

Biological Screening of Brazilian Medicinal Plants

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*In this study, we screened sixty medicinal plant species from the Brazilian savanna ("cerrado") that could contain useful compounds for the control of tropical diseases. The plant selection was based on existing ethnobotanic information and interviews with local healers. Plant extracts were screened for: (a) molluscicidal activity against *Biomphalaria glabrata*, (b) toxicity to brine shrimp (*Artemia salina* L.), (c) antifungal activity in the bioautographic assay with *Cladosporium sphaerospermum* and (d) antibacterial activity in the agar diffusion assay against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus* and *Pseudomonas aeruginosa*. Forty-two species afforded extracts that showed some degree of activity in one or more of these bioassays.*

Keys words: medicinal plants - *Artemia salina* - *Biomphalaria glabrata* - *Cladosporium sphaerospermum* - antibacterial activity - Brazil

The Brazilian savanna, known as "cerrado", comprises a very rich and characteristic flora (Burman 1991) that covers more than 2 million square kilometers of Brazilian inland (Ferri 1973). Many of these plants are used as natural medicines by the people living in the "cerrado" area to treat several tropical diseases including schistosomiasis, leishmaniasis, malaria, fungal and bacterial infections, among others (Ferreira 1980, Corrêa 1984, Grandi et al. 1989, Di Stasi 1989, Hirschmann & Arias 1990, Brandão 1991, Caribé & Campos 1991, Martins et al. 1994, Matos 1994).

Schistosomiasis, caused by the parasite *Schistosoma mansoni*, is an important endemic disease in Brazil and in many other tropical countries (Davis 1996). The life cycle of this parasite involves an intermediate host, represented in Brazil by snails of the genus *Biomphalaria*. Thus, besides chemotherapy of infected people, one of the strategies to combat this disease is to interrupt the parasite's life cycle in endemic areas via control of the snail's population. The search for molluscicidal compounds derived from renewable parts of plants that grow easily in endemic areas could improve

the access of poor communities to molluscicidal agents to treat their water collections. This could enhance their chances to control schistosomiasis (Mott 1987).

Fungi and bacteria cause important human diseases, especially in tropical regions and in immunocompromised or immunodeficient patients. Despite the existence of potent antibiotic and antifungal agents, resistant or multi-resistant strains are continuously appearing, imposing the need for a permanent search and development of new drugs (Silver & Bostian 1993).

In an effort to discover new lead compounds, many research groups screen plant extracts to detect secondary metabolites with relevant biological activities. In this regard, several simple bioassays were developed for screening purposes (Hostettmann 1991), some of them were used in this screening. Thus, *Artemia salina* larvae have been used as a target organism to detect bioactive compounds in plant extracts and toxicity to this crustacean has a good correlation with anti-tumor (McLaughlin 1991) and anti-*Trypanosoma cruzi* (Zani et al. 1995) activities. *T. cruzi* is a protozoan that causes Chagas disease (American trypanosomiasis), an illness that affects approximately 16 million people in tropical and sub-tropical Americas (WHO 1993). The drugs currently in use against this disease, nifurtimox and benznidazole, present several side effects and have limited efficacy, especially in the chronic phase of the disease.

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The aim of this study was to screen for medicinal plant extracts that could be useful for the development of new tools for the control of infectious diseases. While pursuing this goal, we initiated a systematic evaluation of extracts from the "cerrado" plant species in bioassays such as (a) the brine shrimp (*Artemia salina* Leach) lethality assay (BSLA), (b) the assay with the snails *B. glabrata*, (c) the bioautographic antifungal assay with spores of *Cladosporium sphaerospermum* and, (d) the agar diffusion assay using the bacteria *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

MATERIALS AND METHODS

Plant collection - The plants were collected in the vicinities of Belo Horizonte, Minas Gerais, Brazil. They were identified by M Brandão and TMS Grandi. Exsiccates of each species were deposited in the herbaria (Table I) of the following institutions: Fundação Zoobotânica de Belo Horizonte, Empresa de Pesquisas Agropecuária de Minas Gerais and Universidade Federal de Viçosa.

Extraction - The plant parts were dried at 35°C in a convection oven and powdered in a knife mill. The powder was macerated at room temperature for 24 h in CH₂Cl₂ followed by MeOH or in a mixture of CH₂Cl₂-MeOH (1:1, v/v), as indicated in Table II. After filtration, the solvents were removed by rotary evaporation under reduced pressure and at temperatures below 45°C. The yield of the crude extracts are indicated in Table II. In some cases, part of the powder was separated, extracted with H₂O, centrifuged and the supernatant freeze-dried. The extracts were kept at -20°C and the residual solvent of an aliquot removed overnight under high vacuum prior to the bioassays.

Molluscicidal activity - The assay with snails *B. glabrata* was run as described by Alves et al. (1996). Briefly, the crude extracts were dissolved in 500 ml of dechlorinated tap water at an initial concentration of 100 ppm. Ten snails were put into 250 ml of this solution and maintained submerged by a nylon screen during 24 h. After this period, the surviving snails were removed to a flask containing 250 ml of dechlorinated tap water, fed with lettuce, and observed for three days. If all mollusks were killed dilutions were prepared to determine the minimum lethal concentration (LC₁₀₀/24 h). Controls with and without niclosamide (1 ppm) were run in parallel.

Toxicity to *Artemia salina* - The protocol established by McLaughlin (1991) was employed. Briefly, each extract (2 mg) was dissolved in 2 ml of solvent. From these solutions, 500, 50 and 5 µl were transferred to vials in triplicate. The solvent was removed under high vacuum and seawater (5

ml) was added to each vial, resulting in final concentrations of 100, 10 and 1 µg/ml, respectively. Second instar larvae of *A. salina* (ten per vial) were added. After 24 h contact, the survivors were counted and the LC₅₀ calculated using the probit method. Extracts with LC₅₀ ≤ 100 ppm were considered active. Controls with and without thymol (10 ppm) were run simultaneously.

Antifungal activity - The bioautographic assay with *C. sphaerospermum*, developed by Homans and Fuchs (1970), was adopted. Briefly, the extracts (1 mg) were dissolved in a volatile solvent (100 µl) and an aliquot (10 µl) of each solution spotted on TLC plates. After complete solvent removal, a spore suspension of the fungus in nutrient medium was sprayed and the TLC plates incubated in a humid atmosphere for three days at room temperature. The appearance of a growth inhibition zone with the size of the spot (active) or larger than the spot (very active) indicated the presence of fungitoxic substances in the extract. Thymol (50 mg/spot) and solvent (10 µl) were used as controls.

Antimicrobial assay - The agar diffusion assay described by Smânia et al. (1995) was adopted. Briefly, *B. cereus*, *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853), and *S. aureus* (ATCC 25923) were grown in Mueller-Hinton agar and broth (Difco Laboratories). The strains were incubated at 36°C for 18 h, and were diluted to a final concentration of approximately 10⁶ CFU/ml. Each bacterial suspension was spread over the surface of Mueller-Hinton agar containing five wells of 7 mm diameter. The wells were filled with 5 mg of extract dissolved in the medium. The plates were incubated at 36°C for 20 h. Penicillin (30 mg) was used as a positive control. The results were expressed in terms of the diameter of the inhibition zone: < 9 mm, inactive; 9-12 mm, partially active; 13-18 mm, active; > 18 mm, very active.

RESULTS AND DISCUSSION

The sixty plant species evaluated in this screening are listed in Table I. They are distributed among 53 genera and 36 families, with Leguminosae being the most represented within the collection. Table II summarizes the results of the bioassays, listing only the species that presented some activity in at least one bioassay.

Eight species (13%) displayed LC₁₀₀/72h ≤ 100 ppm in the assay with *B. glabrata*. Species from genus *Byrsonima* were especially active and the methanol extract from the leaves of *B. intermedia* Juss, was the most active among all extracts tested, presenting LC₁₀₀ of 20 ppm. According to the World Health Organization guidelines (WHO 1993), this extract can be regarded as a promising

TABLE I
Plant species collected

Entry	Herbarium code	Plant species	Family
1	PAMG ^a 45206	<i>Acosmium dasycarpum</i> (Vogel) Yarkovl	Leg-Caesalpinoideae
2	PAMG 45215	<i>Aeschynomene paniculata</i> Willd.	Leg-Faboideae
3	PAMG 45216	<i>Andira humilis</i> C. Martius	Leg-Faboideae
4	PAMG 45218	<i>Annona crassiflora</i> Mart	Annonaceae
5	PAMG 45235	<i>Austroplenckia populnea</i> Reiss	Celastraceae
6	PAMG 45	<i>Baccharis dracunculifolia</i> DC.	Asteraceae
7	PAMG 45207	<i>Bauhinia curvula</i> Benth.	Leg-Caesalpinoideae
8	PAMG 45217	<i>Bixa orellana</i> L.	Bixaceae
9	PAMG 45219	<i>Bowdichia virgilioides</i> Kunth	Leg-Faboideae
10	VIC ^b 20633	<i>Brosimum gaudichaudii</i> Trecul	Moraceae
11	PAMG 45220	<i>Byrsonima coccolobifolia</i> Kunth	Malpighiaceae
12	PAMG 45223	<i>Byrsonima intermedia</i> A. Juss.	Malpighiaceae
13	PAMG 45245	<i>Byrsonima verbascifolia</i> (L.) Richard	Malpighiaceae
14	PAMG 45208	<i>Cabralea polytricha</i> Jussieu	Meliaceae
15	PAMG 45221	<i>Caryocar brasiliense</i> Cambess.	Caryocaraceae
16	PAMG 45222	<i>Casearia sylvestris</i> Sw	Flacourtiaceae
17	PAMG 45225	<i>Copaifera langsdorffii</i> Desf.	Leg-Caesalpinoideae
18	PAMG 45209	<i>Dalbergia nigra</i>	Leg-Faboideae
19	PAMG 45224	<i>Davilla rugosa</i> Poir	Dilleniaceae
20	PAMG 45226	<i>Didymopanax macrocarpum</i> Seem	Araliaceae
21	PAMG 45227	<i>Dilodendron bipinnatum</i> Radlk	Sapindaceae
22	PAMG 45210	<i>Emmotum nitens</i> Miers	Icacinaceae
23	PAMG 45228	<i>Erythrina mulungu</i> C. Martius	Leg-Faboideae
24	PAMG 45244	<i>Eugenia dysenterica</i> DC	Myrtaceae
25	VIC 20634	<i>Eugenia pitanga</i> (O. Berg) Kiaersk.	Myrtaceae
26	PAMG 45211	<i>Guazuma ulmifolia</i> Lam.	Sterculiaceae
27	BHZB ^c (168)	<i>Hymenaea courbaril</i> L.	Fabaceae
28	PAMG 45229	<i>Hymenaea stigonocarpa</i> C. Martius	Leg-Caesalpinoideae
29	PAMG 45230	<i>Kielmeyera coriacea</i> Mart	Clusiaceae
30	PAMG 45233	<i>Lafoensia pacari</i> St. Hilaire	Lythraceae
31	BHZB (022)	<i>Lantana camara</i> L.	Verbenaceae
32	BHZB (1467)	<i>Leonotis nepetaefolia</i> (L.) R. BR.	Lamiaceae
33	BHZB (46)	<i>Leonurus sibiricus</i> L.	Lamiaceae
34	PAMG 45212	<i>Machaerium opacum</i> Vogel	Leg-Faboideae
35	PAMG 45231	<i>Miconia albicans</i> (SW.) Triana	Melastomataceae
36	BHZB (1573)	<i>Momordica charantia</i> L.	Cucurbitaceae
37	BHZB (2106)	<i>Ocimum gratissimum</i> L.	Lamiaceae
38	PAMG 45234	<i>Ouratea castaneaefolia</i> (DC.) Engl.	Ochnaceae
39	PAMG 45213	<i>Plathymenia foliolosa</i> Benth.	Leg-Mimosoideae
40	BHZB 2107	<i>Pothomorphe umbellata</i> (L.) Miq.	Piperaceae
41	PAMG 45238	<i>Pouteria torta</i> (Mart.) Radlk	Sapotaceae
42	PAMG 45240	<i>Qualea grandiflora</i> Mart.	Vochysiaceae
43	PAMG 45243	<i>Qualea parviflora</i> Mart.	Vochysiaceae
44	PAMG 45214	<i>Roupala heterophylla</i> Pohl	Proteaceae
45	PAMG 45232	<i>Roupala montana</i> Aubl.	Proteaceae
46	PAMG 45236	<i>Rudgea viburnoides</i> (Cham.) Benth	Rubiaceae
47	PAMG 45237	<i>Sabicea brasiliensis</i> Wernham	Rubiaceae
48	PAMG 45246	<i>Sclerolobium aureum</i> (Tul.) Baill.	Fabaceae
49	VIC 20635	<i>Senna occidentalis</i> (L.) Link	Fabaceae
50	PAMG 45247	<i>Solanum lycocarpum</i> St. Hil.	Solanaceae
51	PAMG 45250	<i>Strychnos pseudoquina</i> A. St. Hil.	Loganiaceae
52	PAMG 45251	<i>Stryphnodendron adstringens</i> (Mart.) Cov.	Leg-Mimosoideae
53	PAMG 45252	<i>Styrax camporum</i> Pohl	Styracaceae
54	PAMG 45239	<i>Tabebuia caraiba</i> (Mart.) Bureau	Bignoniaceae
55	BHZB (537)	<i>Tabebuia ochracea</i> (Cham.) Standl.	Bignoniaceae
56	BHZB (1758)	<i>Tamarindus indica</i> L.	Fabaceae
57	PAMG 45241	<i>Vochysia thyrsoidea</i> Pohl	Vochysiaceae
58	PAMG 45242	<i>Xylopia aromatica</i> (Lam) Mart.	Annonaceae
59	PAMG 45248	<i>Zanthoxylum rhoifolium</i> Lam.	Rutaceae
60	PAMG 45249	<i>Zeyhera digitallis</i> (Vell.) Sm. & Sandw.	Bignoniaceae

Plant species were identified by M Brandão or TSM Grandi. *a*: EPAMIG Herbarium; *b*: Federal University of Viçosa Herbarium; *c*: Federal University of Minas Gerais Herbarium.

TABLE II

Activity of plant extracts against *Biomphalaria glabrata*, *Artemia salina*, *Cladosporium sphaerospermum*, *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus* and *Pseudomonas aeruginosa*

Entry plant species	Part ^a (weight, g)	Solvent ^b (yield, % w/w)	Bioassays						
			Bacteria ^f						
			Bgl ^c	Asa ^d	Csp ^e	Sau ^g	Eco ^h	Bce ⁱ	Pae ^j
Controls	niclosamide	W	1	-	-	-	-	-	-
	thymol	D	-	10	2	-	-	-	-
	penicillin	medium	-	-	-	3	3	3	3
1	<i>A. humilis</i>	Leaves (76)	W (3.1)	-	-	-	2	-	-
2	<i>A. crassiflora</i>	Leaves (47)	D/M (11)	-	-	-	2	-	2
		(31)	W (2.7)	-	-	-	2	-	2
3	<i>A. populnea</i>	Leaves (80)	M (13)	-	-	-	2	1	2
4	<i>B. dracunculifolia</i>	Stem (60)	D/M (7.5)	-	-	-	3	-	-
5	<i>B. curvula</i>	Leaves (90)	D (12.2)	-	-	1	3	-	3
		(90)	M (6.3)	-	-	1	3	-	3
6	<i>B. virgilioides</i>	Leaves (58)	D/M (10.6)	-	-	-	2	-	2
		Bark (40)	W (3.5)	-	-	-	3	-	3
7	<i>B. gaudichaudii</i>	Roots (90)	D (5.7)	-	-	2	1	-	-
8	<i>B. coccolobifolia</i>	Leaves (91)	D/M (9.8)	100	-	-	2	-	2
9	<i>B. intermedia</i>	Leaves (54)	M (10.3)	20	-	-	ND	ND	ND
		(17)	W (5.2)	-	-	-	3	-	2
10	<i>B. verbascifolia</i>	Leaves (75)	D/M (9.9)	40	-	-	3	-	3
		Bark (50)	M (6.1)	60	-	-	3	-	3
		(35)	W (2.5)	60	-	-	3	-	3
11	<i>C. polytricha</i>	Fruits (50)	D (12.3)	-	36	-	-	-	-
		(50)	M (5.6)	-	53	-	1	-	-
12	<i>C. brasiliense</i>	Leaves (87)	D/M (10.8)	-	90	-	3	-	2
13	<i>C. sylvestris</i>	Leaves (63)	D/M (9.1)	-	-	-	-	-	2
14	<i>C. langsdorffii</i>	Leaves (54)	D/M (11.3)	-	83	-	3	-	2
15	<i>C. antisiphilitius</i>	Whole (40)	M (9.9)	-	-	-	2	-	2
16	<i>D. nigra</i>	Leaves (70)	M (12.4)	-	-	-	3	2	2
17	<i>D. rugosa</i>	Leaves (54)	D/M (11.8)	100	-	-	3	-	2
18	<i>D. bipinnatum</i>	Fruits (55)	D (13.8)	-	-	1	2	-	1
		Leaves (90)	D (8.6)	-	-	-	3	-	3
19	<i>E. nitens</i>	Leaves (210)	M (11.4)	-	-	1	3	-	1
		Stem (90)	M (7.7)	100	-	-	3	1	2
20	<i>E. disenterica</i>	Leaves (75)	D/M (11.9)	100	-	-	3	-	3
21	<i>E. pitanga</i>	Leaves (50)	D/M (10.2)	-	-	1	3	1	3
		(170)	W (4.5)	-	-	-	2	-	2
22	<i>G. ulmifolia</i>	Bark (325)	M (3.9)	-	-	-	3	-	2
23	<i>H. courbaril</i>	Bark (45)	W (4.2)	-	-	-	3	-	2
24	<i>H. stigonocarpa</i>	Bark (50)	D/M (5.3)	40	-	-	3	-	3
		(145)	M (5.0)	40	-	-	3	-	3
		Leaves (120)	D/M (14.1)	100	-	-	2	-	2
25	<i>L. pacari</i>	Leaves (215)	M (9.4)	-	-	1	3	-	3
26	<i>L. camara</i>	Roots (60)	D (5.3)	-	47	2	3	-	3

cont.

Entry plant species	Part ^a (weight, g)	Solvent ^b (yield, % w/w)	Bioassays						
			Bacteria ^f						
			Bgl ^c	Asa ^d	Csp ^e	Sau ^g	Eco ^h	Bce ⁱ	Pae ^j
27 <i>L. sibiricus</i>	Aerial (90)	D/M (9.9)	-	12	-	-	-	-	-
28 <i>M. opacum</i>	Leaves (50)	D (12.2)	-	-	-	2	-	3	-
29 <i>M. albicans</i>	Leaves (80)	M (10.6)	-	-	-	3	2	2	3
31 <i>P. foliolosa</i>	Leaves (60) (20)	M (11.1)	100	-	-	3	1	2	3
		W (5.5)	-	-	-	3	2	3	3
32 <i>P. torta</i>	Leaves (130)	M (8.9)	ND	-	1	3	1	3	2
33 <i>Q. grandiflora</i>	Bark (60)	M (6.5)	ND	ND	ND	3	-	3	2
34 <i>Q. parviflora</i>	Bark (90) (30)	M (4.2)	-	-	-	3	-	2	2
		W (6.3)	100	-	-	2	-	1	-
35 <i>R. heterophylla</i>	Leaves (80)	M (8.9)	-	-	-	3	-	2	2
36 <i>R. montana</i>	Leaves (20)	W (12.8)	-	-	-	3	2	3	2
37 <i>S. brasiliensis</i>	Aerial (55)	M (10.1)	-	-	-	3	-	2	-
38 <i>S. aureum</i>	Leaves (60)	M (7.3)	-	-	-	3	-	2	1
39 <i>S. adstringens</i>	Bark (160) (20)	M (4.9)	-	-	-	3	1	2	2
		W (5.2)	-	-	-	3	2	3	1
40 <i>S. camporum</i>	Leaves (83)	D/M (11.2)	100	-	-	3	1	3	1
41 <i>T. ochracea</i>	Wood (290) Leaves (26)	D (4.5)	-	-	-	2	-	-	1
		M (8.4)	-	-	-	2	-	-	1
42 <i>X. aromatica</i>	Bark (100) (138)	D/M (5.7)	-	18	-	2	-	2	-
		W (3.4)	-	-	-	3	1	2	2

a: part (weight) extracted; b: D = CH₂Cl₂, M = CH₃OH, D/M = CH₂Cl₂-CH₃OH (1:1); c: *B. glabrata* (LC₁₀₀ in ppm after 24 h exposure); d: *A. salina* (LC₅₀ in ppm after 24 h exposure); e: *C. sphaerospermum* (bioautographic, 100 µg/spot: - : inactive, 1: partially active, 2: active, ND: not determined); f: agar diffusion method with 5 mg/well: - : inactive, 1: partially active, 2: active, 3: very active, ND: not determined; g: *S. aureus*; h: *E. coli*; i: *B. cereus*; j: *P. aeruginosa*; W: water.

candidate for a plant-derived molluscicide. The bark of *B. intermedia* is rich in tannins and its infusion is popularly used as febrifuge (Corrêa 1984). Several classes of compounds have been isolated from this genus, including triterpenes, flavonoids, benzenoids and steroids (Schultes & Raffaaf 1990). Extracts from leaves of *B. verbascifolia* and bark of *Hymenaeae stignocarpa* Mart showed LC₁₀₀ of 40 ppm. The bark of the latter species is reputed as a vermifuge (Caribé & Campos 1991).

From the 60 species evaluated in the BSLA, six (10 %) produced extracts that displayed LC₅₀ ≤ 100 ppm. The CH₂Cl₂-MeOH extracts from the aerial parts of *Leonurus sibiricus* L and from the bark of *Xylopi aromatica* (Lam) Mart were the most active, with LC₅₀ of 12 and 18 ppm, respectively. *L. sibiricus*, is popularly used in Minas Gerais for cold, diarrhea and digestive complaints (Grandi et al. 1989). Investigations of its chemistry and pharmacological properties by Chinese groups resulted in the isolation of bioactive alka-

loids (Luo et al. 1985). Cytotoxic acetogenins, venezinin and asimicin, were isolated from the bark of *X. aromatica* using a BSLA-guided fractionation protocol (Colman-Saizaritoria et al. 1994). Some species from this genus are rich in kaurenoic acid (Alves et al. 1995), known for its activity in the BSLA and against trypanosomes of *T. cruzi* (Zani et al. 1995). Although less potent, extracts from fruits of *Cabralea polythrica*, leaves of *Caryocar brasiliensis* and *Copaifera langsdorffii* and roots of *Lantana camara* were also active in the BSLA. All these plants are used to treat numerous ailments in Brazil (Ferreira 1980, Martins et al. 1994) and were already subjected to phytochemical studies by other groups. The observed activity of *L. camara* in the BSLA could be due to the presence of camaraside and lantanoside, cytotoxic components already isolated from this species (Mahato et al. 1994).

When tested against the spores of phytopathogenic fungus *C. sphaerospermum*, extracts from

eight species (13%) were able to inhibit fungal development. The most active were the CH₂Cl₂ extracts from the roots of *Brosimum gaudichaudii* and *L. camara*. The first is widely used in Brazil for treating skin depigmentation (vitiligo) (Corrêa 1984), an activity attributed to the presence of bergapten and psolaren. These compounds could also account for the observed fungicidal activity of this plant. Previous works showed that the essential oil of *L. camara* is active against fungi (Mahato et al. 1994) and bacteria (*P. aeruginosa* and *S. aureus*) (Ross et al. 1980).

In the agar diffusion assay using four different bacterial strains, more than 60% of plant species afforded extracts with some degree of activity, in particularly against the Gram-positive bacteria *S. aureus* or *B. cereus*. On the other hand, only four plant species (*Dalbergia nigra*, *Lafoensia pacari*, *Miconia albicans*, and *Plathymenia foliolosa*) afforded extracts that were highly active against the more resistant gram negative bacteria *P. aeruginosa*. Moreover, the extracts from *Austroplenckia populnea*, *D. nigra*, *Eugenia pitanga*, *Emmotum nitens*, *Miconia albicans*, *P. foliolosa*, *Pouteria torta*, *Roupala montana*, *Stryphnodendron adstringens* and *X. aromatica*, presented some degree of activity against all bacterial strains, including *E. coli*. However, only extracts from *D. nigra*, *M. albicans*, *P. foliolosa*, *R. montana* and *S. adstringens* caused significant inhibition (13-18 mm inhibition zone) of *E. coli* growth at the dose used (5 mg/well).

Overall, extracts from 70% of the species collected were active in at least one of the bioassays adopted in this screening. If we consider only the most active extracts (bold face in Table II: bgl ≤ 40 ppm, asa ≤ 50 ppm, csp = 2, pae = 3 and eco = 2) and exclude the highly sensitive gram positive bacteria, this proportion is reduced to 23%. This can be considered a very good hit rate, reinforcing the importance of the ethnomedical information in the search of bioactive extracts. Furthermore, many of the highly active extracts presented some specificity against one of the organisms tested and not a generic toxicity. We observed that extraction with CH₂Cl₂ generated two thirds of the fungicidal extracts while none of the CH₂Cl₂ extracts was able to kill snails. Although 10% of the aqueous extracts were active against *B. glabrata*, none of them was fungicidal or toxic to *A. salina*. Finally, the use of methanol provided the majority of the highly active extracts in the bioassay with bacteria. These observations can be rationalized in terms of the polarity of the compounds being extracted by each solvent and, in addition to their intrinsic bioactivity, by their ability to dissolve or diffuse in the different media used in the assays. The more active

extracts were prioritized for the identification of the active components, a work that is already under way.

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REFERENCES

- Alves TMA, Chaves PPG, Nagem TJ, Murta SMF, Ceravolo IP, Romanha AJ, Zani CL 1995. A diterpene from *Mikania obtusata* active on *Trypanosoma cruzi*. *Planta Med* 61: 85-86.
- Alves TMA, Ribeiro A, Mendes NM, Nagem TJ, Hamburguer M, Hostettmann K, Zani CL 1996. Molluscicidal saponins from *Guaiaecum officinale* L. (Zygophyllaceae). *Int J Pharmacog* 34: 81-86.
- Brandão M 1991. Plantas medicamentosas do cerrado mineiro. *Inf Agropec* 15: 15-20.
- Burman A 1991. Saving Brazil's savannas. *New Scientist* 1758: 30-34.
- Caribé J, Campos JM 1991. *Plantas que Ajudam o Homem: Guia Prático para a Época Atual*, Pensamento, São Paulo, p. 6
- Colman-Saizarbitoria T, Zambrano J, Ferrigni NR, Gu ZM, Smith DL, McLaughlin JL 1994. Bioactive annonaceous acetogenins from the bark of *Xylopia aromatica*. *J Nat Prod* 57: 486-493.
- Corrêa MP 1984. *Dicionário das Plantas Úteis do Brasil e das Exóticas Cultivadas*, Ministério da Agricultura, IBDF, Rio de Janeiro, Vols I-VI.
- Davis A 1996. Schistosomiasis. In GC Cook, *Manson's Tropical Diseases*, 20th ed., WB Saunders Company Ltd, London, p. 1413-1456.
- Di Stasi LC 1989. *Plantas Medicinais da Amazônia*, Unesp, São Paulo, p. 39-40.
- Ferreira MB 1980. Plantas portadoras de substâncias medicamentosas, de uso popular, nos cerrados de Minas Gerais. *Inf Agropec* 6: 19-23.
- Ferri, MG 1973. A vegetação de cerrados brasileiros. In E Warming, *Lagoa Santa*, Universidade de São Paulo, São Paulo, p. 287-288.
- Grandi TSM, Trindade JA, Pinto MJF, Ferreira LL, Catella AC 1989. Plantas medicinais de Minas Gerais. *Acta Bot Bras* 3: 185-224.
- Hirschmann GS, Arias AR 1990. A survey of medicinal plants of Minas Gerais, Brazil. *J Ethnopharmacol* 29: 159-172.
- Homans AL, Fuchs A 1970. Directed bioautography on thin layer chromatograms as a method for detecting fungitoxic substances. *J Chromatogr* 51: 327-329.
- Hostettmann K 1991. *Assays for Bioactivity. Methods in Plant Biochemistry*, vol. 6, Academic Press, San Diego, 360 pp.
- Luo S, Mai L, Zhu Z 1985. Separation and determination of alkaloids of *Leonurus sibiricus*. *Chung Yao T'Ung Pao* 10: 32-35.
- Martins ER, Castro DM, Castellani DC 1994. *Plantas Medicinais*, Imprensa Universitária, UFV Viçosa, 220 pp.
- Mahato SB, Sahu NP, Roy SK, Sharma OP 1994. Po-

- tential antitumor agents from *Lantana camara*: structures of flavonois and phenylpropanois glycosides. *Tetrahedron* 50: 9439-9446.
- Matos FJA 1994. *Farmácias Vivas*, EUFC, Fortaleza, 179 pp.
- McLaughlin JM 1991. Crown gall tumours on potato discs and brine shrimp lethality: two simple bioassays for higher plant screening and fractionation. In K Hostettmann, *Assays for Bioactivity*, Academic Press, San Diego, p. 2-32.
- Mott K 1987. *Plant moluscicides*, UNDP/World Bank/WHO/TDR, John Wiley and Sons Ltd., 326 pp.
- Ross SA, El-Keltawi NE, Megalla SE 1980. Antimicrobial activity of some Egyptian aromatic plants. *Fitoterapia* 51: 201-205.
- Schultes RE, Raffauf RF 1990. *The Healing Forest. Medicinal and Toxic Plants of the Northwest Amazônia*, Dioscorides Press, Portland, Oregon, 484 pp.
- Silver LL, Bostian KA 1993. Discovery and development of new antibiotics: the problem of antibiotic resistance. *Antimicrob Agents Chemother* 37: 377-383
- Smânia A, Delle Monache F, Smânia EFA, Gil ML, Benchetrit LC, Cruz FS 1995. Antibacterial activity of substance produced by the fungus *Pycnoporus sanguineus* (Fr.) merr. *J Ethopharmacol* 45: 177-181.
- WHO-World Health Organization 1993. *Tropical Disease Research*, Geneva, 134 pp.
- Zani CL, Chaves PPG, Queiroz R, Mendes NM, Oliveira AB, Cardoso JE, Anjos AMG, Grandi TS 1995. Brine shrimp lethality assay as a prescreening system for anti-*Trypanosoma cruzi* activity. *Phytomedicine* 2: 47-50.