

ANTIFUNGAL PROPERTIES OF PLANTS USED IN BRAZILIAN TRADITIONAL MEDICINE AGAINST CLINICALLY RELEVANT FUNGAL PATHOGENS

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ABSTRACT

Antifungal properties of extracts from eight Brazilian plants traditionally used in popular Brazilian medicine were tested against five clinically relevant *Candida* species, *Cryptococcus neoformans*, and *Sporothrix schenckii*. Results demonstrate that almost all extracts exhibited antifungal activity, at least against one of the microorganisms tested. The ethanolic extract from the leaves of *Schinus terebinthifolius* exhibited potential antifungal activity against *C. glabrata* and *S. schenckii*. Preliminary phytochemical analysis of extract from *S. terebinthifolius* showed the presence of biologically active compounds, namely saponins, flavonoids, triterpenes, steroids and tannins.

Key words: Antifungal properties; medicinal plants; susceptibility test

INTRODUCTION

In developing countries, microorganisms are frequently a cause of prevailing diseases, presenting a serious public health issue in a significant segment of the population as uncovered by either private or official health care systems. The economic crisis, high cost of industrialized medicines, inefficient public access to medical and pharmaceutical care, in addition to the side effects caused by synthetic drugs are some of the factors contributing to the central role of medicinal plants in health care. There is currently an increase in the numbers of immunocompromised individuals due to advances in medical technology and a pan-epidemic of HIV infections. With the rise in-at risk patients, the number of invasive fungal infections has dramatically increased in both developed and developing countries.

Yeasts of the genus *Candida* (in particular *C. albicans*) and of the species *Cryptococcus neoformans* are the fungal agents most frequently involved in the etiology of infectious processes in subjects affected by AIDS. Many studies investigating the

antifungal susceptibility of clinical strains of *Candida* spp. have been performed with a variety of results and these studies point to the emergence of new resistant strains (23). Disseminated cryptococcosis, on the other hand, affects a more limited percentage of patients (6-8%), yet is still a serious life-threatening condition (5).

Sporotrichosis is a chronic cutaneous and subcutaneous disease caused by the dimorphic fungus *Sporothrix schenckii*. New cases are continuously reported from some areas in Mexico, Costa Rica, Guatemala, Colombia, Brazil, Uruguay, South Africa, India and Japan. These regions are considered endemic regions for sporotrichosis despite the uncertainty of the real incidence rate of sporotrichosis. Standard therapy for this disease relies mainly on itraconazole, however, potassium iodide is the drug of choice in developing countries due to its lower cost (4).

In the present scenario, an emergence of multiple drug resistance in human pathogenic fungi and the small number of antifungal classes available stimulated research directed towards the discovery of novel antifungal agents from other sources,

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such as medicinal plants. Despite the great biodiversity of the Atlantic Rain Forest in Brazil, the potential of plant species as sources of new drugs remains unexplored. Less than 20% of all plant species from this environment have been studied for the presence of biologically active substances (8).

In recent years, the antimicrobial properties of medicinal plants have been increasingly reported in different parts of the world. It is expected that plant extracts demonstrating target sites other than those used by currently available antimicrobials will be active against drug resistant microbial pathogens (9).

There is very little information available on the activity of medicinal tropical plants. In the present study, we selected eight Brazilian plants to screen against five clinically relevant *Candida* species, *Cryptococcus neoformans* and *Sporothrix schenckii*. Selection of medicinal plants was based on their traditional use-in-medicine in Brazil (Table 1).

MATERIALS AND METHODS

Plant material

Eight plant samples were used in this study: leaves (312 g) of *Inga dulcis* (Vell.) Mart., leaves (400 g) and stem (250 g) of *Schinus terebinthifolius* Raddi, leaves, stem and flower (149 g) of *Althernanthera brasiliiana* Kuntze, *Piper regnellii* CDC (191 g), *Herissantia crispa* L. Briz (95 g), *Rubus urticaefolius* Poir

(141 g), *Rumex acetosa* L (45 g) and *Baccharis dracunculifolia* DC (483 g). Plants were collected from Santa Catarina State, Brazil in December of 2003 and January of 2004. *I. dulcis* was identified at the Department of Botany of the Federal University of Santa Catarina (UFSC), Florianópolis, SC, and voucher specimens have been deposited in the FLORA-UFSC. Other plant materials were acquired from EPAGRI (Empresa Agropecuária e Extensão Rural de Santa Catarina) germoplasm bank, Itajaí, SC, Brazil. Botanical names, voucher specimens, traditional usage and plant parts used to obtain extracts are listed in Table 1.

Preparation of extracts

Plant extracts were prepared from dried samples (40°C for seven days) and extracted with 80% ethanol (EtOH) over 10 days at room temperature. Following filtration, crude hydro alcoholic extracts were dried under a vacuum. The extracts were subjected to liquid-liquid partition using hexane, dichloromethane (DCM), ethyl acetate (AcOEt) and water. After solvent removal, all fractions were subjected to biological assays against the selected fungi.

Phytochemical analysis

Chemical constituents of the extracts were analyzed using the methodology described by Wagner (28). Thin layer

Table 1. Ethnobotanical information on selected Brazilian medicinal plants.

Botanical name	Voucher number	Vernacular name	Part used	Ethnomedical uses
<i>Inga dulcis</i> (Vell.) Mart Leguminosae	FLOR 33887	“Inga-macaco”	Rind of fruit, fruit	Wounds and in intestinal washes
<i>Schinus terebinthifolius</i> Raddi, Anacardiaceae	44	“Aroeira”	Decoct of stem peel	Cervicitis and chronic cervic vaginitis (1)
<i>Piper regnellii</i> Piperaceae	550	“Caapeba do Paraná”,	Leaves and roots “pariparoba”	Wounds, swellings, and skin irritations (21)
<i>Rubus urticaefolius</i> Rosaceae	31	“Amora-preta”	Leaves and fruit	Affections in the oral cavity and diarrhoeas (25)
<i>Althernanthera brasiliiana</i> Amaranthaceae	382	“Penicilina”	Leaves	Inflammation and diarrheas, cystitis, throat and general infection, antibiotic (2)
<i>Herissantia crispa</i> Malvaceae	316	“Malvaíscó”	Roots and flower	Affections in the respiratory tracts and cough (3)
<i>Rumex acetosa</i> Polygonaceae	514	“Azedeira”	All parts	Diarrhoeas
<i>Baccharis dracunculifolia</i> Asteraceae	138	“Carqueja-vassoura”, “Alecrim do campo”	Leaves, flowers and stem branches	Diseases of the liver, stomach, inflammations

chromatography (TLC) using aluminum-backed (Merck, silica gel 60 F254) was developed with one of three eluents that separate components of plants extracts. Eluents used were: ethyl acetate/methanol/water (40:5.4:5) (polar/neutral); ethyl acetate/formic acid/acetic glacial acid/water (10:1.1:1.1:0.5) (intermediate polarity/acidic); hexane/ethyl acetate (3:1) (non-polar/basic).

To detect the presence of flavonoids, plates were sprinkled with a 5% Aluminum Chloride (AlCl₃) ethanolic solution. The flavonoids appeared as a yellow or greenish fluorescent spot. For triterpene and steroid detection, TLC plates were revealed with a Lieberman-Buchard's reagent, resulting in brown or yellowish coloration. Coumarins and anthraquinones were detected using a 10% KOH solution. The presence of coumarins was observed by development of a blue coloration and anthraquinones by the development of a red color. Both colors were visualized under UV light at 365 nm.

For alkaloids, an orange and brown coloration were observed after sprinkling of Dragendorff's reagent. The presence of saponin was observed by the appearance of blue, violet and sometimes yellow spots using a 10% Vanillin ethanol solution. Polyphenols were observed by the presence of chestnut, violet, green and blue and tannins by bluish or greenish black after revelation with a 2% ferric chloride solution (28).

Fungi

For an antifungal evaluation, strains from the American Type Culture Collection (ATCC, Rockville, MD, USA) were used: *Candida albicans* ATCC 18804, *C. krusei* ATCC 20298, *C. tropicalis* ATCC 750, *C. parapsilosis* ATCC 22019, *C. glabrata* ATCC 2001, *Sporothrix schenckii* ATCC 20679 and *Cryptococcus neoformans* ATCC 32608. All fungal strains were maintained on Sabouraud Dextrose Agar (SDA, Oxoid, Basingstoke, UK) at 4°C and transfers were done at three-month intervals.

Culture media and inoculum

Sabouraud Dextrose Agar was used for the bioautographic test. Synthetic RPMI (Sigma, St. Louis, MO, USA) medium with L-glutamine buffered to pH 7.0 with 0.165 morpholine propanesulfonic acid (MOPS, Sigma) was prepared according to the CLSI M27-A2 document (19) and used for Minimal Inhibitory Concentration (MIC) determination. Fungal cultures, freshly grown at 35°C, and inoculum suspensions were prepared by the spectrophotometric method with a final inoculum of $1.5 \pm 1.0 \times 10^3$ cfu/mL used for susceptibility testing. For the bioautographic test, an appropriately diluted inoculum was obtained from Sabouraud Dextrose Broth and adjusted to a final concentration of 10^5 cfu/mL.

Antifungal assays

Bioautographic assay

Antifungal screening was performed using a modified version of the bioautographic assay described by Rahalison *et al.* (22).

Extracts were dissolved in dimethylsulfoxide (DMSO, Merck, Darmstadt, Germany) at a concentration of 100 µg/mL, and 20 µL of this solution was applied to TLC plates (plates of silica gel of 60F₂₅₄, Merck) with gradual micropipettes. Plates were then submerged twice for five minutes in fungal suspensions, and incubated in a hermetic bell-jar at 35°C for 48 h for *Candida* species and 72 h for *Cr. neoformans* and *S. schenckii*. Subsequently, plates were sprayed with *p*-Iodonitrotetrazolium violet (INT, Sigma) (5 mg/mL) and incubated for 4 hours at $36 \pm 1^\circ\text{C}$. Inhibition zones were observed and measured. DMSO was used as a toxicity control. Amphotericin-B (Sigma) was used as positive control. Culture medium plus microorganisms were included as reference standards. All tests were performed in duplicate.

Susceptibility testing

Broth microdilution testing was performed in accordance with the guidelines of the CLSI M27-A2 document (19). Susceptibility was determined by the microbroth dilution method performed in sterile flat-bottom 96-well microplates. Extracts and fractions were dissolved in DMSO after the addition of RPMI. Serial dilutions were then performed, using RPMI as a diluent, maintaining a constant volume of 1000 µL per tube. The extracts were tested at eight concentrations that varied from 1000 to 7.8 µg/mL. From each dilution, 100 µL volumes were distributed in microplates. As a control for growth and sterility, RPMI alone was used without extracts or solvents. Solvent was added to medium as a control for toxicity. Amphotericin B was included at concentrations of 25 to 0.03 µg/mL, as positive antifungal controls.

After inoculation of fungal strains, plates were incubated at 35°C for 48 hours for *Candida* species and 72 hours for *Cr. neoformans* and *S. schenckii*. All tests were performed in triplicate. The endpoints were determined visually by comparison with the drug-free growth control well. MICs were defined as the lowest extract concentration for which the well was optically clear, and were expressed in µg/mL.

RESULTS AND DISCUSSION

Antifungal activity against at least one of the microorganisms tested was found for each of the eight plant species tested. *S. terebinthifolius* (steam) and *B. dracunculifolia* EtOH extracts as well as *R. urticaefolius* and *P. regnellii* DCM extracts had a broad spectrum of activity, since all of them inhibited at least one of the fungal strains tested.

MIC measurements were carried out with extracts that were efficient against the tested microorganisms by the bioautography method. Greater activity was observed for the AcOEt and DCM extracts of the leaves of *S. terebinthifolius* against *C. krusei*, *C. glabrata* (AcOEt) and *S. schenckii* (DCM), all with MIC values of 30 µg/mL (Table 2). *S. schenckii*

Table 2. Minimal Inhibitory Concentrations ($\mu\text{g/mL}$) of selected Brazilian medicinal plants against opportunistic fungal pathogens.

Plant species	Extracts	C. k	C. t	C. g	C. a	C. p	C. n	S. s
<i>S. terebinthifolius</i> (leaves)	EtOH	250	250	125	-	-	-	15
	AcOEt	30	-	-	60	-	-	-
	DCM	60	125	30	-	-	-	30
<i>S. terebinthifolius</i> (stem)	EtOH	250	>1000*	125	>1000	>1000	>1000	30
	Hexane	-	-	-	-	-	125	15
	AcOEt	60	60	-	-	-	30	60
<i>R. urticaefolius</i>	EtOH	-	125	-	500	-	-	125
	AcOEt	125	-	-	-	125	-	125
<i>B. dracunculifolia</i>	Hexane	>1000	250	1000	>1000	-	30	-
<i>P. regnellii</i>	EtOH	-	60	125	>1000	500	>1000	-
	Hexane	>1000	1000	1000	>1000	1000	60	>1000
	DCM	>1000	>1000	1000	>1000	>1000	30	>1000
<i>H. crispa</i>	EtOH	>1000	>1000	500	-	125	125	-
<i>R. acetosa</i>	EtOH	-	60	-	1000	1000	>1000	1000
Amphotericin-B		0.5	0.25	1.0	1.0	0.5	1.2	2.0

C.k (*C. krusei*), C.t (*C. tropicalis*), C.g (*C. glabrata*), C.a (*C. albicans*), C.p (*C. parapsilosis*), C.n (*Cr. neoformans*), S.s (*S. schenckii*). AcOEt (ethyl acetate), DCM (dichloromethane), EtOH (ethanolic). -: Not tested, absence of halo production in the bioautography test

*>1,000. Considered without activity.

was highly sensitive to EtOH extract from *S. terebinthifolius*, having an MIC of 15 $\mu\text{g/mL}$. The results found in this study, when compared with previous works, show that leaf extract of *S. terebinthifolius* seems to be more potent than many of the plant extracts examined to date for *S. schenckii* (15). In contrast, extracts from the stem of *S. terebinthifolius* were more active against *Cr. neoformans*, considering that the crude and AcOEt extract exhibited a MIC of 30 $\mu\text{g/mL}$ for this yeast.

The antimicrobial activity of aqueous and EtOH extracts from the leaves of *S. terebinthifolius* has been reported in the literature to be active against *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *C. albicans*. However, the aqueous extract was not efficient against *P. aeruginosa* and *E. coli* and the EtOH and aqueous extract were more efficient against *C. albicans* (26). In our study, the aqueous extracts from the leaf or stem of *S. terebinthifolius* exhibited no activity at concentrations lower than 1000 $\mu\text{g/mL}$. Earlier studies showed that *S. terebinthifolius* is indicated for the treatment of stomatitis, bacterial vaginitis (1) and alveolitis or dry socket (16). The composition of the leaf extract of *S. terebinthifolius* obtained by bioprospecting using the methodology described by Wagner (28) revealed the presence of saponins, flavonoids and triterpenes or steroids and tannins.

Lloyd *et al.* (14) has previously reported the presence of sesqui- and triterpenes in *S. terebinthifolius*.

B. dracunculifolia (hexane extract) was more efficient against *Cr. neoformans*, inhibiting the growth of this fungi at a MIC of 30 $\mu\text{g/mL}$. The presence of alkaloids, saponins, flavonoids, anthraquinones and triterpenes or steroids was observed in hexane extract of *B. dracunculifolia*. Other authors have reported the presence of flavones, such as 8-OH flavone and 3,5,7-OH 6,4'-OME flavone (butuletol), in *B. dracunculifolia*. The presence of germacrene-D, bicyclogermacrene, and 4 fl-hydroxygermacra-1 (10), 5E-diene (13) as well as prenylated coumarinic acid derivatives have also been observed in the essential oil of this plant (12).

Hexane and DCM extracts of *P. regnellii* demonstrated high activity against *Cr. neoformans*, inhibiting the growth of this fungus at a MIC of 30 and 60 $\mu\text{g/mL}$. The EtOH extract of this plant also had activity against *C. tropicalis* (MIC 60 $\mu\text{g/mL}$).

Holetz *et al.* (10) demonstrated that extracts of *P. regnellii* had activity against several bacteria, especially *S. aureus* and *B. subtilis*, inhibiting the growth of these bacteria at concentrations of 7.8 $\mu\text{g/mL}$ and 15.6 $\mu\text{g/mL}$, respectively. These values are lower than the values found for the other 12 plants studied. However, *C. albicans* was resistant to the extracts of *P.*

regnellii, agreeing with results obtained in the present study. Some compounds with activity against Gram-positive bacteria have already been isolated from extracts of *P. regnellii*. These compounds include eupomatenoide-6 and eupomatenoide-5 compounds and conocarpan (21).

The chemistry of *Piper* species has been widely investigated and phytochemical investigations from all parts of the world have led to the isolation of a number of physiologically active compounds such as alkaloids/amides, propenylphenols, lignans, neolignans, terpenes, steroids, kawapyrone, piperolides, chalcones, di-hydrochalcones, flavones and flavanones (20).

The antimicrobial properties of different species of the genus *Piper* have also been studied. In a screening for medicinal plants with antimicrobial activity in Colombia, the methanolic extract of the leaf of *P. lanceaefolium* showed activity against *C. albicans*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Mycobacterium phlei*, *B. subtilis* and *S. aureus* (13). *Piper nigrum* (black pepper) is known to have antifungal activity due to lactones, terpenoids, alkaloids and saponins (7). 4,5-Dimethoxy-2,3-(methylenedioxy)-1-allylbenzene, a natural isolate of *P. hispidum* and *P. aduncum*, also has strong antimicrobial activity (18). This natural product and three other related compounds, 4-(5'-hydroxy-5'-nonanyl)-1,2 (methylenedioxy) benzene, 4-(5'-non-4'-enyl)-1,2-(methylenedioxy) benzene, and 6-methoxy-2,3-(methylenedioxy)-4-allylphenol, were synthesized from piperonal and screened for their biological activity. These four compounds showed high levels of antifungal and antibacterial activity against several fungi and bacteria (18).

Most extracts from *R. urticaefolius* had activity against *S. schenckii* with a MIC of 125 µg/mL. According to Richards & Liu (24), the antibacterial activity of the genus *Rubus* was already evidenced for species *Rubus pinfaensis*, as demonstrated by activity against *E. coli*, *B. subtilis*, *P. aeruginosa* and *S. aureus*. Silva & Siqueira (25) observed that extracts of *R. urticaefolius* were capable of inhibiting the growth of Gram-positive and Gram-negative bacteria but were not capable to inhibit the growth of *C. albicans* and *Cr. neoformans*. In the present study, the hexane extract of *R. urticaefolius* presented activity against the growth of *Cr. neoformans* and the hexane and EtOH extracts against *C. albicans*. Both exhibited high MIC values that varied from 500 and 1000 µg/mL. Chemical analysis of the genus *Rubus* have showed the presence of free acids, sugars, peptic substances and ascorbic, folic, acetic, caproic and benzoic acids as well as coumarins (6).

Herissantia crispera extracts were more active against *Cr. neoformans*, with MIC values ranging from 125 µg/mL to 1000 µg/mL. *R. acetosa* extracts exhibited efficient activity against *C. tropicalis* with a MIC of 60 µg/mL. Extracts from the leaf of *I. dulcis* inhibited *C. krusei* (aqueous) and *C. albicans* (AcOEt), but only when a high amount of the extract was used (1000 µg/mL).

The extract of *A. brasiliensis* was inactive against all the microorganisms tested. Souza *et al.* (27) showed that *S. aureus*, *S. epidermidis*, *E. coli* and *Bacillus subtilis* were resistant to the extracts of *A. brasiliensis*. However, other authors found several biological activities in the extract of this plant as inhibitors of lymphocyte cell proliferation (17), and as an antiviral agent against virus herpes simplex 1 (11).

This study provides data about the antimicrobial properties of some tropical plant species using extracts at concentrations that would be able to be studied for therapeutically useful. Some of these extracts may be applied clinically for fungal infection, specially the EtOH extract from the leaf of *S. terebinthifolius* against *S. schenckii*. The results of the present work validate and document, in a systematic way, that most of the plant species studied possess substantial antifungal properties. This explains the use of these plants in folk medicine for the treatment of various diseases, some related to microbial infections.

Further study is necessary for purification, separation, isolation and characterization of the active principles from the hexane fraction obtained from the leaves of *S. terebinthifolius*.

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RESUMO

Atividade Antifúngica de plantas utilizadas na medicina tradicional brasileira contra fungos de relevância clínica

A propriedade antifúngica de extratos de oito plantas utilizadas na medicina tradicional brasileira foi testada contra cinco espécies de *Candida*, com relevância clínica, *Cryptococcus neoformans* e *Sporothrix schenckii*. Os resultados mostraram que todos os extratos exibiram atividade antifúngica contra pelo menos um dos microrganismos testados. O extrato etanólico das folhas de *Schinus terebinthifolius* apresentou potencial atividade antifúngica contra *C. glabrata* e *S. schenckii*. Na análise fitoquímica preliminar dos extratos de *S. terebinthifolius* observou-se a presença de compostos biologicamente ativos como, flavonóides, triterpenos, esteróides e taninos.

Palavras-chave: Propriedade antifúngica; plantas medicinais; teste de susceptibilidade

REFERENCES

- Amorim, M.M.R.; Santos, L.C. (2003). Treatment of bacterial vaginosis with *Schinus terebinthifolius* Raddi vaginal gel: a randomised controlled trial. *Rev. Bras. Ginecol. Obstet.*, 25, 95-102.
- Biavatti, M.W.; Bellaver, M.H.; Volpato, L.; Costa, C.; Bellaver, C. (2003). Preliminary studies of alternative feed additives for broilers: *Alternanthera brasiliana* extract, propolis extract and linseed oil. *Rev. Bras. Cienc. Avic.*, 5, 147-151.
- Bovine, M.G.; Okano-Carvalho, R.M.; Vieira, M.F. (2001). Malvaceae A. Juss. no Parque Estadual do Rio Doce, Minas Gerais, Brasil. *Rodriguésia*, 52, 17-47.
- Bustamante, B.; Campos, P.E. (2001). Endemic sporotrichosis. *Curr Opin Infect Dis*, 14: 145-149.
- Chen, E.S.; Blank and Casadevall, A. (1996). Extracellular proteinase activity of *Cryptococcus neoformans*. *Clin. Diagn. Lab. Immunol.*, 3, 570-574.
- Costa, A.F. (1994). Farmacognosia. Lisboa: Fundação Calouste Gulbenkian press.
- Cowan, M.M. (1999). Plant products as antimicrobial agents. *Clin. Microbiol. Rev.*, 12, 564-82.
- Elisabetsky, E.; Souza, G.C. (2002). Etnofarmacologia como ferramenta na busca de substâncias ativas. In: C.M.O. Simões, *Farmacognosia da planta ao medicamento*, UFRG e UFSC, Porto Alegre/Florianópolis, pp.107-122.
- Gibbons, S. (2003). An overview of plant extracts as potential therapeutics. *Expert Opin. Ther. Pat.*, 13, 489-497.
- Holetz, F.B.; Pessini, G.L.; Sanches, N.G.; Cortez, D.A.G.; Nakamura, C.V.; Dias, B.D. (2002). Screening of Some Plants Used in the Brazilian Folk Medicine for the Treatment of Infectious Diseases. *Mem. Inst. Oswaldo Cruz*, 97, 1027-1031.
- Lagrotta, M.H.C.; Wigg, M.D.; Santos, M.M.G.; Miranda, M.M.F.S.; Camara, F.P.; Couceiro, J.N.S.S.; Costa, S.S. (1994). Inhibitory activity of extracts of *Alternanthera brasiliana* (Amaranthaceae) against the herpes simplex virus. *Phytother. Res.*, 8, 358-361.
- Loayza, I.; Abujder, D.; Aranda, R.; Jakupovic, J.; Collin, G.; Deslauriers, H.; Jeand, F.I. (1995). Essential Oils of *Baccharis salicifolia*, *B. latifolia* and *B. dracunculifolia*. *Phytochemistry*, 38, 381-389.
- Lopez, A.; Hudson, J.B.; Towers, G.H.N. (2001). Antiviral and antimicrobial activities of Colombian medicinal plants. *J. Ethnopharmacol.*, 77, 189-196.
- Loyd, H.A.; Taysir, M.J.; Evans, L.; Morton, J.F. (1977). Terpenes of *Schinus terebinthifolius*. *Phytochemistry*, 16, 1301-1302
- Masoko, P.; Picard, J.; Eloff, J.N. (2005). Antifungal activities of six South African *Terminalia* species (Combretaceae). *J. Ethnopharmacol.*, 99, 301-308.
- Melo Jr., E.J.M.; Raposo, M.J.; Neto, J.A.; Diniz, M.F.A.; Marcelino, C.A.C.; Sant'Ana, A.E.G. (2002). Medicinal plants in the healing of dry socket in rats: Microbiological and microscopic analysis. *Phytomedicine*, 9, 109-116.
- Moraes, V.L.; Santos, L.F.; Castro, S.B.; Loureiro, L.H.; Lima, O.A.; Souza, M.L.; Yien, L.M.; Rossi-Bergmann, B.; Costa, S.S. (1994). Inhibition of lymphocyte activation by extracts and fractions of *Kalanchoe*, *Alternanthera*, *Paullinia* and *Mikania* species. *Phytomedicine*, 1, 199-204.
- Muraleedharan, G.N.; Basil, A.B. (1990). Antimicrobial *Piper* Metabolite and Related Compounds. *J. Agric. Food Chem.*, 38, 1093-1096.
- Manual Clinical and Laboratory Standards Institute. (2002) Reference method for broth dilution antifungal susceptibility testing of yeasts. Approved standard M27-A2. Second edition. National Committee for Clinical Laboratory Standards, Wayne, Pa.
- Parmar, V.S.; Subhash, C.J.; Kirpal, S.B.; Rajni, J.; Poonam, T.; Amitabh, J.; Om, D.T.; Ashok, K.P.; Jesper, W.; Carl, E.O.; Per, M.B. (1997). Phytochemistry of the Genus *Piper*. *Phytochemistry*, 46, 591-673.
- Pessini, G.L.; Dias, B.P.F.; Nakamura, C.V.; Cortez, D.A.G. (2003). Antibacterial activity of extracts and neolignans from *Piper regnellii* (Miq.) C. DC. var. *pallidum* (C. DC.) Yunck. *Mem. Inst. Oswaldo Cruz*, 98, 1115-1120.
- Rahalison, L.; Hamburger, M.; Monod, M.; Frenk, E.; Hostettmann, K. (1993) Antifungal test in Phytochemical investigations: Comparison of bioautographic methods using phytopathogenic and human pathogenic fungi. *Planta Med.*, 60, 41-44.
- Resende, J.C.P.; Resende, M.A. (1999). *In vitro* antifungal susceptibility of clinical isolates of *Candida* spp. from hospitalised patients. *Mycoses*, 42: 641-644.
- Richards, R.M.E.; Liu, X. (1994). Antibacterial activity of compounds from *Rubus pinfaensis*. *Planta Med.*, 4, 20-4.
- Silva, J.P.; Siqueira, A.M. (2000). Acción antibacteriana de extratos hidroalcohólicos de *Rubus urticaefolius*. *Rev. Cubana Plant. Med.*, 5, 26-9.
- Schomourlo, G.; Mendonça-Filho, R.R.; Alviano, C.S.; Costa, S.S. (2006). Screening of antifungal agents using ethanol precipitation and bioautography of medicinal and food plants. *J. Ethnopharmacol.*, 96, 563-568.
- Souza, G.C.; Haas, A.P.S.G.; Poser, L.; Schapoval, E.S.; Elisabetsky, E. (2004). Ethnopharmacological studies of antimicrobial remedies in the south of Brazil. *J. Ethnopharmacol.*, 90, 135-143.
- Wagner, H.; Bladt, S. (1984). Plant Drug Analysis. A Thin Layer Chromatography Atlas. 2 Ed. Springer. München.