

Antimicrobial activity of *Byrsonima* species (Malpighiaceae)

Daniele C. Michelin,¹ Miriam Sannomiya,² Maria E. Figueiredo,² Daniel Rinaldo,² Lourdes C. dos Santos,² Alba R. M. Souza-Brito,³ Wagner Vilegas,² Hérica R. N. Salgado*¹

¹Departamento de Fármacos e Medicamentos, Faculdade de Ciências Farmacêuticas, Universidade Estadual Paulista, 14801-902 Araraquara-SP, Brazil,

²Departamento de Química Orgânica, Instituto de Química, Universidade Estadual Paulista, 14800-900 Araraquara-SP, Brazil,

³Instituto de Biologia, Departamento de Fisiologia e Biofísica, Universidade Estadual de Campinas, Caixa Postal 6109, 13083-970 Campinas-SP, Brazil

RESUMO: “Atividade antimicrobiana de espécies de *Byrsonima* (Malpighiaceae)”. Espécies de *Byrsonima*, família Malpighiaceae, são popularmente conhecidas como “murici”. Existem várias propriedades atribuídas às folhas de espécies de *Byrsonima* incluindo febrífuga, no tratamento de disfunções gastrointestinais e doenças de pele. Neste trabalho avaliamos a atividade antimicrobiana dos extratos das folhas de *Byrsonima fagifolia*, *B. basiloba* e *B. intermedia* usando o método de difusão em disco. Os resultados obtidos mostraram que os extratos metanólicos das folhas apresentaram atividade antimicrobiana contra todos os microrganismos testados.

Unitermos: Malpighiaceae, *Byrsonima*, atividade antimicrobiana.

ABSTRACT: *Byrsonima* species, family Malpighiaceae, is popularly known as “murici”. There are several properties attributed to the leaves of *Byrsonima* species including febrifuge, to treat gastrointestinal dysfunctions and skin diseases. In this work, the antimicrobial activity of *Byrsonima fagifolia*, *B. basiloba* and *B. intermedia* extracts obtained from the leaves were evaluated by using the disc-diffusion method. The results obtained showed that the methanol extracts of leaves had presented antimicrobial activity against all the microorganisms tested.

Keywords: Malpighiaceae, *Byrsonima*, antimicrobial activity.

INTRODUCTION

Medicinal plants are natural resources, leading to valuable herbal products, which are often used in the treatment of various diseases.

The Cerrado, a Brazilian savanna, comprises a very rich and characteristic flora. Many of these plants are used as natural medicines by the people living in this region to treat several tropical diseases including schistosomiasis, leishmaniasis, malaria, fungal and bacterial infections (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).

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 incessantly appearing, imposing the need for 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

based on ethnopharmacological uses (Bezerra et al., 2006; Funke & Melzig, 2006; Leitão et al., 2006; Lima et al., 2006; Barbosa-Filho et al., 2007; Saúde-Guimarães & Faria, 2007; Rodríguez et al., 2008). *Byrsonima* species are widely distributed throughout “Cerrado” and in the region Northeast of Brazil. Leaves are used against fever and ulcers, including dermal and gastrointestinal diseases (Silva et al., 2001; Lopez et al., 2001; Agra et al., 2007 and 2008). Chemical investigation of species belonging in to this genus resulted in the isolation of steroids, triterpenes, flavonoids, proanthocyanidins and sulphonoglycolipids (Rastrelli et al., 1997; Mendes et al., 1999; Bejar et al., 1995). We have previously reported the isolation and/or identification of quercetin derivatives, galloyl quinic acids, proanthocyanidins besides the dimeric flavonoid amentoflavone and minor amounts of galloyl hexose and galloyl saccharose in the *B. crassa* leaves extract (Sannomiya et al., 2004; Sannomiya et al., 2005a; Sannomiya et al., 2005b; Sannomiya et al., 2005c). Some biological activities of *Byrsonima* species have been previously investigated. The methanol and hydromethanol extracts from *B. basiloba* (formerly documented as *B. cinera*) leaves have presented anti-diarrhea activity (Figueiredo et al.,

2005). The polar extracts of *B. crassa* leaves showed a potential antiulcerogenic effect in mice (Sannomiya et al., 2005a). The methanol extract of *B. crassifolia* leaves showed strong giardicidal activity (Amaral et al., 2006). The ethyl acetate of *B. crassifolia* roots was active against *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Shigella flexneri*, *Staphylococcus aureus*, *S. epidermidis*, *Streptococcus pneumoniae* and *Micrococcus luteus* (Martínez-Vasquez et al., 1999). The preliminary biological screening indicated that EMeOH and EMeOH 80%, including both the EtOAc and aqueous fractions of EMeOH, showed antimicrobial activity against *Staphylococcus epidermidis*, *Bacillus cereus*, *B. subtilis*, *Salmonella* sp., *Proteus mirabilis*, *Enterococcus faecalis*, *Shigella* sp. and *Candida albicans* (Sannomiya et al., 2005c). Despite the popular use of *B. basiloba*, *B. intermedia* and *B. fagifolia* as medicinal plants, there are no data about the antimicrobial effect of their leaf extracts. Thus, the interest in these plants is justifiable because of their potential medicinal value. The present study has the aim to evaluate the antimicrobial activity of *Byrsonima* species extracts obtained from the leaves by using the disc-diffusion method.

MATERIAL AND METHODS

Microorganisms

Eight microbial species were analyzed, which were taken from international collections. The bacteria *Bacillus subtilis* (ATCC 9372), *Bacillus cereus* (ATCC 14579), *Shigella* spp (IAL 1578), *Staphylococcus epidermidis* (ATCC 12226), *Proteus mirabilis* (CDC 305), *Salmonella* spp (ATCC 19196), *Enterococcus faecalis* (ATCC 29212), and the yeast *Candida albicans* (ATCC 10231).

Plant material

Leaves of *Byrsonima fagifolia* Niedenzu (IK) were collected by Dr. Clélia A. Hiruma-Lima at Porto Nacional, State of Tocantins, Brazil. The plant was identified by Prof. Dr. Eduardo Ribeiro dos Santos, Department of Botany, Universidade do Tocantins - State of Tocantins, where a voucher specimen was deposited (n° 6398). The plant material of *Byrsonima basiloba* A. Juss. (n° 24163) and *B. intermedia* A. Juss. (n° 24164) were collected by Luiz Fernando Rolim de Almeida at Pratânia, State of São Paulo, Brazil and authenticated by Dr. Clemente José Campos and deposited at the Herbarium of Unesp - Botucatu.

Extract preparation

The air-dried and powdered leaves of all *Byrsonima* species were extracted separately and

exhaustively with CHCl_3 , MeOH and 80% MeOH successively at room temperature (48 h for each solvent). Solvents were evaporated at 60 °C under reduced pressure affording the extracts coded as ECHCl₃, EMeOH and EMeOH 80% (Table 1).

Phytochemical screening

The chromatographic analyses were made by TLC on Si gel eluted with the mobile phases: CHCl_3 /MeOH (70:30, v:v) and CHCl_3 /MeOH/ H_2O (80:18:2, v/v).

Chromatographic evaluation of the MeOH and 80% MeOH extracts by TLC revealed with NP/PEG (diphenylaminoborate/polyethyleneglycol) reagent (Wagner et al., 1984) produced intense orange and yellow spots characteristic of flavonoids and plates revealed with anisaldehyde/sulfuric acid solution also produced reddish and gray spots, suggesting the presence of catechin derivatives and phenolic compounds, respectively. We also spotted authentic standards (Sigma) of quercetin, myricetin, kaempferol, (+)-catechin, (-)-epicatechin, gallic acid and methyl gallate.

The detection of the tannins were realized according to the proceedings described by Simões et al. (2001) using the reaction the reaction with iron salts Schneider (1990) and with the gelatin.

Iodine vapors and saturated solution of H_2SO_4 /CeSO₄ (saponins and terpenes) and anisaldehyde/ H_2SO_4 solution (flavonoids, terpenes, saponins, gallic acid derivatives and catechins) (Wagner et al., 1984) were also used.

The classes of compounds found in the EMeOH and MeOH 80% leaves extracts of *Byrsonima* species are indicated in Table 2.

Disc-diffusion method

The dried plant extracts of leaves and barks were dissolved in the same solvent (MeOH and CHCl_3) to a final concentration of 30 mg/mL. After, they were sterilized by filtration through 0.45 µm Millipore filters. Antimicrobial tests were carried out by disc-diffusion method (Bauer et al., 1966).

The microorganism cultures were grown in Brain Heart Infusion liquid medium at 37 °C. After 6 h of growth, each microorganism culture, at a concentration of 10⁶ cells/mL, was inoculated on the surface of Mueller-Hinton agar plates (100 µL). Subsequently, filter papers discs (6 mm in diameter) saturated with extracts (20 µL) were placed on the surface of each inoculated plate, in Brain Heart Infusion solid medium. The plates were incubated at 35 °C for 24 h for bacteria and for 48 h for *C. albicans*. After this period, the zones of growth inhibition around the discs were measured. Overall, cultured microorganisms with inhibition zones equal to

or greater than 7 mm were considered susceptible to the tested extract (Nascimento et al., 2000).

The negative control was the solvent used and the positive control was ciprofloxacin (5 µg/disc) for bacteria and ketoconazole (40 µg/disc) for *C. albicans*. All determinations were made in duplicate.

Minimum inhibitory concentration

The minimum inhibitory concentration (MIC) was determined by the dilution method according to National Committee for Clinical Laboratory Standards (NCCLS, 2003). The bacteria were grown in nutrient broth (Brain Heart Infusion liquid medium) for 6 h. After that 20 µL of 10⁶ cells/mL were inoculated in tubes containing nutrient broth supplemented with eight different concentrations (0.75, 1.50, 3.0, 6.0, 12.0, 15.0, 18.0 and 24.0 mg/mL) of the extracts. The tubes were incubated at 37 °C for 24 h for bacteria and for 48 h, for *C. albicans*. The lowest concentrations, which did not show any growth of tested organism after macroscopic evaluation were determined as MIC. All determinations were made in duplicate (Nascimento et al., 2000).

RESULTS AND DISCUSSION

As shown in Table 1, the extracts of the leaves of *Byrsonima basiloba*, *B. intermedia* and *B. fagifolia* were prepared by maceration with chloroform, methanol and hydromethanol solvents.

The results of the phytochemical screening of these species (Table 2) showed the presence of catechins, tannins, gallic acid derivatives and flavonoids in the *B. basiloba* and *B. intermedia* species. Previously we have reported the isolation of (+)-catechin and quercetin-3-*O*- α -L-arabinopyranoside from the EMeOH of the leaves of *B. basiloba* (Figueiredo et al., 2005). However, *B.*

fagifolia presented only gallic acid derivatives and flavonoids in its composition. This result confirms those related before with the analytical approach based on the liquid chromatography/electrospray ionization tandem mass spectrometry from the infusion of the *B. fagifolia*. The analysis permitted the detection of five flavonoids glycosides and twenty galloyl quinic acid derivatives (Sannomiya et al., 2007).

In this work were evaluated the antimicrobial activity of all the extracts prepared from these *Byrsonima* species against seven bacteria and one yeast. The EMeOH and EMeOH 80% extracts were proved to be good solvents in extracting inhibitory compounds from tested plants. The other hand the ECHCl₃ extracts of all species showed inactive against to the microorganisms evaluated.

In the assay against the microorganisms using the agar diffusion method (Table 3), the mean zones of inhibition obtained were between 7 to 14 mm. The MICs values observed was between 1.5 and 12.0 mg/mL. In fact, lowers MIC values were associated with the EMeOH from *B. intermedia* against *E. faecalis* (1.5 mg/mL) and *B. subtilis* (3.0 mg/mL).

The EMeOH 80% extract of the leaves from *B. fagifolia* showed to be most active against *B. cereus*, *E. faecalis* and *Shigella* with MICs values of 3.0 mg/mL than EMeOH with MICs values of 6.0, 7.5 and 6.0, respectively (Table 3). The presence of galloyl quinic acid derivatives on this extract can be responsible to the observed activity. This class of compounds has been recognized as possessing high activity against bronchial hyper-reactivity, allergic reactions, antiherpetic, anti-human immunodeficiency virus (HIV) reverse transcriptase, and anti-HIV activity (Bokech et al., 1996; Nishizawa et al., 1989).

Theses results can be explained based on the larger amount of the galloyl quinic acids derivatives

Table 1. Extracts obtained from *Byrsonima* species.

Species	ECHCl ₃ (g) (yield %)	EMeOH (g) (yield %)	EMeOH 80% (g) (yield %)
<i>B. basiloba</i> (1000 g)	77.7 (7.7%)	94.2 (9.4%)	63.1 (6.1%)
<i>B. intermedia</i> (590 g)	16.7 (2.8%)	85.2 (14.4%)	29.6 (5.0%)
<i>B. fagifolia</i> (1000 g)	47.3 (4.7%)	24.6 (2.5%)	12.9 (1.3%)

Table 2. Phytochemical screening of *Byrsonima* species.

Test	<i>B. fagifolia</i>			<i>B. basiloba</i>			<i>B. intermedia</i>		
	ECHCl ₃	EMeOH	EMeOH 80%	ECHCl ₃	EMeOH	EMeOH 80%	ECHCl ₃	EMeOH	EMeOH 80%
Catechins	-	-	-	-	+	+	-	+	+
Tannins	-	-	-	-	+	+	-	+	+
GAD	-	+	+	-	+	+	-	+	+
Flavonoids	-	+	+	-	+	+	-	+	+
Saponins	-	-	-	-	-	-	-	-	-
Terpenes	+	-	-	+	-	-	+	-	-

absent = (-); present = (+); ECHCl₃ = chloroform extract; EMeOH = methanol extract; EMeOH 80% = hydromethanol extract; GAD = gallic acid derivatives.

Table 3. Antimicrobial activities of polar extracts from *Byrsonima* species.

Species	<i>B. intermedia</i>						<i>B. basiloba</i>						<i>B. fagifolia</i>									
	EMeOH		EMeOH 80%		EMeOH		EMeOH 80%		EMeOH		EMeOH 80%		EMeOH		EMeOH 80%		Cipro		Keto			
Concentration (mg/mL)	50	75	100	50	75	100	50	75	100	50	75	100	50	75	100	50	75	100	50	75	100	
Microorganisms																						
<i>Se</i>	11	11	12	12	12	12	12	12	12	12	13	14	10	10	10	8	9	9	9	25	NT	NT
<i>Bc</i>	11	11	12	9	10	11	10	11	12	10	11	11	9	9	9	9	9	9	9	20	NT	NT
<i>Bs</i>	10	10	11	10	11	11	11	11	11	11	11	12	10	11	12	8	9	9	9	20	NT	NT
<i>S</i>	10	10	11	11	11	11	11	11	12	11	12	12	10	10	11	9	9	9	9	22	NT	NT
<i>Pm</i>	10	10	11	8	8	9	7	7	7	7	8	8	9	9	10	8	8	8	22	NT	NT	NT
<i>Ef</i>	11	13	13	9	9	9	8	10	10	8	9	9	7	7	7	7	7	7	22	NT	NT	NT
<i>Shi</i>	10	11	11	10	11	11	11	14	14	11	11	11	9	10	11	9	9	10	22	NT	NT	NT
<i>Ca</i>	11	11	12	9	10	11	10	11	12	10	11	12	10	10	11	9	9	10	NT	NT	NT	16

Se = *Staphylococcus epidermidis*; *Bc* = *Bacillus cereus*; *Bs* = *Bacillus subtilis*; *S* = *Salmonella*; *Pm* = *Proteus mirabilis*; *Ef* = *Enterococcus faecalis*; *Shi* = *Shigella*; *Ca* = *Candida albicans*; Diameter of zone (mm); NT = not tested; Cipro = ciprofloxacin; Keto = ketoconazole.

Table 4. Minimum inhibitory concentration (MIC) exhibited by *Byrsonima* species.

Microorganisms	MIC (mg/mL)					
	<i>B. basiloba</i>		<i>B. intermedia</i>		<i>B. fagifolia</i>	
	MeOH	MeOH 80%	MeOH	MeOH 80%	MeOH	MeOH 80%
<i>Se</i>	6.0	6.0	6.0	6.0	6.0	6.0
<i>Bs</i>	6.0	6.0	3.0	7.5	6.0	6.0
<i>Bc</i>	6.0	6.0	6.0	7.5	6.0	3.0
<i>Ef</i>	6.0	6.0	1.5	6.0	7.5	3.0
<i>Shi</i>	6.0	6.0	6.0	12.0	6.0	3.0
<i>Pm</i>	9.0	7.5	9.0	7.5	6.0	6.0
<i>S</i>	7.5	6.0	6.0	6.0	6.0	7.5
<i>Ca</i>	7.5	7.5	6.0	9.0	6.0	6.0

Se = *Staphylococcus epidermidis*; *Bc* = *Bacillus cereus*; *Bs* = *Bacillus subtilis*; *S* = *Salmonella*; *Pm* = *Proteus mirabilis*; *Ef* = *Enterococcus faecalis*; *Shi* = *Shigella*; *Ca* = *Candida albicans*.

on the EMeOH 80% than EMeOH. This difference can be expected by the higher polarity of the solvent extraction.

The results appear promising for a possible use of the *B. intermedia* and *B. fagifolia* or their components as antimicrobial agents. However, further studies are necessary to evaluate the toxic effects of these extracts.

Further phytochemical studies are in progress to establish which are the compounds responsible for the bioactivity of these medicinal plants and thus ascertain the value of the ethnobotanical approach for the screening of plants as potential sources of bioactive substances.

ACKNOWLEDGEMENTS

We are grateful to Fundação Amparo à Pesquisa do Estado de São Paulo (FAPESP) for a grant to M.S. and to M.E.F. and for fundings from the Biota-Fapesp Program and to Conselho Nacional de Desenvolvimento Científico (CNPq) for a grant to D.C.M., to H.R.N.S. and to W.V.

REFERENCES

- Agra MF, França PF, Barbosa-Filho JM 2007. Synopsis of the plants known as medicinal and poisonous in Northeast of Brazil. *Rev Bras Farmacogn* 17: 114-140.
- Agra MF, Silva KN, Basílio IJLD, França PF, Barbosa-Filho JM 2008. Survey of medicinal plants used in the region Northeast of Brazil. *Rev Bras Farmacogn* 18: 472-508.
- Amaral FMM, Ribeiro MNS, Barbosa-Filho JM, Reis AS, Nascimento FRF, Macedo RO 2006. Plants and chemical constituents with giardicidal activity. *Rev Bras Farmacogn* 16 (Supl.): 696-720.
- Barbosa-Filho JM, Nascimento-Júnior FA, Tomaz ACA, Athayde-Filho PF, Silva MS, Cunha EVL, Souza MFV, Batista LM, Diniz MFFM 2007. Natural products with antileprotic activity. *Rev Bras Farmacogn* 17: 141-148.
- Bauer AW, Kirby MDK, Sherris JC, Truck M 1966. Antibiotic

susceptibility testing by a standardized single disk method. *Am J Clin Pathol* 45: 493-496.

- Bejar E, Amarquaye A, Chec C-T, Malone MH, Fong HHS 1995. Constituents of *Byrsonima crassifolia* and their spasmogenic activity. *Int J Pharmacogn* 33: 25-32.
- Bezerra JL, Costa GC, Lopes TC, Carvalho ICDS, Patrício FJ, Sousa SM, Amaral FMM, Rebelo JMM, Guerra RNM, Ribeiro MNS, Nascimento FRF 2006. Avaliação da atividade leishmanicida *in vitro* de plantas medicinais. *Rev Bras Farmacogn* 16 (Supl.): 631-637.
- Bokesch HR, McKee TC, Currens MJ, Gulakowski RJ, McMahan JB, Cardellina II JH, Boyd MR 1996. HIV-inhibitory gallotannins from *Lepidoptryx staudtii*. *Nat Prod Lett* 8: 133-136.
- Brandão M 1991. Plantas medicamentosas do cerrado mineiro. *Inf Agropec* 15: 15-20.
- Caribé J, Campos JM 1991. *Plantas que Ajudam o Homem: Guia Prático para a Época Atual*. São Paulo: Pensamento.
- Corrêa MP 1984. *Dicionário das Plantas Úteis do Brasil e das Exóticas Cultivadas*. Rio de Janeiro: Ministério da Agricultura IBDF.
- Di Stasi LC 1989. *Plantas Medicinais da Amazônia*. São Paulo: Unesp, p. 39-40.
- Ferreira MB 1980. Plantas portadoras de substâncias medicamentosas, de uso popular, nos cerrados de Minas Gerais. *Inf Agropec* 6: 19-23.
- Figueiredo ME, Michelin DC, Sannomiya M, Silva MA, Santos LC, Almeida LFR, Souza-Brito ARM, Salgado HRN, Vilegas W 2005. Avaliação química e anti-diarreica das folhas de *Byrsonima cinera* DC. (Malpighiaceae). *Rev Bras Cienc Farm* 41: 79-83.
- Funke I, Melzig MF 2006. Traditionally used plants in diabetes therapy - phytotherapeutics as inhibitors of α -amylase activity. *Rev Bras Farmacogn* 16: 1-5.
- 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.
- Leitão SG, Castro O, Fonseca EM, Julião LS, Tavares ES, Leo RRT, Vieira RC, Oliveira DR, Leitão GG, Martino V, Sulsen V, Barbosa YAG, Pinheiro DPG, Silva PEA, Teixeira DF, Lourenço MCS 2006. Screening

- of Central and South American plant extracts for antimycobacterial activity by the Alamar Blue test. *Rev Bras Farmacogn* 16: 6-11.
- Lima MRF, Ximenes CPA, Luna JS, Sant'Ana AEG 2006. The antibiotic activity of some Brazilian medicinal plants. *Rev Bras Farmacogn* 16: 300-306.
- Lopez A, Hudson JB, Towers GHN 2001. Antiviral and antimicrobial activities of Colombian medicinal plants. *J Ethnopharmacol* 77: 189-196.
- Martinez-Vasquez M, Gonzalez-Esquinca AR, Cazares-Luna L, Moreno-Gutierrez MN, Garcia-Argãez AN 1999. Antimicrobial activity of *Byrsonima crassifolia* (L.) H.B.K. *J Ethnopharmacol* 66: 79-82.
- Martins ER, Castro DM, Castellani DC 1994. *Plantas Mediciniais*. Viçosa: Imprensa Universitária UFV.
- Matos FJA 1994. *Farmácias Vivas*. Fortaleza: EUFC.
- Mendes CC, Cruz FG, David JM 1999. Triterpenos esterificados com ácidos graxos e ácidos triterpênicos isolados de *Byrsonima microphylla*. *Quim Nova* 22: 185-188.
- Nascimento GGF, Locatelli J, Freitas PC, Silva GL 2000. Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. *Braz J Microbiol* 31: 247-256.
- NCCLS 2003. National Committee for Clinical Laboratory Standards - *Performance Standards for Antimicrobial Disc Susceptibility Tests 2003*. Approved Standard M2-A7. Pennsylvania: Wayne.
- Nishizawa M, Yamagashi T, Dutschman GE, Parker WB, Bodner AJ, Kilkuskie RE, Cheng Y-C, Lee K-H 1989. Anti-Aids agents. 1. Isolation and characterisation of four new tetragalloylquinic acids as a new class of HIV reverse transcriptase inhibitors from tannic acid. *J Nat Prod* 52: 762-768.
- Rastrelli L, De Tommasi N, Berger I, Caceres A, Saravia A, De Simone F 1997. Glycolipids from *Byrsonima crassifolia*. *Phytochemistry* 45: 647-650.
- Rodríguez M, Hasegawa M, González-Mújica F, Motta N, Castillo A, Castillo J, Zea E, Mora K, Sousa L, González A, Camejo D 2008. Antidiabetic and antiradical activities of plants from Venezuelan Amazon. *Rev Bras Farmacogn* 18: 331-338.
- Sannomiya M, Rodrigues CM, Coelho RG, Dos Santos LC, Hiruma-Lima CA, Souza-Brito ARM, Vilegas W 2004. Application of preparative high-speed counter-current chromatography for the separation of flavonoids from the leaves of *Byrsonima crassa* Niedenzu (IK). *J Chromatogr A* 1035: 47-51.
- Sannomiya M, Fonseca VB, Da Silva MA, Rocha LRM, Dos Santos LC, Hiruma-Lima CA, Souza-Brito ARM, Vilegas W 2005a. Flavonoids and antiulcerogenic activity from *Byrsonima crassa* leaves extracts. *J Ethnopharmacol* 97: 1-6.
- Sannomiya M, Montoro P, Piacente S, Pizza C, Souza-Brito ARM, Vilegas W 2005b. Application of liquid chromatography/electrospray ionization tandem mass spectrometry to the analysis of polyphenolic compounds from an infusion of *Byrsonima crassa* Niedenzu. *Rapid Commun Mass Spectrom* 19: 2244-2250.
- Sannomiya M, Michelin DC, Rodrigues CM, Dos Santos LC, Salgado HRN, Hiruma-Lima CA, Souza-Brito ARM, Vilegas W 2005c. *Byrsonima crassa* Niedenzu (IK): antimicrobial activity and chemical study. *Rev Cienc Farm Básica Apl* 26: 71-75.
- Sannomiya M, Dos Santos LC, Carbone V, Napolitano A, Piacente S, Pizza C, Souza-Brito ARM, Vilegas W 2007. Liquid chromatography/electrospray ionization tandem mass spectrometry profiling of compounds from the infusion of *Byrsonima fagifolia* Niedenzu. *Rapid Commun Mass Spectrom* 21: 1393-1400.
- Saúde-Guimarães DA, Faria AR 2007. Substâncias da natureza com atividade anti-*Trypanosoma cruzi*. *Rev Bras Farmacogn* 17: 455-465.
- Schneider G 1990. *Arzneidrogen. Ein Kompendium für Pharmazeuten, Biologen und Chemiker*. Mannheim: Wissenschaftsverlag.
- Silva SR, Silva AP, Munhoz CB, Silva Jr MC, Medeiros MB 2001. *Guia de Plantas do Cerrado utilizadas na Chapada dos Veadeiros*. Brasília: WWF.
- Silver LL, Bostian KA 1993. Discovery and development of new antibiotics: the problem of antibiotic resistance. *Antimicrob Agents Chemother* 37: 377-383.
- Simões CMO, Schenkel EP, Gosmann G, Mello JCP, Mentz LA, Petrovick PR 2001. *Farmacognosia, da Planta ao Medicamento*. 3. ed, Porto Alegre / Florianópolis: Ed. Universidade/ UFRGS, Ed. da UFSC.
- Wagner H, Bladt H, Zgainski EM 1984. *Plant Drug Analysis*. Berlin: Springer.