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Artigo

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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 gastrintestinais 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.,

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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* (Martinéz-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 (nº 6398). The plant material of *Byrsonima basiloba* A. Juss. (nº 24163) and *B. intermedia* A. Juss. (nº 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 $ECHCl_3$, 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/$CeSO_4$ (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 ºC. After 6 h of growth, each microorganism culture, at a concentration of 10^6 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 ºC 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 10^6 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 ºC 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 $ECHCl_3$ 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 %)
B. basiloba (1000 g)	77.7 (7.7%)	94.2 (9.4%)	63.1 (6.1%)
B. intermedia (590 g)	16.7 (2.8%)	85.2 (14.4%)	29.6 (5.0%)
B. fagifolia (1000 g)	47.3 (4.7%)	24.6 (2.5%)	12.9 (1.3%)

Table 2. Phytochemical screening of *Byrsonima* species.

Test	*B. fagifolia*			*B. basiloba*			*B. intermedia*		
	$ECHCl_3$	EMeOH	EMeOH 80%	$ECHCl_3$	EMeOH	EMeOH 80%	$ECHCl_3$	EMeOH	EMeOH 80%
Catechins	-	-	-	-	+	+	-	+	+
Tannins	-	-	-	-	+	+	-	+	+
GAD	-	+	+	-	+	+	-	+	+
Flavonoids	-	+	+	-	+	+	-	+	+
Saponins	-	-	-	-	-	-	-	-	-
Terpenes	+	-	-	+	-	-	+	-	-

absent = (-); present = (+); $ECHCl_3$ = chloroform extract; EMeOH = methanol extract; EMeOH 80% = hydromethanol extract; GAD = gallic acid derivatives.

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

Species	*B. intermedia*						*B. basiloba*						*B. fagifolia*							
Extract/positive control/negative control	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	5	40
Microorganisms	Inhibition zones (mm)																			
Se	11	11	12	12	12	12	12	12	12	12	13	14	10	10	10	8	9	9	25	NT
Bc	11	11	12	9	10	11	10	11	12	10	11	11	9	9	9	9	9	9	20	NT
Bs	10	10	11	10	11	11	11	11	11	11	11	12	10	11	12	8	9	9	20	NT
S	10	10	11	11	11	11	11	11	12	11	12	12	10	10	11	9	9	9	22	NT
Pm	10	10	11	8	8	9	7	7	7	7	8	8	9	9	10	8	8	8	22	NT
Ef	11	13	13	9	9	9	8	10	10	8	9	9	7	7	7	7	7	7	22	NT
Shi	10	11	11	10	11	11	11	14	14	11	11	11	9	10	11	9	9	10	22	NT
Ca	11	11	12	9	10	11	10	11	12	10	11	12	10	10	11	9	9	10	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)					
	B. basiloba		*B. intermedia*		*B. fagifolia*	
	MeOH	MeOH 80%	MeOH	MeOH 80%	MeOH	MeOH 80%
Se	6.0	6.0	6.0	6.0	6.0	6.0
Bs	6.0	6.0	3.0	7.5	6.0	6.0
Bc	6.0	6.0	6.0	7.5	6.0	3.0
Ef	6.0	6.0	1.5	6.0	7.5	3.0
Shi	6.0	6.0	6.0	12.0	6.0	3.0
Pm	9.0	7.5	9.0	7.5	6.0	6.0
S	7.5	6.0	6.0	6.0	6.0	7.5
Ca	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.

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