ANTIMICROBIAL EFFECTS OF SOME BRAZILIAN MEDICINAL PLANTS AGAINST INTESTINAL DISORDERS

Airton Luiz Gonçalves

Médico; Doutor em Microbiologia Aplicada pela Universidade Estadual Paulista – UNESP. E-mail: airtonlgoncalves@ig.com.br

Antonio Alves Filho

Biólogo no Laboratório Evangélico de Análises Clínicas. E-mail: alvesfilho.a@hotmail.com

Hercules Menezes

Biólogo; Docente no Departamento de Bioquímica e Microbiologia na Universidade Estadual Paulista – UNESP. E-mail: hermes@rc.unesp.br

RESUMO: Extratos hidroalcoólicos obtidos de 22 espécies de árvores medicinais brasileiras foram testados para a sua atividade antimicrobiana (AA), contra três diferentes microrganismos isolados de inoculi obtidos de fezes diarreicas (Escherichia coli enteropatogênica, Shigella sonnei e Salmonella spp). O método de difusão em ágar foi utilizado para os ensaios de antibiose. Dos 66 ensaios realizados, 8% apresentaram alta AA. Excepcional AA foi observada em Anacardium occidentale L. (Anacardiaceae), Ilex paraguariensis A.St.Hil. (Aquifoliacea), Myroxylon peruiferum L.f. (Fabaceae), Mimosa tenuiflora (Wild.) Poiret (Leguminosae), contra Shigella sonnei e Salmonella spp, bem como extrato de Eugenia uniflora L. (Myrtaceae) contra Escherichia coli enteropatogênica, Shigella sonnei e Salmonella spp. Ensaios de antibiose com antibióticos disponíveis comercialmente foram conduzidos simultaneamente contra as bactérias acima mencionadas, para comparar com o potencial AA dos extratos de árvores estudados. Os resultados obtidos até o momento indicam um excelente potencial terapêutico para essa planta.

PALAVRAS-CHAVE: Atividade Antimicrobiana; Árvores Medicinais Brasileiras; Antibiose.

ANTIMICROBIAL EFFECTS OF SOME BRAZILIAN MEDICINAL PLANTS ON INTESTINAL DISORDERS

ABSTRACT: Hydroalcoholic extracts from 22 species of Brazilian medicinal trees were screened for their antimicrobial activity (AA) against 3 different microorganisms isolated from diarrheic feces inocula (Escherichia coli enteropathogenic, Shigella sonnei and Salmonella spp). The agar diffusion method was used for antibiosis assay purposes. In 66 (100%) samples tested, 16 (24%) exhibited high AA. Exceptional AA was observed in Anacardium occidentale L. (Anacardiaceae), Ilex paraguariensis A.St.Hil. (Aquifoliacea), Myroxylon peruiferum L.f. (Fabaceae), Mimosa tenuiflora (Wild.) Poiret (Leguminosae), against Shigella sonnei and Salmonella spp, and Eugenia uniflora L. (Myrtaceae) extract against Escherichia coli enteropathogenic, Shigella sonnei and Salmonella spp. Antibiosis assays with commercially available antibiotics were simultaneously conducted against the above-mentioned bacteria so that the AA potential of the medicinal trees extracts could be compared. Results indicate the plants' excellent therapeutic potential.

KEYWORDS: Antimicrobial Activity; Brazilian Medicinal Trees; Antibiosis.

INTRODUCTION

The use of medicinal plants, especially in developing countries, contributes significantly to primary health care (WHO, 2001).

Enterocolitis with diarrhoea, fever and dehydration is the most common disease in developing countries, particularly in children, due to deficient sanitary conditions (CACERES et al., 1990).

During the last decade, infectious diseases and particularly infectious diarrhoea have threatened the life of millions of people around the world (ASHBOLT, 2004). This situation has been compounded by an increase in the development of antimicrobial resistance by different microorganisms, including bacteria and parasites, and the high cost of antimicrobials. The increasing prevalence of multidrug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raise the threat of untreatable bacterial infections and urge the search for new infection-fighting strategies (ETKIN, 2001). The development of novel, efficient and inexpensive drugs is thus of great importance.

The highly rich and diverse biota of the Brazilian tropical forest includes a number of plants that are used in natural medicine for treating tropical diseases, including bacterial infections. Ethnobotanic studies conducted in rural communities in different regions of Brazil pointed to gastrointestinal and respiratory disorders as the most frequent illness treated with medicinal plants (KUBO, 1997; GARLET, 2000; ALICE et al., 1991). Traditional medicine is highly acknowledged and different communities use a wide variety of plants to treat gastrointestinal disorders, such as diarrhoea and infection by intestinal parasites, which are particularly prevalent in these areas (MACGAW; JAGER; VAN STEDEN, 2000).

Plants have an almost limitless ability to synthesize secondary metabolites. In many cases, these substances are the plant's defense mechanisms against predation by microorganisms, insects and herbivores (SCHULTES, 1978).

Contrary to synthetic drugs, antimicrobial compounds of plant origin are not associated with many side effects and have an enormous therapeutic potential to heal several infectious diseases (ELOFF, 1988).

Plants which have been used as medicine for hundreds of years constitute an obvious choice for study.

In an effort to discover new compounds, many research groups screen plant extracts to detect secondary metabolites with relevant biological activities and, consequently, several simple bioassays were developed for such screening purposes (HAMBURGUER; HOSTETMANN, 1991; CRAGG; NEWMAN; SNADER, 1997).

In current study, 22 species of Brazilian medicinal native trees used in folk medicine in the region have been investigated. Plant selection was based on existing ethnobotanic information and all species have been used in the treatment of infectious disease. Their in vitro antimicrobial activity was thus analyzed.

2 MATERIALS AND METHODS

2.1 PLANT MATERIAL

The plants were collected in the vicinity of Iracemápolis, Limeira and Rio Claro SP Brazil. They were identified by researchers that undertook the collection and their identification was confirmed by scientists of the Botany Department of the State University of São Paulo (Unesp), Rio Claro SP Brazil. Exsiccates of each species were deposited in the herbarium of Yodervas Ltda institution Campinas SP Brazil. Traditional practices by herbalists in the state of São Paulo provide the basis of selecting the part of the plant to be tested. Table 1 shows the 22 species, family, local name, part tested and traditional usage.

Table 1 Plant species, local name, tested part and popular usages

VEGETAL SPECIES	LOCAL NAME	TESTED PART	POPULAR USAGES*
Anacardium occidentale L	Cajueiro	Chestnut-caju	Diarrhoea, bronchitis, stomatitis, pharyngitis (5)
Schinus terebithifolia Raddi	Aroeira	Bark-trunk	Uterine and vaginal infections, wounds, hemorrhoids, stomatitis, pharyngitis (3)
Ilex paraguariensis A.St-Hil.	Erva-mate	Leaf	Wounds, abscesses, skin diseases, dyspepsia, myalgia (5,7)
Tabebuia avellanedae Lor.ex Griseb	Ipê-roxo	Bark-trunk	Abscesses, stomatitis, pharyngitis, uterine and vaginal infections, anti-inflammatory, diuretic (7)
Bixa orellana L.	Urucum	Seed	Diarrhoea, cold, pharyngitis, bronchitis, burns (5,7)
Cereus jamacaru DC.	Cactus-flor	Flower	Cough, bronchitis, febrifuge, wounds, abscesses, kidney stone (5)
Bauhinia forficata Link	Pata-de-vaca	Leaf	Cystitis, intestinal parasites, diarrhoea, Kidney stones (5)
Copaifera langsdorffii Desf.	Copaíba	Bark-trunk	Abscesses, wounds, would healing, anti-inflammatory (6)
Hymenaea courbaril L.	Jatobá	Bark-trunk	Diarrhoea, cough, bronchitis, external mycoses, wounds, cystitis, prostatitis (6)
Cecropia hololeuca Miq.	Umbaúba	Leaf	Diarrhoea, cystitis, prostatitis (5)
Maytenus ilicifolia Reissek	Espinheira-santa	Leaf	Gastric and duodenal ulcers, gastritis, wounds, skin diseases (7)
Trichilia catigua A.Juss.	Catuaba	Bark-trunk	Sexual impotence, neurasthenia, wounds, viral disease of skin (herpes), abscesses (4)
Myroxylon peruiferum L.f.	Bálsamo-do-perú	Bark-trunk	Rheumatism, bronchitis, wounds, abscesses, tuberculosis, scabies, mycoses and skin parasites, laryngitis, diarrhoea, uterine and vaginal infections, urinary infections (6,7)
Pterodon emarginatus Vogel	Sucupira	Fruit-sâmara	Rheumatism, diabetes, skin diseases, repellent insects and insect bites (5)
Casearia sylvestris Sw.	Guaçatonga	Leaf	Burns, wounds, herpes, skin diseases, stomatitis, pharyngitis (5)

Ocotea odorifera (Vell.) Rohwer	Sassafrás	Bark-trunk	Rheumatism, anti-inflammatory, diuretic, syphilis, insect repellent insect (5)
Mimosa tenuiflora (Wild.) Poiret	Tepezcuite	Bark-trunk	Wounds, stomatitis, gastritis, gastric dyspepsia, anti-inflammatory, wound heeling (1)
Stryphnodendron adstringens (Mart) Coville	Barbatimão	Bark-trunk	Uterine and vaginal infections, hemorrhoids, wounds, conjunctivitis, diarrhoea, pharyngitis (5)
Anadenanthera colubrina (Vell.) Brenan	Angico	Bark-trunk	Cough, bronchitis, uterine and vaginal infections, gonorrhea (5)
Eugenia uniflora L.	Pitangueira	Fruit	Rheumatism, diarrhoea, febrifuge, intestinal parasites, bronchitis, cough (5)
Psidium guajava L.	Goiabeira	Leaf	Diarrhoea, stomatitis, pharyngitis, uterine and vaginal infections, wounds (2)
Genipa americana L.	Genipapo	Fruit	Gonorrhea, diarrhoea, wounds, pharyngitis, syphilis (5,6)

* Reference for usage: 1: Anton and collaborators (1993); 2: Cáceres and collaborators (1990); 3: Gruenwald, Brendler and Jaenickke (2000); 4: Manabe (1992); 5: Mors, Rizzini and Pereira (2000); 6: Schultes and Raffauf (1990); 7: Taylor (1998).

2.2 PREPARATION OF EXTRACTS

Orientation of the A process of the Brazilian Pharmacopeia (BRASIL, 1988), adapted for Younes, Varella and Suffredini (2000), was followed for the preparation of hydro-alcoholic extracts. The drying of the vegetal material was initially undertaken at room temperature and completed in a greenhouse at 50°C until a text-standard of 20% humidity is obtained. The material was ground in a grain mill and the powder was added to the hydro-alcoholic solution (ethanol 70%) at the ratio of 10% ($m.v^{-1}$). Each solution was stored for 25 days at room temperature and then filtered.

2.3 MICROORGANISMS USED AND GROWTH CONDITIONS

Inocula were obtained from clinically infected spots obtained from diarrheic feces. Three different microorganisms were isolated (Escherichia coli enteropathogenic, Shigella sonnei and Salmonella spp). The bacteria cultures were grown in nutrient agar (Oxoid n.2) at 35°C. After 24 hours of growth each microorganism were diluted to a final concentration of approximately 10^6 CFU/mL. An aliquot of 100 mL of this suspension was sown in petri plates with 15 mL of Mueller-Hinton agar, with a thickness of approximately 4 mm (SHADOMY; SPNEL-INGROF, 1980).

2.4 ANTIMICROBIAL SUSCEPTIBILITY TESTING

The agar diffusion method described by Bauer and collaborators (1966) for antibiosis assay purposes was adopted. Subsequently, 6mm-diameter filter paper discs were saturated either with the extract (10 ml) or with commercially available antibiotics (CECOM Ltda.): ampicillin 10 mg (AMP), ciprofloxacin 5 mg (CIP), ceftriaxone 30 mg (CRO), cefotaxime 30 mg (CTX), sulfamethoxazole 25 mg (SFT), chloramphenicol 30 mg (CLO), amoxicillin 30 mg (AMC) were tested against the microorganisms as follows Escherichia coli enteropathogenic (AMP, CTX, CLO, CIP); Shigella sonnei (AMP, CRO, SFT, CLO, CIP); Salmonella spp (AMC, CLO, AMP, SFT, CRO, CIP). The plates were incubated at 36°C for 24 h and the results were expressed in terms of the diameter of the inhibition zone: < 9 mm [absence of susceptibility (R)]; ³ 9 mm [susceptibility (S)].

3 RESULTS AND DISCUSSION

Whereas Table 2 shows the results of antibiosis assays summarized and grouped according to species and family, Table 3 gives the result of the commercial antibiotic control test for each microorganism.

Table 2 Results of the antimicrobial activity of 22 extracts from Brazilian native trees against 3 different microorganisms

Family	**Specie**	Ece	Ss	Sl
Anacardiaceae	*A.occidentale*	R	S18	S16
	S.terebinthifolia	R	R	R
Aquifoliaceae	*I.paraguariensis*	R	S14	S15
Bignoniaceae	*T.avellanedae*	R	R	R
Bixaceae	*B.orellana*	R	R	R
Cactaceae	*C.jamacaru*	R	R	R
Caesalpiniaceae	*B.forficata*	R	R	R
	C.langsdorffii	R	S14	R
	H.courbaril	S28	R	R
Cecropiaceae	*C.hololeuca*	S11	R	R
Celastraceae	*M.ilicifolia*	R	R	R
Erythroxylaceae	*T.catigua*	R	R	R
Fabaceae	*M.peruiferum*	R	S12	S14
	P.emarginatus	R	R	R
Flacourtiaceae	*C.sylvestris*	R	R	R
Lauraceae	*O.odorifera*	R	R	R
Leguminosae	*M.tenuiflora*	R	S20	S18
Mimosoideae	*S.adstringens*	R	S30	R
	A.colubrina	R	R	R
Myrtaceae	*E.uniflora*	S14	S34	S32
	P.guajava	R	R	S11
Rubiaceae	*G.americana*	R	R	R

Ece=Escherichia coli enteropathogenic, *Ss=Shigella sonnei, Sl=Salmonella* spp, R=resistant (development of the inhibition halo was not extant), S=susceptibility (development of the inhibition halo) in mm, including the 6mm disc.

Table 3 Results of commercial antibiotic control test for each microorganism

Microorganism	**Resistant Control**	**Sensitive Control**
Escherichia coli enteropathogenic	AMP	SFT, CTX, CLO, CIP
Shigella sonnei	AMP	CLO, SFT, CRO, CIP
Salmonella spp	AMC	CLO, AMP, SFT, CRO, CIP

AMP=(ampicillin 10 mg), SFT=(sulfamethoxazole 25mg), CTX=(cefotaxime 30 mg), CLO=(chloramphenicol 30 mg), CIP=(ciprofloxacin 5 mg), CRO=(ceftriaxone 30 mg), AMC=(amoxicillin 30 mg). Resistant Control (development of the inhibition halo was not extant), Sensitive Control (development of the inhibition halo, variable in accordance with the antibiotic employed).

* Measures standards in mm of halos of commercial antibiotic inhibition in the test of sensitive control: SFT(17), CFX(23), CLO(22), CIP(18), CRO(20), AMP(23).

Sixteen samples (24%) exhibited high AA out of the 66 (100%) tested samples. Ten (45%) of plant extracts showed some degree of activity against at least one of the microorganisms.

The most susceptible microorganism was Shigella sonnei which was inhibited by seven (32%) extracts; Salmonella spp was inhibited by six (27%) extracts; Escherichia coli enteropathogenic was inhibited by three (13%) extracts.

The families Anacardiaceae (Anacardium occidentale), Aquifoliaceae (Ilex paraguariensis), Fabaceae (Myroxylon peruiferum), Leguminosae (Mimosa tenuiflora) and Myrtaceae (Eugenia uniflora) had the highest AA.

Although the literature indicated antimicrobial activity (AA) of the hydro-alcoholic extracts of the species: Schinus terebinthifolia (BANDEIRA; WANICK, 1974), Tabebuia avellanedae (OLIVEIRA; ALMEIDA; SILVA FILHO, 1990), Bixa orellana (MORS; RIZZINI; PEREIRA, 2000), Cereus jamacaru (BRUHN; LINDGREN, 1976), Bauhinia forficata (MIYAKE, 1986), Maytenus ilicifolia (OLIVEIRA, 1991), Trichila catigua (MANABE, 1992), Pterodon emarginatus (FASCIO et al., 1976), Casearia sylvestris (BASILE, 1990), Ocotea odorifera (LORENZI, 1992), Anadenanthera colubrina (MORS; RIZZINI; PEREIRA, 2000) and Genipa americana (GUARNACCIA et al., 1972), current results (Table 2) failed to demonstrate any antibacterial activity in the extracts of the above mentioned plants.

Since all the microorganisms used in this study presented resistance to some commercial antibiotics (Table 3), this fact suggested that they were also resistant to these plants.

Extracts of Hymenaea courbaril and Cecropia hololeuca only had AA against Escherichia coli enteropathogenic. The extracts of Copaifera langsdorffii and Stryphnodendron adstringens had AA only against Shigella sonnei and Psidium guajava extract only against Salmonella spp. Hymenaea courbaril is known for its terpens and phenolics compounds with proven AA (MARSAIOLI, 1975). Cecropia hololeuca was not described in any of the consulted literature; Copaifera langsdorffii is related as also active against Escherichia coli and Staphylococcus aureus (EL NUNZIO, 1985). Consulted literature shows that Stryphnodendron adstringens is active against Staphylococcus aureus, Escherichia coli, Bacillus subtilis. Although Pseudomonas aeruginos (ALVES et al., 2000) showed AA against Shigella sonnei, it failed to inhibit Escherichia coli enteropathogenic. In spite of the fact that Psidium guajava is cited as sensitive to Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa and Candida albicans (HOLETZ et al., 2002; GNAN; DEMELLO, 1999) as results in Table 2 show, sensitivity of Salmonella spp, has no evidenced activity against Escherichia coli enteropathogenic.

High AA was obtained by extract of Anacardim occidentale, Ilex paraguariensis, Myroxylon peruiferum and Mimosa tenuiflora against Shigella sonnei and Salmonella spp. Anacardium occidentale is cited in literature as sensitive to Proteus mirabilis, Shigella sonnei and Staphylococcus aureus (LORENZI, 1992). Ilex paraguariensis and Myroxylon peruiferum are not described in any consulted literature and Mimosa tenuiflora extract is cited in literature for its activity against Streptococcus spp and Staphylococcus spp (HEINRICH et al., 1992).

When compared to other extracts, the extract of Eugenia uniflora showed a higher AA. It is cited as having activity against Streptococcus spp, Escherichia coli and Bacillus cereus (ALVES et al., 2000), as shown on Table 2, and AA against Escherichia coli enteropathogenic, Shigella sonnei and Salmonella spp.

4 CONCLUSIONS

Owing to the high number of plants with AA, further studies on a greater variety of species of native trees and with isolated microorganisms of a greater number of clinical infections, coupled to the identification of compounds for this activity, will confirm the therapeutic potential of these and other Brazilian native plants.

REFERENCES

ALICE, C. B. et al.. Screening of plants used in south Brazilian folk medicine. **Journal of Ethnopharmacology**, v. 35, p. 165-171, 1991.

ALVES, T. M. A. et al.. Biological screening of Brazilian medicinal plants. **Memórias do Instituto Oswaldo Cruz**, v. 95, n. 3, p. 367-373, 2000.

ANTON, R. et al.. Pharmacognosy of Mimosa tenuiflora (Willd.) Poiret. **Journal of Ethnopharmacology** v. 38, p. 153-157. 1993.

ASHBOLT, J. N.. Microbial contamination of drinking water and disease outcomes in developing regions. **Toxicology**, v. 198, p. 229-238, 2004.

BANDEIRA, J. A.; WANICK, M. C.. Ação antinflamatória e cicatrizante de Schinus aroeira Vell., em pacientes com cervicite e cervicovaginite. **Revista do Instituto de Antibióticos**, Recife, v. 12, p. 105-106, 1974.

BASILE, A. C.. Pharmacological assay of Casearia sylvestris. 1. Preventive anti-ulcer activity and toxicity of the leaf crude extract. **Journal of Ethnopharmacology**, v. 30, p. 187-197, 1990.

BAUER, A. W. et al.. Antibiotic susceptibility testing by a standardized single disc method. **American Journal of Clinical Pathology** v. 45, p. 493-496. 1966.

BRUHN, J. C.; LINDGREN, J. E.. Cactaceae alkaloids. XXIII. Alkaloids of Pachycereus-aborginum and Cereus jamacaru. **Lloydia**, v. 39, p. 175-177, 1976.

CÁCERES, A. et al.. Plants used in Guatemala for the treatment of gastrointestinal disorders. 1. Screening of 84 plants against enterobacteria. **Journal of Ethnopharmacology**, v. 30, p. 55-73, 1990.

CRAGG, G. M.; NEWMAN, D. J.; SNADER, K. M.. Natural products in drug discovery and development. **Journal of Natural Products**, v. 60, p. 52-60, 1997.

EL NUNZIO, M. J.. Óleo de copaíba e seu emprego cosmético. **Aerosol e Cosméticos**, v. 7, n. 41, p. 7-9, 1985.

ELLOF, J. N.. Which extractant should be used for the screening and isolation of antimicrobial components from plants? **Journal of Ethnopharmacology**, v. 60, p. 1-8, 1998.

ETKIN, N.. Perspectives in Ethnopharmacology: forging a closer link between bioscience and traditional empirical knowledge. **Journal of Ethnopharmacology**, v. 76, p. 177-182, 2001.

FASCIO, M. et al.. Diterpenoid furans from Pterodon species. **Phytochemistry**, v. 15, p. 201-203, 1976.

BRASIL. **Farmacopéia Brasileira**. 4. ed. São Paulo, SP: Atheneu, 1988.

GARLET, T. M. B.. **Levantamento das plantas medicinais utilizadas no município de Cruz Alta RS Brazil**. 2000. 220fls. Dissertação (Mestrado em Botânica) - Universidade Federal do Rio Grande do Sul. Porto Alegre, RS: UFRGS, 2000.

GNAN, S. O.; DEMELLO, M. T.. Inhibition of Staphylococcus aureus by aqueous goiaba extracts. **Journal of Ethnopharmacology**, v. 68, p.103-108, 1999.

GRUENWALD, J.; BRENDLER, T.; JAENICKKE, C.. **Physicians Desk References**. New Jersey: Med. Econ. Co., 2000.

GUARNACCIA, R. et al.. Geniposidic acid, a glucoside from Genipa americana. **Tetrahedron Letters**, v. 50, p. 5125-5127, 1972.

HAMBURGER, H.; HOSTETTMANN, K.. The link between phytochemistry and medicine. **Phytochemistry**, v. 30, p. 3864-3874, 1991.

HEINRICH, M. M. et al.. Parasitological and microbiological evaluation of mixed Indian medical plants. **Journal of Ethnopharmacology**, v. 36, p. 81-85, 1992.

HOLETZ, F. B. et al.. Screening of some plants used in the Brazilian folk medicine for the treatment of infectious diseases. **Memórias do Instituto Oswaldo Cruz**, v. 97, n. 7, p. 1027-1031, 2002.

KUBO, R. R.. **Levantamento das plantas de uso medicinal em Coronel Bicaco, RS**. 1997. 163fls. Dissertação (Mestrado) - Universidade Federal do Rio Grande do Sul. Porto Alegre, RS: UFRGS, 1997.

LORENZI, H.. **Árvores brasileiras** – manual de identificação e cultivo de plantas arbóreas nativas no Brasil. Nova Odessa, SP: Ed. Plantarum, 1992.

MANABE, H.. Effects of catuaba extracts on microbial and HIV infection. **In Vitro**, v. 6, n. 2, p. 161-165, 1992.

MARSAIOLI, A. J.. Diterpenes in the bark of Hymenaea courbaril. **Phytochemistry**, v. 14, p. 1882-1883, 1975.

MACGAW, L. J.; JAGER, A. K.; VAN STEDEN, J. Antibacterial, antihelmintic and anti-amoebic activity in South African medicinal plants. **Journal of Ethnopharmacology**, v. 72, n. 1/2, p. 247-263, 2000.

MIYAKE, E. T.. Caracterização farmacognóstica de pata-de-vaca (Bauhinia forficata), **Revista Brasileira de Farmacognosia**, v. 1, n. 1, p. 56-68, 1986.

MORS, W. B.; RIZZINI, C. T.; PEREIRA, N. A.. **Medicinal plants of Brazil**. Michigan: Reference Publications, 2000.

OLIVEIRA, A. B.; ALMEIDA, E. R.; SILVA FILHO, A. A.. Estrutura química e atividade biológica de naftoquinonas de Bigniaceas brasileiras. **Quimica Nova**, v. 13, n. 4, p. 302-307, 1990.

OLIVEIRA, M. G. Pharmacologic and toxicologic effects of two Maytenus species in laboratory animals. **Journal of Ethnopharmacology**, v. 51, p. 113-117, 1991.

SCHULTES, R. E.. **The kingdom of plants**. Thonson, W.A.R.: Mc Graw-Hill, 1978.

SCHULTES, R. E.; RAFFAUF, F.. **The healing forest. Medicinal and toxic Plants of the Northwest Amazonia**. Portland, USA: R.F. Dioscorides Press, 1990.

SHADOMY, S.; SPINEL-INGROF, A.. Susceptibility testing: with antifungal drugs. In: LENNETTE, E.. **Manual of clinical of microbiology**. 3rd ed. Washington: American Society for Microbiology, 1980. cap. 62, p. 647-653.

TAYLOR, L.. **Herbal secrets of the Rainforest**. Rocklin, CA: Prima Publishing Inc., 1998.

YOUNES, R. N.; VARELLA, A. D.; SUFFREDINI, I. B.. Extração e rastreamento de novas drogas em plantas brasileiras. **Acta Oncológica Brasileira**, v. 20, p. 15-19, 2000.

WHO - World Health Organization, **General Guidelines for Methodologies on Research and Evaluation of Traditional Medicine**. Geneva: WHO, 2001.

Recebido em: 08 Julho 2010
Aceito em: 26 Junho 2011