Brazil) through Prosul 490204/2005-8 is also acknowledged. This work is part of the collaborative project CYTED-X.11:PIBATUB.

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