

## Screening of Some Plants Used in the Brazilian Folk Medicine for the Treatment of Infectious Diseases

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*Extracts of 13 Brazilian medicinal plants were screened for their antimicrobial activity against bacteria and yeasts. Of these, 10 plant extracts showed varied levels of antibacterial activity. Piper regnellii presented a good activity against Staphylococcus aureus and Bacillus subtilis, a moderate activity on Pseudomonas aeruginosa, and a weak activity against Escherichia coli. Punica granatum showed good activity on S. aureus and was inactive against the other standard strains. Eugenia uniflora presented moderate activity on both S. aureus and E. coli. Psidium guajava, Tanacetum vulgare, Arctium lappa, Mikania glomerata, Sambucus canadensis, Plantago major and Erythrina speciosa presented some degree of antibacterial activity. Spilanthes acmella, Lippia alba, and Achillea millefolium were considered inactive. Five of the plant extracts presented compounds with Rf values similar to the antibacterial compounds visible on bioautogram. Of these, three plants belong to the Asteraceae family. This may mean that the same compounds are responsible for the antibacterial activity in these plants. Anticandidal activity was detected in nine plant extracts (P. guajava, E. uniflora, P. granatum, A. lappa, T. vulgare, M. glomerata, L. alba, P. regnellii, and P. major). The results might explain the ethnobotanical use of the studied species for the treatment of various infectious diseases.*

Key words: medicinal plants - antibacterial activity - anticandidal activity - bioautography

The use of medicinal plants in the world, and especially in South America, contributes significantly to primary health care. Many plants are used in Brazil in the form of crude extracts, infusions or plasters to treat common infections without any scientific evidence of efficacy. Pharmacological studies done with essential oils from 15 species of aromatic plants obtained in Northeast of Brazil have shown activity coherent with the use of these plants in folk medicine. These studies have dealt with the effect of these oils on muscle contraction and with their antispasmodic, analgesic, anti-inflammatory, anticonvulsant and antibacterial activity (Leal-Cardoso & Fonteles 1999).

Recently, Nakamura et al. (1999) reported the antibacterial activity of both essential oil and purified active compound of *Ocimum gratissimum*, traditionally used in Brazilian folk medicine to treat different diseases, e. g. upper respiratory tract infections, diarrhoea, skin diseases, pneumonia, and also as a treatment for cough, fever and conjunctivitis. The compound that showed antibacterial activity for gram-positive and gram-negative bacteria in the essential oil was identified as eugenol by spectral data.

More recently, Alves et al. (2000) had screened 60 medicinal plant species from the Brazilian savanna for molluscicidal activity, toxicity to brine shrimp, antifungal activity and antibacterial activity. In this study, 42 species afforded extracts that showed some degree of activity in one or more of these bioassays.

Plants which have been used as medicines over hundreds of years, constitute an obvious choice for study. It is interesting to determine whether their traditional uses are supported by actual pharmacological effects or merely based on folklore. In the present study, we chose 13 plants currently used in the folk medicine in our region. All of them have been used in the treatment of infectious disease, so that the in vitro antimicrobial activity was investigated.

### MATERIALS AND METHODS

*Plant collection* - The plants were collected in March 2001, in Maringá, PR, Brazil. The plants were identified by the same researchers that accomplished the collection, deposited and authenticated at Herbarium of State University of Maringá, Maringá, PR, Brazil (Table I).

*Preparation of extracts* - The plant parts selected were ground, submitted to a maceration process with ethanol-water (90-10%) for 48 h at 25°C and protected from sunlight. The hydroalcoholic extract obtained was evaporated under vacuum, lyophilised and the residue directly assayed against the microorganisms presented below.

*Microorganisms used and growth conditions* - The test organisms included the bacteria *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 15442, *Bacillus subtilis* ATCC 6623, and *Staphylococcus aureus* ATCC 25923. The yeasts *Candida albicans*, *C. krusei*, *C. parapsilosis*, and *C. tropicalis* were obtained from local

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clinical microbiology laboratories. 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 minimum inhibitory concentrations (MICs) of all extracts and reference antibiotics (tetracycline, vancomycin, penicillin, and nistatin; Sigma Chemical Co., St. Louis, MO) were determined by microdilution techniques in Mueller-Hinton broth (Merck) for bacteria and RPMI-1640 (Sigma Chemical Co.) for fungi (NCCSL 1999, 2000). Each extract (2 mg/ml) was aseptically mixed with inoculum prepared in the same media at a density adjusted to the tube 0.5 of McFarland' scale (10<sup>6</sup> yeasts or 10<sup>8</sup> bacterial cells), and

diluted 1:10 for the broth microdilution procedure. Microtiter trays 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 produced an 80% reduction in visible growth compared with control. MBCs were defined as the lowest concentration yielding negative subcultures or only one colony.

*Thin layer chromatography* - Kieselgel GF<sub>254</sub> plates, 20 x 20 cm, 1 mm thick, were used. Plant extracts were applied and the chromatogram developed using CHCl<sub>3</sub>/MeOH/H<sub>2</sub>O (65:35:5) as solvent. TLC plates were run in duplicate and one set was used as the reference chromatogram. Spots and bands were visualized by UV irra-

TABLE I  
Traditional use of species selected for antimicrobial investigation

Species (family) (Herbarium number according to collector)	Local name	Part tested	Popular use
<i>Arctium lappa</i> Willd (Asteraceae) (8426)	Bardana, Bardana-maior, Orelha-de-gigante	Leaf	Leaves and stem are used to treat abscess, skin injuries, insect bites, mycosis, and genital affections (1)
<i>Tanacetum vulgare</i> L. (Asteraceae) (8425)	Tanaceto, Erva dos vermes	Leaf	Leaves, flowers, and seeds are recommended as anti-inflammatory and helminth infections (2)
<i>Erythrina speciosa</i> Andrews (Leguminosae Papilionidae) (8416)	Mulungu	Stem	The traditional usage indicates that <i>Erythrina</i> species could have analgesic, anti-inflammatory and antibacterial activity (3)
<i>Psidium guajava</i> L (Myrtaceae) (8423)	Goiabeira	Leaf	Leaf, root, and bark extracts are used for treatment of diarrhoea, leukorrhea, cholera, external ulcers, and skin diseases (4)
<i>Mikania glomerata</i> Spreng (Asteraceae) (8420)	Guaco	Leaf	Leaves infusion used as antiseptic, anti-inflammatory, and antibacterial (4, 5, 6)
<i>Spilanthes acmella</i> Mart. (Asteraceae) (8418)	Agrião-do-Brasil, Jambu	Leaf	A decoction or infusion of the leaves and flowers is recommended for stammering, toothache, stomatitis and throat complaints (4)
<i>Lippia alba</i> (Mill.) N.E.Br. (Verbenaceae) (8421)	Erva-cidreira, Erva-cidreira-brasileira, Alecrim-do-campo, Chá-da-febre	Leaf	Its leaves are employed as an fusion or decoction to treat gastrointestinal disorders, dysentery, colds and cough, as well as febrifuge (4)
<i>Achillea millefolium</i> L. Asteraceae (Asteraceae) (8424)	Mil-folhas, Erva-de-cortadura	Leaf	Flowers and leaves are used to treat wounds, ulcers, diarrhoea, skin injuries, and gastrointestinal disorders (3, 4)
<i>Piper regnellii</i> Miq. (Piperaceae) (8392)	Pariparoba, Capeva	Leaf	Leaf and root are used in the form of crude extracts, infusions or plasters to treat common infections (1)
<i>Eugenia uniflora</i> L (Myrtaceae) (8419)	Pitanga	Leaf	Leaves are used for treatment of throat complaints (4)
<i>Punica granatum</i> L (Punicaceae) (8417)	Romã	Fruit	Fruit is used against aphtha, diarrhoea, intestinal parasites (4, 6)
<i>Sambucus canadensis</i> L. (Caprifoliaceae) (8422)	Sabugueiro	Leaf	Leaf, flower, and fruit extracts of parts of these plants have been used for respiratory and pulmonary disorders (cold, coughs, etc.) (4, 6)
<i>Plantago major</i> L. (Plantaginaceae) (8427)	Tanchagem, Erva-de-orelha	Leaf	Leaves and seeds are used as antiseptic, anti-inflammatory, and antibacterial (4, 6, 8)

Reference for uses: 1: Alzugaray (1983); 2: Corrêa (1984); 3: Cruz (1979); 4: Silva & Sant'ana(1995); 5: Oliveira & Kisue (1989); 6: Biazzi (1996); 7: Neto (1987); 8: Zatta (1998)

diation (254 and 366 nm) and H<sub>2</sub>SO<sub>4</sub> spray reagent. The other set was used for bioautography.

**Bioautography** - Chromatogram developed as described above was placed in plate square with cover and a inoculum of *S. aureus* containing 10<sup>6</sup> CFU/ml in molten Mueller-Hinton agar was distributed over the plates. After solidification of the medium, the TLC plate was incubated overnight at 37°C. Subsequently bioautogram was sprayed with an aqueous solution of 2,3,5-triphenyltetrazolium chloride (TTC) and incubated at 37°C for 4 h. Inhibition zones indicated the presence of active compounds.

## RESULTS AND DISCUSSION

The ethnobotanical screening tests of hydroalcoholic extracts of 13 plants against both gram-positive and gram-negative bacteria and yeasts by using the microdilution technique are given in Table II. It was considered that if the extracts displayed an MIC less than 100 µg/ml, the antimicrobial activity was good; from 100 to 500 µg/ml the antimicrobial activity was moderate; from 500 to 1000 µg/ml the antimicrobial activity was weak; over 1000 µg/ml the extract was considered inactive. *Piper regnellii* presented a good activity against the gram-positive bacteria *S. aureus* and *B. subtilis* with MIC of 7.8 and 15.6 µg/ml, respectively, a moderated activity on the gram-negative *P. aeruginosa* with MIC of 250 µg/ml, and a weak activity against the gram-negative *E. coli* with MIC 1000 µg/ml. *Punica granatum* showed good activity on *S. aureus* with MIC of 62.5 µg/ml and was inactive against the other standard strains at concentrations ≥ 1000 µg/ml. On the other hand, *Psidium guajava* afforded extract with moderate activity against gram-positive bacteria and on the gram-negative bacteria *E. coli*, while the extracts of *Arctium lappa*, *Tanacetum vulgare* and *Mikania glomerata* presented some degree of activity against both gram-positive and gram-negative bacteria. *Eugenia uniflora* presented moderate activity on both *S. aureus* and *E. coli*. *Sambucus canadensis* shown moderate activity against *B. subtilis*, and *Plantago major* was weakly active on *S.*

*aureus*. *Spilanthes acmella*, *Lippia alba*, and *Achillea millefolium* were considered inactive. The minimal bactericidal concentrations were within two-twofold dilution of the MICs for these organisms. The most susceptible bacterium was *S. aureus*.

Different results were obtained for the 13 studied species for antifungal activity against the yeasts *C. albicans*, *C. krusei*, *C. parapsilosis*, and *C. tropicalis*. In the anti-yeast assay, *P. guajava* extract displayed good activity against all the yeasts tested at concentrations of 15.6 to 125 µg/ml. Both *E. uniflora* and *P. granatum* afforded extracts with good activity against some yeast, but were inactive on *C. albicans*. The extracts of *L. alba* and *P. major* showed moderate response against *C. krusei*, and a weak activity on *C. tropicalis*, but did not show any activity against *C. albicans* and *C. parapsilosis*. *M. glomerata* and *P. regnellii* yielded extracts with moderate activity against *C. krusei* and *C. tropicalis*, while *A. millefolium* extract was weakly active against *C. krusei*. *E. speciosa*, *S. acmella*, and *S. canadensis* were inactive for all the yeasts tested.

To obtain some information on the active components, plant extracts were analyzed by TLC on silica gel. TLC plates were run in duplicate and one set was used as the reference chromatogram, and the other set was assayed for bioautography (Figure). Panel A shows the chromatogram of plant extracts sprayed with Vanillin/Sulphuric acid. Panel B shows the appearance of same chromatogram after treatment with bacterial inoculum, indicating the location of bacterial inhibition zone. Two antibacterial compounds were detected in the origin. These compounds were present in the extracts from *E. uniflora* (Line 10) and *P. granatum* (Line 11). *P. guajava* presented antibacterial compound (s) with *Rf* of 0.68 (Line 4). All other antibacterial compounds presented similar *Rf* values (0.85-0.93), and were detected in the extracts from *A. lappa* (Line 1), *T. vulgare* (Line 2), *E. speciosa* (Line 3), *M. glomerata* (Line 5) and *P. regnellii* (Line 9). In the solvent system used for screening, it forms a streak which can extend over more

TABLE II  
Minimal inhibitory concentration (MIC) of 13 Brazilian medicinal plants

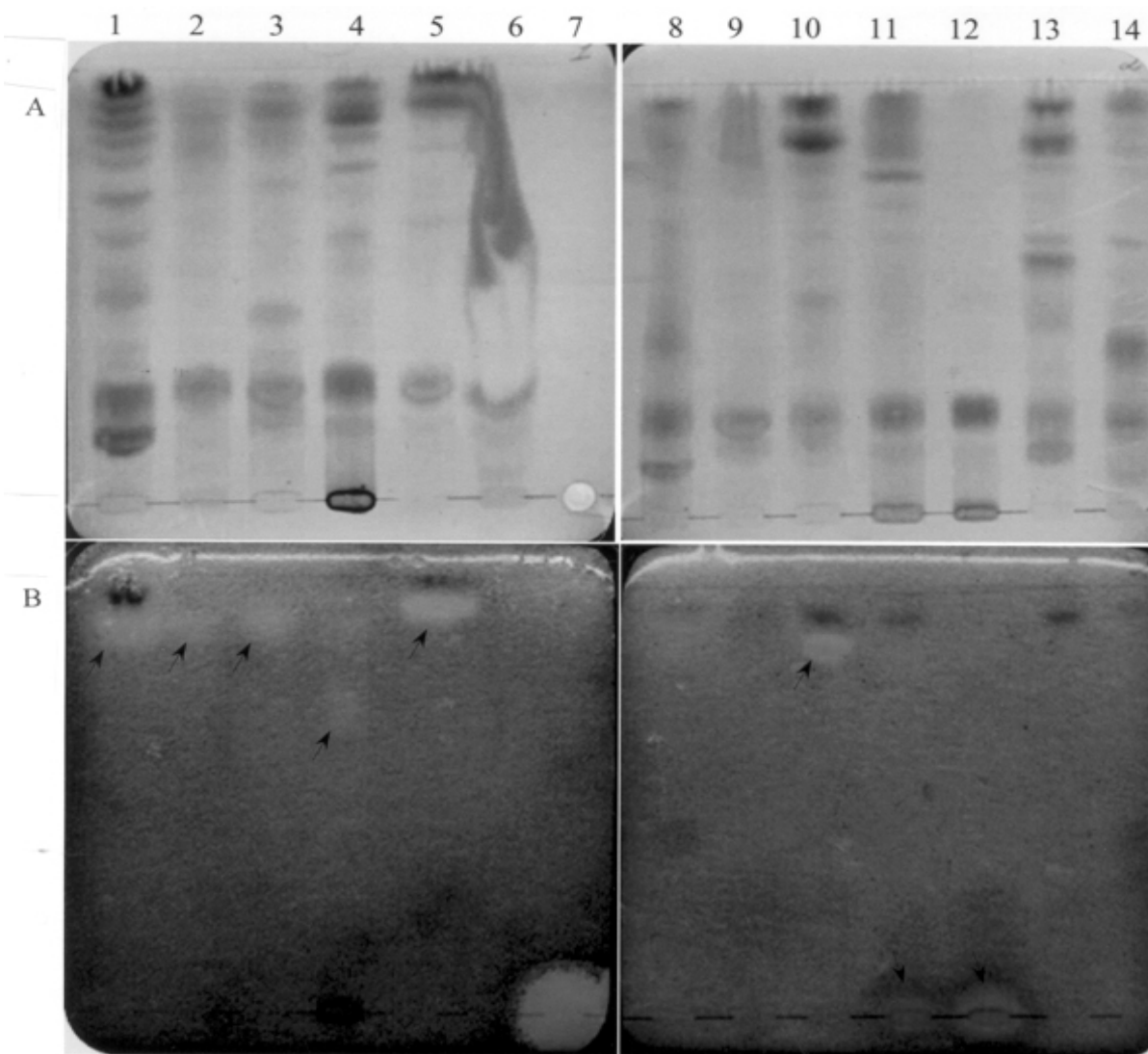
Plants	MIC (µg/ml)							
	Antibacterial activity				Antifungal activity			
	<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Candida albicans</i>	<i>C. krusei</i>	<i>C. parapsilosis</i>	<i>C. tropicalis</i>
<i>Arctium lappa</i>	500	500	500	500	> 1000	> 1000	> 1000	> 1000
<i>Tanacetum vulgare</i>	> 1000	500	500	500	> 1000	500	> 1000	500
<i>Erythrina speciosa</i>	500	250	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000
<i>Psidium guajava</i>	250	500	500	> 1000	125	<b>15.6</b>	<b>62.5</b>	<b>15.6</b>
<i>Mikania glomerata</i>	500	250	500	> 1000	> 1000	500	> 1000	500
<i>Spilanthes acmella</i>	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000
<i>Lippia alba</i>	> 1000	> 1000	> 1000	> 1000	> 1000	125	> 1000	1000
<i>Achillea millefolium</i>	> 1000	> 1000	> 1000	> 1000	> 1000	1000	> 1000	> 1000
<i>Piper regnellii</i>	<b>7.8</b>	<b>15.6</b>	1000	250	> 1000	125	> 1000	500
<i>Eugenia uniflora</i>	250	> 1000	500	> 1000	> 1000	<b>31.2</b>	125	<b>31.2</b>
<i>Punica granatum</i>	<b>62.5</b>	> 1000	> 1000	> 1000	> 1000	<b>15.6</b>	12.5	<b>15.6</b>
<i>Sambucus canadensis</i>	> 1000	250	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000
<i>Plantago major</i>	1000	> 1000	> 1000	> 1000	> 1000	125	> 1000	1000

than one  $R_f$  unit, which may overlap with other active compounds in the extracts. Also, due to clustering of bioautography zones detected in the origin, it was difficult to distinguish individual compounds. Use of additional, more polar solvent system to develop TLC plates in future work on individual plants will allow better separation on these zones. In the Mirtaceae family there is a large variety of active principles against microorganisms including essential oils, flavonoids, (Slowing et al. 1994, Lis-Balchin et al. 2000, Hernández et al. 2000) and tannins (Scalbert 1991, Djipa et al. 2000). The presence of flavonoids and tannins in Punicaceae family can be responsible by antimicrobial activity (Sahar et al. 1997). Since the antimicrobial activity in species of the Piperaceae family has been found with the amides, essential oil, lignans, phenylpropanoids, alkaloids, neolignans and chromene (Masuda et al. 1991, Benevides et al. 1999, Costantin et al. 2001, Dorman & Deans 2000), its is possible that these compounds could

be responsible by the antimicrobial properties reported here.

Under the conditions employed here, all test samples have more potent inhibitory effects on gram-positive bacteria in comparison to gram-negative bacteria. According to the TLC separation, five plant extracts yielded components with  $R_f$  values similar to the antibacterial compounds visible on bioautograms. In addition, many of these inhibition zones were associated with dark blue spots which had been detected under UV radiation (data not shown). Of these, three plants belong to the Asteraceae family. This may mean that the same compounds are responsible for the antibacterial activity in these plants.

In the present study hydroalcoholic extracts of some Brazilian medicinal plants were tested against bacteria and yeasts. Ten out of 13 plant extracts from eight families displayed some degree of antibacterial activity, in particular against the gram-positive bacteria *S. aureus* and *B.*



Thin layer chromatography plates of 13 Brazilian medicinal plants were run in duplicate and one set was visualized by Vanillin/Sulfuric acid spray reagent (A). The other set was used for bioautography with *Staphylococcus aureus* (B). 1: *Arctium lappa*; 2: *Tanacetum vulgare*; 3: *Erythrina speciosa*; 4: *Psidium guajava*; 5: *Mikania glomerata*; 6: *Spilanthes acmella*; The reference compound was vancomycin; 7: *Lippia alba*; 8: *Achillea millefolium*; 9: *Piper regnellii*; 10: *Eugenia uniflora*; 11: *Punica granatum*; 12: *Sambucus canadensis*; 13: *Plantago major*. Arrows indicate antibacterial activity.

*subtilis*, representing more than 77% of the screened extracts. This is to be expected because the outer membrane of gram-negative bacteria is known to present barrier to penetration of numerous antibiotic molecules, and the periplasmic space contains enzymes, which are able of breaking down foreign molecules introduced from outside (Duffy & Power 2001). It was interesting that the extracts of *P. regnellii* showed response on both gram-positive and gram-negative bacteria and yeasts. In spite of *P. guajava* exhibited good activity against all the yeasts and a moderate activity against gram-positive bacteria, the inhibitory activity against gram-negative bacteria displayed by the extract was low.

Although this study investigated the in vitro antimicrobial activity, the results substantiate the ethnobotanical use of the 13 studied species for the treatment of various bacteria-related diseases. However, in vivo data may be helpful in determining the potential usefulness of these plants for treatment of infectious diseases. In terms of conservation, the results show that leaf material is useful for antimicrobial uses, and it could be used without any detrimental effect on the plant.

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