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# SCREENING OF BRAZILIAN PLANTS FOR ANTIMICROBIAL AND DNA-DAMAGING ACTIVITIES. I. ATLANTIC RAIN FOREST – ECOLOGICAL STATION JURÉIA-ITATINS.

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## Abstract

**I. Atlantic Rain Forest – Ecological Station Juréia-Itatins)** Crude extracts from 88 plant species occurring in São Paulo State (Brazil), collected in an Atlantic Forest region, have been screened for antimicrobial and DNA-damaging activities. Of the 114 extracts assayed for antibacterial activity, only the extracts from leaves and stems of *Aspidosperma ramiflorum* (Apocynaceae) showed a slight activity against *Escherichia coli*. In the antifungal assay with *Candida albicans*, no active extract was observed, while in the bioautography assay with *Cladosporium sphaerospermum* and *C. cladosporioides* 12% were active. However, only the extract of *Psychotria mapoureooides* (Rubiaceae) stems showed a strong inhibition of both fungi. The DNA-damaging assay with mutant strains of *Saccharomyces cerevisiae* resulted in 17.5 % of active extracts. The majority (55 %) of the active extracts showed selectivity for the DNA-repair mechanism of topoisomerase II and only 20 % showed a selective response for the mechanism of topoisomerase I.

**Key words:** Atlantic Rain forest, Antibacterial activity, Antifungal activity, antitumoral activity, plants screening.

## Resumo

**(Triagem de plantas nativas do Brasil para atividades antimicrobiana e de Danos no DNA I. Mata Atlântica – Estação Ecológica Juréia-Itatins).** Oitenta e oito espécies nativas do estado de São Paulo foram coletadas numa região de Mata Atlântica e ensaiadas quanto a sua atividade antimicrobiana e capacidade de causar danos no DNA. Dos 114 extratos submetidos aos ensaios para atividade antibacteriana, apenas os extratos de folhas e galhos de *Aspidosperma ramiflorum* (Apocynaceae) apresentaram uma atividade fraca contra *Escherichia coli*. No ensaio antifúngico com *Candida albicans*, não foram observados extratos ativos. Por outro lado, no ensaio de bioautografia com *Cladosporium sphaerospermum* e *C. cladosporioides* 12% dos extratos apresentaram atividade. Contudo, nesse ensaio, somente o extrato dos ramos de *Psychotria mapoureooides* (Rubiaceae) inibiu fortemente o crescimento de ambas espécies do fungo. O ensaio para danos no DNA com cepas mutantes de *Saccharomyces cerevisiae* apresentou 17.5 % de extratos ativos. A maioria dos extratos ativos (55 %) apresentou resultados seletivos para danos dependentes da topoisomerase II como mecanismo de reparo do DNA e somente 20 % foram seletivos para o mecanismo da topoisomerase I.

**Palavras-chave:** Mata Atlântica, Atividade antibacteriana, Atividade antifúngica, Atividade antitumoral, Bioprospecção vegetal

## 1. Introduction

Brazil is one of the countries with the highest plant biodiversity distributed in different biomes. Only in São Paulo State, two of the most important biomes are found namely, Atlantic Forest and Cerrado. The Atlantic forest plays an important role in the overall balance of São Paulo ecosystem and recently has been considered one of the five hotspots for biodiversity in the world (Myers et al., 2000). However, less than 5% of its original vegetation remains dispersed in several thousand fragments (SMA, 1996; Tabarelli et al., 1999). As the Atlantic Forest landscapes become increasingly fragmented, populations of forest species are reduced, ecosystem inputs and outputs are altered resulting in a progressive erosion of biological diversity (Terborgh & Winter, 1980; Tilman et al., 1994). Due to its intense fragmentation the Atlantic forest can be considered the most endangered biome in Brazil.

Previous studies showed that tropical forests contain more than half of the world's estimated 500,000 plant species and less than 1% of these plants have been researched for biological activity (Conte, 1996). These species may contain three to four times the number of active chemical constituents than their temperate counterparts. Very few studies on medicinal plants have been performed in areas such as the Mata Atlantica, Caatinga, Pantanal, and Cerrado. Recently, an ethnobotanical survey took place in rural and urban areas of three cities in the Atlantic forest region of São Paulo State in which 628 medicinal uses were described for 114 plant species. The survey demonstrated that the majority of the plants were employed for respiratory and gastrointestinal diseases and as analgesics. On the other hand, the majority of the plants cultivated for medicinal usage were exotic (Di Stasi et al., 2002).

As vast amount of the native Brazilian plant species have not yet been chemically or biologically evaluated, the interdisciplinary project BIOTA/SP (Conservation and Utilization of São Paulo Biodiversity) aims the complete description of the biodiversity in the State including a systematic biological investigation. Following the objectives of the BIOTA/SP program, crude extracts were screened for antibacterial, antifungal, and DNA-damaging activities.

## 2. Material and methods

### 2.1. Study Area

The Ecological Station Juréia-Itatins (ESJI) is located between the parallels 24°17'-24°40'S and 47°00'-47°36'W. The Station includes part of the cities of Iguape, Peruibe, Itariri, Pedro de Toledo and Miracatu, in the region of the Valley of the Ribeira do Iguape, the southern coast of the State of São Paulo. It lays 210 km away from the city of São

Paulo and about 100 km of Cubatão, the ESJI is limited to the north for the Mountain range of Itatins and the Southeast for the Atlantic Ocean. The park has the form of an inverted triangle, with 90 km of width and 45 km of extension from North to South, and it is cut by the river "Una do Prelado", that, in its 80 km extension, runs together to the Atlantic coast. (Figure 1)

### 2.2. Plant material

The plants analysed were collected at the ESJI in August 2001, identified by Dr. I. Cordeiro (Instituto de Botânica-SP) and a voucher specimen was deposited at the Herbarium of the Instituto de Botânica de São Paulo (SP), and the collection numbers can be found in Table 1.

### 2.3. Extraction

The plants were dried in the shadow at room temperature. The dry material was separated (stems and leaves) and ground. The ground material (30-60 g) was extracted with 60-80 ml ethanol 92°GL in an automatic extractor (ASE 300, Dionex) at 70°C with an extraction cycle of 15 min. The extracts were concentrated under vacuum in a rotatory evaporator, to eliminate the residual water the extracts were further dried in a steam bath at 50°C. The yields varied from 2 – 10 g.

### 2.4. Antimicrobial activity:

The crude ethanol extracts were suspended in a solution of ethanol:Tween 20:water (1:1:98 v/v/v) to a concentration of 10 mg/mL. The suspension obtained was employed for the antimicrobial assay. All the extracts were tested with Gram-positive model bacteria, *Staphylococcus aureus* subsp. *aureus* (ATCC 25923), a Gram-negative model, *Escherichia coli* (ATCC 25922) and yeast *Candida albicans* (ATCC 10231). For each assay, the microorganisms were incubated in inclined tubes (Antibiotic agar nº 1, Merck) for 24 h. After this period, a microorganism suspension was prepared in saline solution (0.9 %) to yield a transmittance of 20% at 560 nm. The media was prepared by pouring a basal layer of 10 mL of Antibiotic agar nº 1 (Merck) in Petri dishes of 100-mm diameter. After solidification, a superficial layer composed for 4 mL of Antibiotic agar nº 1 and 1 mL of microorganism suspension was spread above the basal layer. When the plates were solid, wells of 3.5 or 5.0 mm diameter were drilled in the media surface. The samples (40 µL) and positive control (20 µL of Chloramphenicol 1 mg/mL for bacteria, and 20 µL of Nystatin 1 mg/mL for the yeast) were pipetted into the wells. The plates were incubated for 24 h and inhibition zones were measured with a digital paquímetro (Dorman & Deans, 2000).

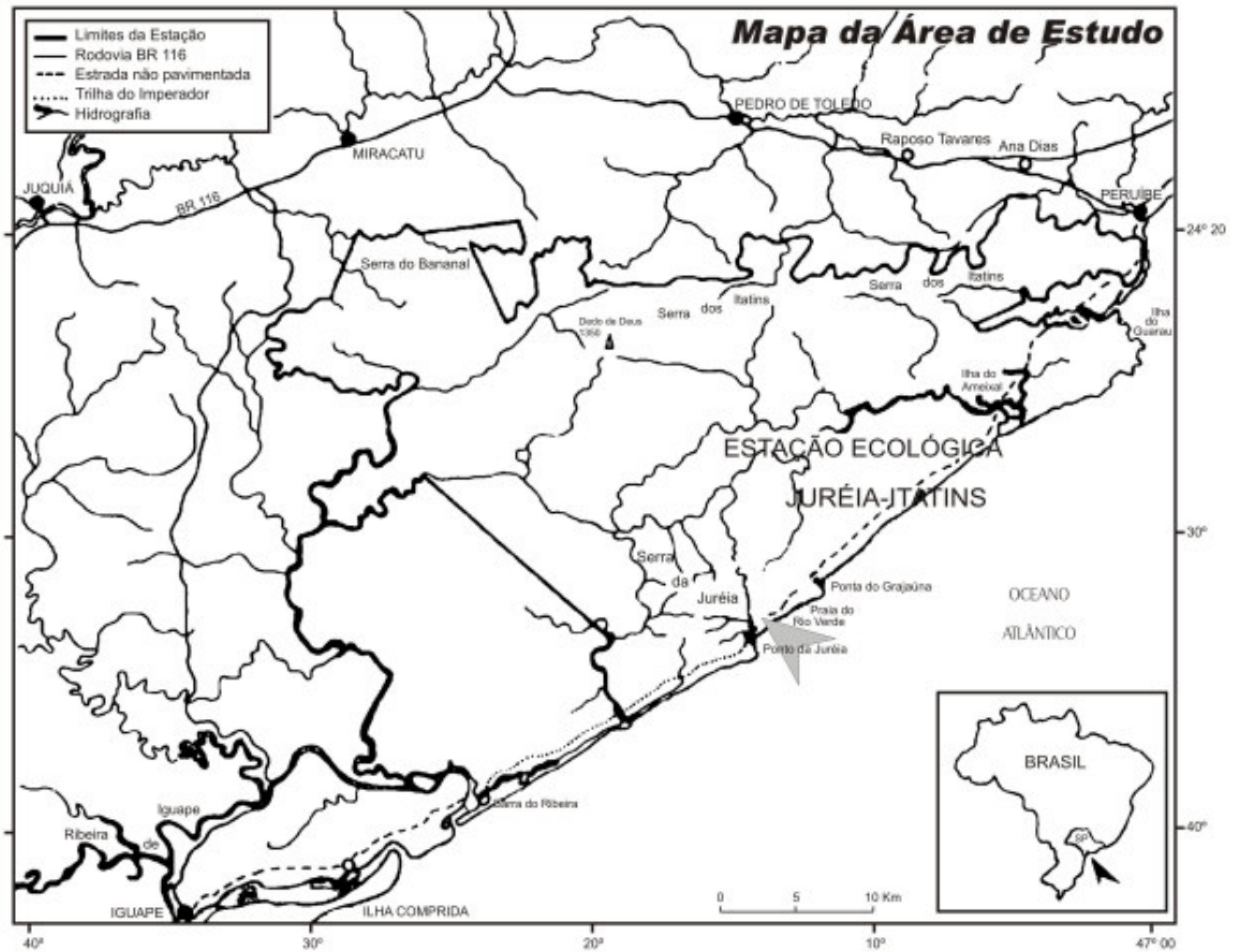


Figure 1 – Location of the study area, Ecological Station Juréia-Itatins, in the state of São Paulo (Brazil).

**Table 1-** Antibacterial, Antifungal and Antitumoral activities of native plants from the Southeastern Brazilian Rain Forest. (i = inactive; \* = weak; \*\* = moderate; \*\*\*= strong)

Family	Species	Collection number	Plant Part	Bioautography Assay (TLC)				DNA Damaging Assay			Antimicrobial Assay		
				<i>C. sphaerospermum</i>		<i>C. cladosporioides</i>		Inhibition zone (mm)			Inhibition zone (mm)		
				Rf	Potential	Rf	Potential	Rad+	Rad52Y	RS321	<i>E. coli</i>	<i>S. aureus</i>	<i>C. albicans</i>
<b>Anacardiaceae</b>	<i>Tapirira guianensis</i> Aubl.	Cordeiro 2552	leaves	-	i	-	i	i	i	i	i	i	i
<b>Annonaceae</b>	<i>Annona cacans</i> Warm.	Cordeiro 2553	leaves	-	i	-	i	i	i	i	i	i	i
	<i>Guatteria elliptica</i> R.E. Fries	Cordeiro 2254	leaves	-	i	-	i	11,5	8	i	i	i	i
	<i>Rollinia sericea</i> R.E. Fries	Cordeiro 2555	leaves	trail	*	-	i	15	8	i	i	i	i
	<i>Xylopia langsdorfiana</i> A. St. Hil. & Tul.	Cordeiro 2556	leaves	origin/trail/0.69	*/ */ *	-	i	i	12	8	i	i	i
<b>Apocynaceae</b>	<i>Aspidosperma olivaceum</i> Müll. Arg.	Silva 269	leaves	-	i	-	i	i	i	i	i	i	i
	<i>Aspidosperma ramiflorum</i> Müll. Arg.	Cordeiro 2732	stems	-	i	0.08	*	21	16	i	2.5	i	i
			leaves	0.09	*	0.08	*	21	16	15	1.4	i	i
<i>Malouetia arborea</i> (Vell.) Miers	Cordeiro 2558	leaves	-	i	-	i	i	i	i	i	i	i	
<b>Aquifoliaceae</b>	<i>Ilex theezans</i> Mart.	Cordeiro 2559	leaves	i	i	-	i	i	i	i	i	i	i
<b>Bignoniaceae</b>	<i>Tabebuia obtusifolia</i> (Cahm) Bureau	Silva 270	leaves	0.59/0.71/0.81	***/*	-	i	10	i	i	i	i	i
	<i>Tabebuia obtusifolia</i> (Cahm) Bureau	Cordeiro 2562	leaves	-	i	-	i	10	i	i	i	i	i
	<i>Tabebuia serratifolia</i> (Vahl.) G. Nicholson	Cordeiro 2563	leaves	origin	*	origin	*	i	i	i	i	i	i
<b>Bombacaceae</b>	<i>Eriotheca pentaphylla</i> (Vell.) A. Robyns	Silva 264	leaves	origin	*	-	i	i	i	13	i	i	i
	<i>Quararibea turbinata</i> Poir.	Silva 298	leaves	-	i	-	i	i	i	i	i	i	i
<b>Boraginaceae</b>	<i>Cordia sellowiana</i> Cham.	Cordeiro 2567	leaves	-	i	origin	*	i	i	i	i	i	i

Family	Species	Collection number	Plant Part	Bioautography Assay (TLC)				DNA Damaging Assay			Antimicrobial Assay			
				<i>C. sphaerospermum</i>		<i>C. cladosporioides</i>		Inhibition zone (mm)			Inhibition zone (mm)			
				Rf	Potential	Rf	Potential	Rad+	Rad52Y	RS321	<i>E. coli</i>	<i>S. aureus</i>	<i>C. albicans</i>	
<b>Bromeliaceae</b>	<i>Pitcairnia flammea</i> Lindl.	Silva 292	leaves	-	i	-	i	i	i	i	i	i	i	
<b>Burseraceae</b>	<i>Protium widgrenii</i> Engl.	Cordeiro 2310	leaves	-	i	-	i	i	i	i	i	i	i	
<b>Chrysobalanaceae</b>	<i>Couepia venosa</i> Prance.	Cordeiro 2574	leaves	-	i	-	i	i	i	i	i	i	i	
	<i>Hirtella hebeclada</i> Moric. ex DC.	Silva 277	leaves	-	i	-	i	11	i	i	i	i	i	i
			stems	-	i	-	i	i	i	i	i	i	i	i
			leaves	origin	*	-	i	10	i	i	i	i	i	i
	<i>Licania hoehnei</i> Pilg.	Cordeiro 2576	leaves	-	i	-	i	i	i	i	i	i	i	
<i>Parinari excelsa</i> Sabine	Cordeiro 2579	leaves	i	-	origin	*	11.5	11	i	i	i	i		
<b>Clusiaceae</b>	<i>Calophyllum brasiliense</i> Camb.	Silva 274	stems	-	i	origin	***	i	i	i	i	i	i	
			leaves	-	i	origin	*	i	i	i	i	i	i	
	<i>Clusia criuva</i> Vesque	Silva 291	leaves	-	i	-	i	i	i	10.5	i	i	i	
			stems	-	i	-	i	i	i	i	i	i	i	
<b>Costaceae</b>	<i>Costus spiralis</i> (Jacq.) Roscoe	Silva 271	leaves	-	i	origin	*	i	i	i	i	i	i	
<b>Cyatheaceae</b>	<i>Cyathea corcovadensis</i> (Raddi) Domin	Cordeiro 1578	leaves	-	i	-	i	i	i	9	i	i	i	
<b>Erythroxylaceae</b>	<i>Erythroxylum cuspidifolium</i> Mart.	Cordeiro 2583	leaves	origin	*	origin	*	8	i	i	i	i	i	

Family	Species	Collection number	Plant Part	Bioautography Assay (TLC)				DNA Damaging Assay			Antimicrobial Assay		
				<i>C. sphaerospermum</i>		<i>C. cladosporioides</i>		Inhibition zone (mm)			Inhibition zone (mm)		
				Rf	Potential	Rf	Potential	Rad+	Rad52Y	RS321	<i>E. coli</i>	<i>S. aureus</i>	<i>C. albicans</i>
<b>Euphorbiaceae</b>	<i>Actinostemon concolor</i> (Spreng.) Müll. Arg.	Cordeiro 2584	leaves	-	i	-	i	i	i	i	i	i	i
			stems	-	i	-	i	i	i	i	i	i	i
	<i>Hyeronima alchorneoides</i> Allemao	Silva 273	leaves	-	i	-	i	i	i	i	i	i	i
			leaves	-	i	-	i	i	9	10	i	i	i
	<i>Pausandra morisiana</i> Radek	Cordeiro 2587	leaves	-	-	origin	*	i	i	i	i	i	i
			leaves	-	i	-	i	i	i	i	i	i	i
	<i>Pera glabrata</i> (Schott) Poepp. ex Baill	Cordeiro2589	leaves	-	i	origin	*	9	i	i	i	i	i
			leaves	-	i	-	i	9	10	i	i	i	i
	<i>Tetrorchidium rubrivenium</i> Poepp.	Silva 290	leaves	-	i	-	i	9	10	i	i	i	i
			stems	origin	*	0.25	**	i	i	i	i	i	i
<i>Tetrorchidium rubrivenium</i> Poepp.	Cordeiro 2306A	leaves	-	i	-	i	i	i	i	i	i	i	
		leaves	-	i	0.03	***	i	i	i	i	i	i	
<b>Flacourtiaceae</b>	<i>Casearia decandra</i> Jacq.	Cordeiro 2590	leaves	-	i	0.03	***	i	i	i	i	i	i
			leaves	trail	*	origin	*	11	8	9	i	i	i
<b>Gesneriaceae</b>	<i>Sinningia schiffneri</i> Fritsch	Silva 293	leaves	-	i	-	i	i	i	i	i	i	i
			leaves	i	-	i	-	i	i	i	i	i	i
	<i>Sinningia mauroana</i> A. Chautems	Silva 296	leaves	i	-	i	-	i	i	i	i	i	i

Family	Species	Collection number	Plant Part	Bioautography Assay (TLC)				DNA Damaging Assay			Antimicrobial Assay			
				<i>C. sphaerospermum</i>		<i>C. cladosporioides</i>		Inhibition zone (mm)			Inhibition zone (mm)			
				Rf	Potential	Rf	Potential	Rad+	Rad52Y	RS321	<i>E. coli</i>	<i>S. aureus</i>	<i>C. albicans</i>	
<b>Lacistemaceae</b>	<i>Lacistema lucidum</i> Schnizl.	Silva 268	leaves	-	i	origin	*	i	i	i	i	i	i	
<b>Lauraceae</b>	<i>Cryptocaria saligna</i> Mez.	Cordeiro 2603	leaves	0.49/0.57/0.77	*/ */ *	-	i	i	i	i	i	i	i	
	<i>Nectandra membracea</i> (Sw.) Griseb.	Cordeiro 2605	leaves	0.48	*	0.48	**	10	i	i	i	i	i	
	<i>Ocotea odorifera</i> (Vell.) J.G. Rohwer	Cordeiro 2730	leaves	0.8	*	-	i	i	i	i	i	i	i	
				stems	-	i	origin/ 0.72	***/ *	i	14	i	i	i	i
	<i>Ocotea dispersa</i> (Nees) Mez.	Cordeiro 2606	leaves	trail	*	origin	*	10	i	12	i	i	i	
	<i>Ocotea odorifera</i> (Vell.) J.G. Rohwer	Cordeiro 2608	stems		-	i	0.72	*	i	i	i	i	i	i
				leaves	0.75	**	0.73	**	i	i	11	i	i	i
	<i>Ocotea velloziana</i> Mez.	Cordeiro 2612	leaves	-	i	-	i	i	i	i	i	i	i	
<b>Lecythidaceae</b>	<i>Cariniana estrellensis</i> (Raddi) Kuntze	Cordeiro 2613	leaves	-	i	origin	**	i	i	i	i	i	i	
<b>Leguminosae</b>	<i>Hymenaea courbaril</i> L.	Cordeiro 2618	leaves	-	i	-	i	11	12	i	i	i	i	
	<i>Inga edulis</i> Mart.	Cordeiro 2620	leaves	-	i	-	i	i	i	i	i	i	i	
	<i>Inga laurina</i> (Sw.) Willd.	Cordeiro 2619	leaves	-	i	origin	*	8	i	9	i	i	i	
	<i>Machaerium nictitans</i> Benth.	Cordeiro 2625	leaves	-	i	-	i	12	i	8	i	i	i	
	<i>Ormosia arborea</i> (Vell.) Harms	Cordeiro 2626	leaves	-	i	-	i	i	i	i	i	i	i	

				Bioautography Assay (TLC)				DNA Damaging Assay			Antimicrobial Assay		
Family	Species	Collection number	Plant Part	<i>C. sphaerospermum</i>		<i>C. cladosporioides</i>		Inhibition zone (mm)			Inhibition zone (mm)		
				Rf	Potential	Rf	Potential	Rad+	Rad52Y	RS321	<i>E. coli</i>	<i>S. aureus</i>	<i>C. albicans</i>
<b>Leguminosae</b> (cont.)	<i>Piptadenia gonoacantha</i> (Mart.) J.F. Macbride	Cordeiro 2627	leaves	origin/ trail	*/ *	-	i	i	i	10	i	i	i
	<i>Pterocarpus rohrii</i> Vahl.	Cordeiro 2628	leaves	-	i	-	i	i	i	13.5	i	i	i
<b>Malpighiaceae</b>	<i>Barnebya dispar</i> (Griseb.)W.R. Anderson & B. Gates	Cordeiro 2730A	leaves	origin	**	origin	*	i	i	i	i	i	i
	<i>Heteropteris chrysophylla</i> (Lam.) Kunth.	Silva 289	leaves	origin	**	origin	**	8	i	i	i	i	i
			stems	-	i	-	i	i	i	i	i	i	i
<b>Malvaceae</b>	<i>Hibiscus pernambucensis</i> Arruda	Silva 288	leaves	origin	*	origin	*	i	8	i	i	i	i
<b>Marcgraviaceae</b>	<i>Norantea brasiliensis</i> Choisy	Silva 295	leaves	-	i	-	i	i	i	i	i	i	i
			stems	-	i	-	i	i	i	10	i	i	i
<b>Melastomataceae</b>	<i>Miconia pyrifolia</i> Naud.	Cordeiro 2632	leaves	-	i	-	i	i	i	i	i	i	i
<b>Meliaceae</b>	<i>Cabrlea canjerana</i> (Vell.) Mart.	Cordeiro 2633	leaves	-	i	-	i	12	i	i	i	i	i
	<i>Guarea macrophylla</i> Vahl.	Silva 284	leaves	-	i	-	i	12	8	i	i	i	i
	<i>Trichilia lepidota</i> Mart.	Cordeiro 2636	leaves	-	i	-	i	i	9	12	i	i	i

Family	Species	Collection number	Plant Part	Bioautography Assay (TLC)				DNA Damaging Assay			Antimicrobial Assay		
				<i>C. sphaerospermum</i>		<i>C. cladosporioides</i>		Inhibition zone (mm)			Inhibition zone (mm)		
				Rf	Potential	Rf	Potential	Rad+	Rad52Y	RS321	<i>E. coli</i>	<i>S. aureus</i>	<i>C. albicans</i>
<b>Moraceae</b>	<i>Brosimum guianense</i> (Aubl.) Huber	Cordeiro 2640	leaves	-	i	origin	*	i	i	i	i	i	i
		Cordeiro 2645	leaves	-	i	-	i	i	i	i	i	i	i
	<i>Ficus pulchella</i> Schott		stems	-	i	origin	*	i	i	i	i	i	i
		Cordeiro 2644	leaves	origin	*	origin	*	i	i	i	i	i	i
	<i>Ficus insipida</i> Willd.		stems	-	i	-	i	i	i	12.5	i	i	i
		Cordeiro 2571	leaves	origin	*	origin	*	i	10	i	i	i	i
	<i>Sorocea bonplandii</i> (Baill.) W.C. Burger, Lang. & Wess. Boer	Cordeiro 2642	leaves	-	i	0.09	*	i	i	i	i	i	i
		Silva 278	leaves	-	i	-	i	14	i	i	i	i	i
<b>Myristicaceae</b>	<i>Virola gardneri</i> (A.DC.) Warb.	Cordeiro 2648	leaves	-	i	-	i	i	i	i	i	i	
			stems	-	i	origin	*	i	i	i	i	i	
<b>Myrsinaceae</b>	<i>Myrsine umbellata</i> Mart.	Cordeiro 2306	leaves	origin	*	origin	*	i	11	i	i	i	i
<b>Myrtaceae</b>	<i>Campomanesia phaea</i> (Berg.) L.R. Landrum	Cordeiro 2657	leaves	-	i	-	i	i	i	9	i	i	i
			stems	-	i	-	i	i	i	11	i	i	i
<b>Nyctaginaceae</b>	<i>Guapira opposita</i> (Vell.) Reitz.	Silva 297	leaves	origin/ trail	*/ *	origin	**	i	i	i	i	i	i
<b>Ochnaceae</b>	<i>Ouratea multiflora</i> (Pohl.) Engl.	Cordeiro 2695	leaves	-	i	origin	*	11	11	i	i	i	i

Family	Species	Collection number	Plant Part	Bioautography Assay (TLC)				DNA Damaging Assay			Antimicrobial Assay		
				<i>C. sphaerospermum</i>		<i>C. cladosporioides</i>		Inhibition zone (mm)			Inhibition zone (mm)		
				Rf	Potential	Rf	Potential	Rad+	Rad52Y	RS321	<i>E. coli</i>	<i>S. aureus</i>	<i>C. albicans</i>
<b>Olacaceae</b>	<i>Heisteria silvianni</i> Schwacke	Cordeiro 2308	leaves	origin/ 0.72	*/ *	origin	*	i	9	10	i	i	i
			stems	-	i	-	i	i	i	i	i	i	i
	<i>Tetrastylidium grandiflorum</i> (Baill.) Sleumer	Cordeiro 2696	leaves	origin	*	-	i	9	11	i	i	i	i
<b>Proteaceae</b>	<i>Roupala brasiliensis</i> Klotzsch	Cordeiro 2698	leaves	-	i	-	i	i	i	10	i	i	i
<b>Rhamnaceae</b>	<i>Rhamnidium glabrum</i> Reiss.	Cordeiro 2699	leaves	-	i	-	i	i	i	i	i	i	i
<b>Rubiaceae</b>	<i>Amaioua intermedia</i> Mart.	Silva 279	leaves	-	i	-	i	i	i	i	i	i	i
			stems	-	i	origin	*	i	10	10	i	i	i
	<i>Psychotria mapoureoides</i> DC.	Cordeiro 2705	leaves	-	i	-	i	i	i	i	i	i	i
stems			origin/ 0.62	***/ *	origin	***	i	i	11	i	i	i	i
	<i>Psychotria mapoureoides</i> DC.	Cordeiro 2705A	leaves	-	i	-	i	i	i	i	i	i	i
	<i>Psychotria nuda</i> (Cham. & Schltdl.) Wawra	Silva 276	leaves	-	i	-	i	i	i	i	i	i	i
	<i>Rudgea recurva</i> Müll. Arg.	Cordeiro 2707	leaves	-	i	-	i	10	i	i	i	i	i
	<i>Rustia formosa</i> (Cham. & Schltdl.) Klotzsch	Cordeiro 2708	leaves	-	i	-	i	i	i	i	i	i	i
<b>Rutaceae</b>	<i>Conchocarpus fontanesianus</i> (A.St.-Hill.) J.A. Kallunki & J.R. Pirani	Cordeiro 1581A	leaves	origin/0.58/0.69	*/ */ *	origin/0.58/0.69	*/ */ *	i	i	i	i	i	i
			<i>Metrodorea nigra</i> A.St.-Hill.	Cordeiro 2709	leaves	origin	*	-	i	i	i	i	i

Family	Species	Collection number	Plant Part	Bioautography Assay (TLC)				DNA Damaging Assay			Antimicrobial Assay		
				<i>C. sphaerospermum</i>		<i>C. cladosporioides</i>		Inhibition zone (mm)			Inhibition zone (mm)		
				Rf	Potential	Rf	Potential	Rad+	Rad52Y	RS321	<i>E. coli</i>	<i>S. aureus</i>	<i>C. albicans</i>
<b>Sapindaceae</b>	<i>Cupania oblongifolia</i> Mart.	Cordeiro 2711	leaves	-	i	-	i	i	i	i	i	i	i
	<i>Matayba juglandifolia</i> Radlk.	Cordeiro 2712	leaves	-	i	origin	**	i	i	i	i	i	i
	<i>Matayba elaeagnoides</i> (Cambess) Radlk.	Silva 283	leaves	-	i	-	i	i	10	i	i	i	i
			stems	-	i	-	i	i	i	i	i	i	i
<b>Sapotaceae</b>	<i>Chrysophyllum flexuosum</i> Mart.	Cordeiro 2713	leaves	-	i	origin	*	8	11	8	i	i	i
	<i>Chrysophyllum flexuosum</i> Mart.	Silva 272	leaves	-	i	-	i	i	i	i	i	i	i
	<i>Chrysophyllum inornatum</i> Mart.	Silva 280	leaves	0.05	*	origin	*	9	i	i	i	i	i
	<i>Chrysophyllum inornatum</i> Mart.	Cordeiro 2302	leaves	-	i	origin	*	i	i	i	i	i	i
	<i>Ecclinusa ramiflora</i> Mart.	Cordeiro 2715	leaves	0.56	*	-	i	i	i	i	i	i	i
	<i>Pouteria psamophila</i> (Mart.) Radlk.	Cordeiro 2718	leaves	-	i	origin	*	i	i	i	i	i	i
	<i>Pouteria grandiflora</i> (A.DC.) Radlk.	Cordeiro 1576	leaves	0.71	*	-	i	i	i	i	i	i	i
<b>Violaceae</b>	<i>Amphirrhox longifolia</i> (A. St.-Hil.) Spreng.	Cordeiro 2722	leaves	origin	*	origin	*	9	i	i	i	i	i

## 2.5. TLC bioautography assay:

*Cladosporium cladosporioides* (Fres.) de Vries SPC 140 and *Cladosporium sphaerospermum* Penz. SPC 491 were maintained at the Instituto de Botânica-SMA. Ten microliters of a solution corresponding to 400 µg of crude extracts were applied on Al-backed silica Gel GF<sub>254</sub> TLC sheets (Merck) and eluted with CHCl<sub>3</sub>: MeOH (9:1 V/V). After the elution, the solvent was completely removed. The plates were, then, sprayed with a spore suspension of *C. sphaerospermum* or *C. cladosporioides* in a glucose and salt solution (Homans & Fuchs, 1970; Rahalison et al., 1994) and incubated for 48 h at 28°C. After incubation, clear inhibition zones appeared against a dark background chromatogram. Nystatin (1.0 µg) and miconazole (0.5 µg) were used as positive controls.

## 2.6. DNA-Damaging Assay:

Due to the large number of extracts that required testing, a pre-screening using strains of topoisomerase-deficient *Saccharomyces cerevisiae* (rad52Y, topoisomerase I, and RS321N, topoisomerase II) was conducted by means of an agar well diffusion assay. Provided there is no inhibition of growth of the wild type (RAD+) strain, differential inhibition of growth in any of the mutant strains (rad52Y or RS321N) acts as an indicator of DNA-damaging activity (Gunatilka et al., 1992).

The RS321N, rad52Y, and RAD+ strains of *Saccharomyces cerevisiae* were kindly donated from Dr. David G. I. Kingston (Virginia Polytechnic Institute and State University) and Dr. Randall K. Johnson (SmithKline Beecham Pharmaceuticals). Individual strains of yeast were seeded onto 2% nutrient agar plates. Samples were solubilized in 1:1 DMSO-MeOH to a concentration of 4 mg/mL and 100 µL were placed in agar wells made through the removal of 6 mm plugs from the media. Activity was measured as the zone (mm) surrounding the well where no yeast growth was visible. Camptothecin and streptonigrin were used at 5 µg/mL as controls.

## 3. Results and discussion

The results of this screening are summarized in Table 1. All the 114 extracts were assayed by the agar diffusion method for antibacterial activity against a Gram + and a Gram – model bacterium at a doses of 400 µg/well. In the assay conditions, only the crude extracts of *Aspidosperma ramiflorum* leaves and stems presented a slight activity against *E. coli*. In the same test conditions with the yeast *C. albicans*, none of the assayed extracts were active. *Aspidosperma* species are known for the accumulation of indole alkaloids, mainly those from the aspidospermatane group. In *A. ramiflorum* were previously isolated two bis-indole alkaloids, ramiflorine A and B, β-yohimbine and 10-

methoxygeissoschizol (Marques et al., 1996), no further biological investigation has been reported. The crude alkaloid extracts and isolated compounds of *A. excelsum* and *A. marcgravianum* demonstrated an antibacterial activity against *Bacillus subtilis* and *S. aureus*, both Gram + bacteria, but not against the Gram – bacteria, *E. coli* and *Pseudomonas aeruginosa*, and the yeast *C. albicans* (Verpoorte et al., 1982, 1983).

On the other hand, when the extracts were assayed for antifungal activity with filamentous fungi, *Cladosporium sphaerospermum* and *C. cladosporioides*, in a bioautography assay, thirteen extracts showed a positive response (moderate to strong) to either one of the species or for both species. From the 88 plant species evaluated, only five showed a specific activity against *C. cladosporioides*: *Casearia decandra* (leaves), one of the specimens of *Ocotea odorifera* (stems), *Calophyllum brasiliense* (stems), *Cariniana estrellensis* (leaves) and *Matayba juglandifolia* (leaves). *Casearia* species are known to accumulate cytotoxic and DNA-damaging clerodane diterpenoids (Carvalho et al., 1998; Sai Prakash et al., 2002), which might be related to the antifungal activity determined. *Ocotea* species are recognised for the accumulation of lignans with anti-inflammatory activity (Jager et al., 1996; Jesus-Morais et al., 2000). In *O. odorifera* were isolated safrole and methyl-eugenol and some terpenoids (Lordello et al., 2000), these compounds may be responsible for antifungal activity. The phenolic compounds found in *C. brasiliense* were related to different biological activities such as, gastroprotective, inhibition of sulfotransferases and anti-nociceptive (Sartori et al., 1999; da Silva et al., 2001; Messia-Vela et al., 2001). However, no reports on antimicrobial activity for this species were found. Only one report was found on *Cariniana* species, which demonstrated an anti-tyrosinase activity for *C. brasiliensis* extracts (Baurin et al., 2002). An antitumor coumarin, Cleomiscosin A, has been isolated from *Matayba arborescens* (Arisawa et al., 1984), similar compounds might be present in the *M. jugandifolia* extracts that could be related with the fungitoxic effect observed.

A specific activity against *C. sphaerospermum* was observed only in the leaves of one specimen of *Tabebuia obtusifolia*. *Tabebuia* species are known for the accumulation of bioactive naphthoquinones (Pinto et al., 2000; Ueda et al., 1994; Grazziotin et al., 1992).

Of the thirteen active extracts, seven showed response against both fungi: *Tetrorchidium rubrivenium* stems, *Nectandra membranacea* leaves, *Ocotea odorifera* leaves; *Barnebya dispar* leaves, *Heteropteris chrysophylla* leaves, *Guapira opposita* leaves and *Psychotria mapoureoides* stems. There were no reports on the chemical composition of the two Malpighiaceae species analysed, *B. dispar* and *H. chrysophylla*, nor on the Euphorbiaceae *T. rubrivenium*. The Lauraceae species, *N. membranacea* and

one of the *O. odorifera* specimens, are known to accumulate phenolic compounds and alkaloids that may be related to the antifungal activity (Lorenzo *et al.*, 2001; Moreno *et al.*, 1991). Antibacterial and antifungal activities have already been reported for extracts of *Psychotria* species (Locher *et al.*, 1995; Jayasinghe *et al.*, 2002) as well as the presence of cytotoxic compounds (Roth *et al.*, 1986; Adjibade *et al.*, 1989). The *Psychotria* genus is known to produce several indole alkaloid skeletons (Verotta *et al.*, 1998; Kerber *et al.*, 2001), which might be related with the activities detected in *P. mapoureoides*.

The DNA-damaging assay with mutant strains of *S. cerevisiae* resulted in 17.5% active extracts (inhibition zone  $\geq 10$  mm) with selectivity for the DNA-repair mechanisms of topoisomerase I and/or topoisomerase II. From the twenty active extracts, eleven were selective for topoisomerase II (*Campomanesia phaea*-stems, *Clusia criuva*-leaves, *Eriotheca pentaphylla*-leaves, *Ficus insipida*-stems, *Norantea brasiliensis*-stems, *Ocotea odorifera*-leaves, *Piptadenia gonoacantha*-leaves, *Psychotria mapoureoides*-stems, *Pterocarpus rohrii*-leaves, and *Roupala brasiliensis*-leaves), four selective for topoisomerase I (*Myrsine umbellata*-leaves, *Pourouma guianensis*-leaves, *Ocotea odorifera*-stems, *Matayba elaeagnoides*-leaves), and five showed activity for both DNA-repair mechanisms (*Amaioua intermedia*-stems, *Heisteria silvianni*-leaves, *Hyeronima alchorneoides*-leaves, *Trichilia lepidota*-leaves, and *Xylopia langsdorfiana*-leaves).

The extracts from leaves of *P. rohrii* and *E. pentaphylla* showed the highest specific activity for topoisomerase II mechanism. No reports on the chemical composition or biological activity of *P. rohrii* are available. However, savinin, a lignan isolated from *P. santalinus*, was able to inhibit the production of the Tumor Necrosis Factor- $\alpha$  and T cell proliferation without displaying cytotoxicity (Cho *et al.*, 2001). Additionally, extracts of *P. santalinus* also showed a strong inhibitory activity of the enzyme COX-2 and a moderate inhibition of NO synthase (Hong *et al.*, 2002).

The extracts of stems from one specimen of *Ocotea odorifera* showed the highest activity with the yeast strain deficient in the topoisomerase I repair mechanism. This result might be related with the accumulation of alkaloids in some Lauraceae species. In *O. lecoxylon* was isolated an aporphine alkaloid which inhibited human topoisomerase I (Zhou *et al.*, 2000) and in crude extracts of *N. grandiflora* which were active against sarcoma 180 and Erlich's carcinoma (Moreno *et al.*, 1993).

From the 88 plant species assayed, 56 showed some biological activity. As the assays were performed with crude extracts, it is necessary to check if these activities are only due to synergistic effect or to a single compound. In order to perform this evaluation bio-guided fractionation of the active extracts are underway in our laboratories. Addition-

ally, attempts are being made to evaluate the active fractions with cancer cell lines and human or crop pathogenic fungal strains.

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