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Modulatory Effect of *Byrsonima basiloba* Extracts on the Mutagenicity of Certain Direct and Indirect-Acting Mutagens in *Salmonella typhimurium* Assays

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ABSTRACT *Byrsonima basiloba* A. Juss. species is a native arboreal type from the Brazilian "cerrado" (tropical American savanna), and the local population uses it to treat diseases, such as diarrhea and gastric ulcer. It belongs to the Malpighiaceae family, and it is commonly known as "murici." Considering the popular use of *B. basiloba* derivatives and the lack of pharmacological potential studies regarding this vegetal species, the mutagenic and antimutagenic effect of methanol (MeOH) and chloroform extracts were evaluated by the Ames test, using strains TA97a, TA98, TA100, and TA102 of *Salmonella typhimurium*. No mutagenic activity was observed in any of the extracts. To evaluate the antimutagenic potential, direct and indirect mutagenic agents were used: 4 nitro-*o*-phenylenediamine, sodium azide, mitomycin C, aflatoxin B_1, benzo[*a*]pyrene, and hydrogen peroxide. Both the extracts evaluated showed antimutagenic activity, but the highest value of inhibition level (89%) was obtained with the MeOH extract and strain TA100 in the presence of aflatoxin B_1. Phytochemical analysis of the extracts revealed the presence of *n*-alkanes, lupeol, ursolic and oleanolic acid, (+)-catechin, quercetin-3-*O*-α-L-arabinopyranoside, gallic acid, methyl gallate, amentoflavone, quercetin, quercetin-3-*O*-(2″-*O*-galloyl)-β-D-galactopyranoside, and quercetin-3-*O*-(2″-*O*-galloyl)-α-L-arabinopyranoside.

KEY WORDS: • *Ames test* • *antimutagenic* • **Byrsonima** • *mutagenic*

INTRODUCTION

Compounds capable of promoting mutation are found in our daily diet, as well as in air and water pollution, drinks, medications, and solar radiation. It is known that mutations are a major factor for the onset of cancer. Chemoprevention, *i.e*., prevention against cancer development by chemical substances that act as antimutagenic agents and present the capacity to interact with mutagenic compounds or their metabolites and reduce their effects, is one possible alternative for preventing cancer.[1]

Exposure to environmental mutagenic substances can influence not only the development of cancer, but also cardiovascular and neurodegenerative diseases.[2–4]

The species *Byrsonima basiloba* A. Juss., belonging to the Malpighiaceae family, is commonly known as "murici,"[5] and the local population uses its derivative products for the treatment of diseases, such as diarrhea and gastric ulcer. However, no effective biological activity has been detected up to now, since only a few studies have been performed regarding this species. Previously, our group reported the antidiarrheal activity of methanolic and hydromethanolic extracts obtained from the leaves of *Byrsonima cinera* (now formally documented as *B. basiloba*) determined by intestinal motility in Swiss mice. This assay showed a significant reduction in gastrointestinal motility of both extracts evaluated. A chemical study of the methanolic extract of this species revealed (+)-catechin and quercetin-3-*O*-α-L-arabinopyranoside.[6]

Other species of the *Byrsonima* genus are characterized by the presence of sulfonoglycolipids, phytosterols, triterpenes, aromatic esters, amino acids, proanthocyanidins, and flavonoids.[7]

Considering the popular use of *B. basiloba*, as well as the limited number of pharmacological potential studies regarding this botanical species, the mutagenic and antimutagenic effects of methanol (MeOH) and chloroform ($CHCl_3$) extracts were evaluated using different mutagens with direct (sodium azide [SAZ], mitomycin C (MMC), hydrogen peroxide, and 4-nitro-*o*-phenylenediamine [NPD]) and indirect {aflatoxin B_1 (AFL), benzo[*a*]pyrene (B[*a*]P), and hydrogen

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peroxide (H_2O_2)} action. This study was based on the *Salmonella* reversion assay, which is widely used for the detection of antimutagenic agents, especially those present in plant extracts.[8–11]

MATERIALS AND METHODS

Chemicals

Dimethyl sulfoxide (DMSO), MeOH, $CHCl_3$, nicotinamide adenine dinucleotide phosphate sodium salt, D-glucose-6-phosphate disodium salt, magnesium chloride, L-histidine monohydrate, D-biotin, SAZ, 2-aminoanthracene, NPD, MMC, B[*a*]P, and AFL were purchased from Sigma Chemical Co. (St. Louis, MO). Oxoid nutrient broth no. 2 (Oxoid, London, UK) and Difco (Detroit, MI) Bacto Agar were used as bacterial media. D-Glucose, magnesium sulfate, citric acid monohydrate, anhydrous dibasic potassium phosphate, sodium ammonium hydrogen phosphate, monobasic sodium phosphate, dibasic sodium phosphate, sodium chloride, and H_2O_2 were purchased from Merck (Whitehouse Station, NJ).

Vegetal material

Leaves of *B. basiloba* A. Juss. were collected at Pratânia, SP, Brazil by Luiz Fernando R. de Almeida of the Botucatu Institute of Biosciences of São Paulo State University (IBB-UNESP), Botucatu, SP, Brazil, and identified by Dr. Jose Clemente Campos, also of the IBB-UNESP. Exsiccates of voucher number 24163 can be found in the UNESP Herbarium, Botucatu.

Ames mutagenicity assay

The *Salmonella* mutagenicity assay was performed using the preincubation method,[12] for 20–30 minutes with *Salmonella typhimurium* strains TA100, TA98, TA97a, and TA102, with and without metabolic activation. The metabolic activation mixture (S9 mix) was freshly prepared before each test using an Aroclor-1254-induced rat liver fraction purchased (lyophilized) from Moltox Molecular Toxicology Inc. (Boone, NC). The *S. typhimurium* strains were kindly provided by Dr. B. Ames of the University of California, Berkeley, CA.

Five different doses of the compounds extracted from the leaves of *B. basiloba* were evaluated in this assay; all of them were diluted in DMSO. The $CHCl_3$ extract was tested in the mutagenicity assay at doses of 1.99, 3.97, 7.95, 11.92, and 15.90 mg per plate, and the MeOH extract at doses of 3.65, 7.30, 14.60, 21.90, and 29.20 mg per plate. For the antimutagenicity assay the following concentrations were used: 0.99, 1.99, 3.97, 7.95, and 15.90 mg per plate for the $CHCl_3$ extract and 0.46, 0.91, 1.83, 3.65, and 7.30 mg per plate with indirect mutagens and 1.82, 3.65, 7.30, 14.60, and 21.90 mg per plate with direct mutagens for the MeOH extract.

The concentrations used were based on the bacterial toxicity of each preparation, estimated in a preliminary test. In all subsequent assays, the upper limit of the dose range tested was either the highest nontoxic dose or the lowest toxic dose determined in this preliminary assay. Toxicity was apparent either as a reduction in the number of His^+ revertants or as an alteration in the auxotrophic background (*i.e.*, background lawn).

Each concentration of the tested mixtures was added to 500 μL of pH 7.4 buffer or 500 μL of S9 mix (to test the influence of metabolic activation) and 100 μL of bacterial culture and then incubated at 37°C for 20–30 minutes. After this time 2 mL of top agar was added, and the mixture was poured onto a plate containing minimal agar. The plates were incubated at 37°C for 48 hours, and the His^+ revertant colonies were manually counted. All the experiments were performed in triplicate.

The standard mutagens used as positive controls in experiments without S9 mix were NPD (10 μg per plate) for TA98 and TA97a, SAZ (1.25 μg per plate) for TA100, and daunomycin (3 μg per plate) for TA102. 2-Aminoanthracene (0.125 μg per plate) was used in the experiments with metabolic activation for all strains. DMSO served as the negative (solvent) control (100 μL per plate).

Statistical analysis was performed with the Salanal computer program, adopting the model of Bernstein *et al.*[13] The mutagenic index was also calculated for each dose, as the average number of revertants per plate divided by the average number of revertants per plate of the negative (solvent) control. A sample was considered positive when the mutagenic index was ≥ 2 for at least one of the tested doses and when the response was dose-dependent.[14]

Ames antimutagenicity assay

According to the methodology of preincubation in plates, developed by Maron and Ames,[12] different concentrations of MeOH and $CHCl_3$ extracts of *B. basiloba* were mixed with 0.1 mL of bacterial culture and the mutagenic agent (SAZ, NPD, MMC, H_2O_2, AFL, or B[*a*]P) and then incubated at 37°C for 20–30 minutes. After incubation, 2 mL of top agar was added and supplemented with traces of histidine and biotin, and the content of each tube was lightly homogenized and poured onto a plate with minimum glucose agar. After the solidification of top agar, the plates were incubated for 48 hours at 37°C, and the number of revertant colonies per plate was counted. The entire assay was performed in triplicate. The percentage of mutagenicity inhibition was calculated according to Tachino *et al.*,[15] where:

$$\text{Inhibition} = \left(1 - \frac{\text{revertants induced per plate with inhibitor}}{\text{revertants induced per plate without inhibitor}} \right) \times 100$$

A non-antimutagenic effect was considered when a value lower than 25% was obtained, a moderate effect when a value between 25% and 40% was obtained, and strong antimutagenicity at values greater than 40%.[16]

Cell viability was also determined for each antimutagenesis experiment to evaluate the potential bactericidal effect of the mutagens. The responses were considered cytotoxic when the percentage of sample survival was less than 60% of the total observed for the negative control.[17]

Phytochemical analysis

The powdered leaves (800 g) were extracted exhaustively and successively with $CHCl_3$ and MeOH at room temperature, 48 hours for each solvent. The solvents were evaporated at 60°C under reduced pressure, providing the $CHCl_3$ (94.5 g) and MeOH (79.3 g) extracts, respectively. The yields (wt/wt) for the $CHCl_3$ and MeOH extracts from the dried powders of *B. basiloba* leaves were 11.8% and 9.9%, respectively.

A portion of $CHCl_3$ extract (30.0 g) was filtered through a silica column (15 cm × 5.0 i.d.) to separate the compounds according to their polarity. This column initiated the elution with a pure *n*-hexane solvent, then with dichloromethane, and finally methanol. After evaporation of the remaining solvent, the hexane fraction (HF) (0.4 g), dichloromethane fraction (DF) (12.0 g), and MeOH fraction (MF) (10.3 g) were obtained, respectively. Part of the HF was chromatographed through a chromatography column (silica gel 60) with hexane (100%) that produced an *n*-alkane mixture of C_{30}–C_{34} consisting almost exclusively of triacontane (C_{30}) and dotriacontane (C_{32}). The MF was analogously fractionated by silica gel column chromatography using $CHCl_3$ as an eluent, gradually increasing the polarity with MeOH. The fractions were guided to provide ursolic and oleanolic acids. The DF resulted in an exclusive mixture of lupeol. These triterpenes were identified by comparison of their spectroscopic data with those reported in the literature.[18]

n-*Alkane identification: gas chromatography analyses*

Gas chromatography analyses were performed using a Varian (Walnut Creek, CA) CP 3380 gas chromatograph equipped with a fused silica CBP-5 capillary column (25 m × 0.33 mm i.d.; film thickness 0.5 m) coupled to a flame ionization detector. Hydrogen was used as the carrier gas (60 kPa), and the injection split ratio was 1:30. The injection temperature was 250°C; the column temperature was maintained at 50°C for 1 minute and then increased to 300°C at 10°C/minute, where this temperature was maintained for 10 minutes; the detector temperature was 280°C. *n*-Alkane standards and the fraction isolated from the HF of 1 μL were manually injected using a 10-μL Hamilton (Reno, NV) syringe.

Phytochemical screening and compound isolation from the MeOH extract

The fresh MeOH extract proved positive for steroids, flavonoids, and proanthocyanidins.[19]

A portion (10.0 g) of the MeOH extract of *B. basiloba* was partitioned with a mixture of ethyl acetate/H_2O (1:1, vol/vol). The ethyl acetate fraction (4.0 g) was subjected to gel permeation chromatography on a Sephadex LH-20 (Pharmacia, Piscataway, NJ) column (57 × 3 cm), eluted with MeOH. Fractions (15 mL) were collected and checked by thin-layer chromatography on silica gel plates eluted with a mixture of $CHCl_3$/MeOH/H_2O (80:18:2, by volume) and revealed either with natural products/polyethyleneglycol reagent or with anisaldehyde/sulfuric acid solution.[20] Fractions 28–32 (60.0 mg) were successively fractionated by column chromatography on silica gel (Merck, 10.0 × 1.5 cm i.d.) eluted with $CHCl_3$/MeOH (90:10, vol/vol), providing gallic acid and methyl gallate (18.0 mg). Fractionation was realized by cellulose column chromatography (12.0 × 1.5 i.d.) using MeOH/H_2O (80:20) to yield the pure biflavonoid amentoflavone (8.0 mg).

The nuclear magnetic resonance spectra in DMSO-d_6 were obtained using a Varian INOVA 500 spectrometer, operating at 500 MHz for ^{1}H and 150 MHz for ^{13}C. Chemical shifts are given as δ (ppm) using trimethylsilane as an internal standard. Thin-layer chromatography analyses were performed on silica gel Si F254 (Merck) plates eluted with $CHCl_3$/MeOH (80:20, vol/vol). The plates were visualized using ultraviolet light (254 nm).

High-performance liquid chromatography analysis

The high-performance liquid chromatography quantitative analysis of the MeOH extract was performed with a liquid chromatograph equipped with a ProStar 210 dual solvent pump, a ProStar 330 photodiode array detector (Varian, Palo Alto, CA), and a Rheodyne (Cotati, CA) model 7125 sample injector with a 20-μL sample loop. The analytical column was a Phenomenex (Torrance, CA) Luna C2 RP18 (250 × 4.6 mm i.d.; particle size 5 μm) equipped with a Phenomenex Security Guard (4.0 × 2.0 mm). The mobile phase compositions used were water (eluent A) and acetonitrile (eluent B), both containing 0.05% trifluoroacetic acid. The gradient program was as follows: 27–34% B (3 minutes), 34–65% B (42 minutes), and 65–100% (5 minutes). Total run time was 50 minutes. The flow rate of the mobile phase was 1.0 mL/minute. Star LC Workstation software was used both for the operation of the detector and for data processing. These experiments permitted the identification of the flavonoids quercetin-3-*O*-(2″-*O*-galloyl)-β-D-galactopyranoside, quercetin-3-*O*-(2″-*O*-galloyl)-α-L-arabinopyranoside, and quercetin. Compound identification was performed by comparison retention time, by spiking with known standards, and by comparison with previously isolated compounds under the same conditions. Methods using external standards were used to quantify each compound. For calibration curves, appropriate volumes of the standard stock solutions in rutin (1.0 mg/mL) were diluted with 75% MeOH. For quantitation, peak areas were compared with the calibration curves. Flavonol glycosides and aglycones were calculated as rutin in the concentration range of 10–500 μg/mL ($y = -4.81 \times 10^5 + 1.91 \times 10^5 x$, $R^2 = 0.9999$, $n = 7$, $\lambda = 360$ nm). The high-performance liquid chro-

Table 1. Mutagenic Activity Expressed by the Number of Revertants and Mutagenic Index in Strains TA98, TA100, TA97a, and TA102 of *S. typhimurium* Exposed to Several Doses of $CHCl_3$ and MeOH Extracts of *B. basiloba*, With (+S9) or Without (−S9) Metabolic Evaluation

Treatment (mg/plate)	*TA98*		*TA100*		*TA97a*		*TA102*	
	−S9	*+S9*	*−S9*	*+S9*	*−S9*	*+S9*	*−S9*	*+S9*
$CHCl_3$								
DMSO	37.0 ± 3.61	28.7 ± 4.5	106.3 ± 19.5	97.7 ± 4.7	112.5 ± 7.8	156.0 ± 11.5	291.3 ± 6.7	266.3 ± 31.6
1.99	43.7 ± 12.2 (1.2)	32.7 ± 3.5 (1.2)	119.3 ± 9.8 (1.1)	109.0 ± 10.5 (1.2)	120.0 ± 4.2 (1.1)	162.0 ± 8.9 (1.0)	248.0 ± 32.6 (0.8)	312.7 ± 10.4 (1.2)
3.97	36.3 ± 2.52 (1.0)	27.7 ± 3.5 (1.3)	123.3 ± 8.9 (1.2)	107.7 ± 16.6 (1.1)	133.0 ± 5.7 (1.2)	173.3 ± 10.7 (1.1)	274.3 ± 18.0 (0.9)	304.0 ± 21.0 (1.1)
7.95	41.0 ± 5.2 (1.1)	31.3 ± 7.5 (1.1)	130.0 ± 6.9 (1.2)	116.3 ± 24.6 (1.2)	126.5 ± 6.4 (1.1)	169.3 ± 20.0 (1.1)	201.7 ± 14.3 (0.7)	315.7 ± 18.5 (1.2)
11.92	34.7 ± 7.64 (0.9)	38.7 ± 2.5 (1.0)	126.3 ± 5.1 (1.2)	101.0 ± 13.1 (1.0)	138.0 ± 5.7 (1.2)	195.3 ± 24.7 (1.2)	232.0 ± 7.9 (0.8)	324.7 ± 22.2 (1.2)
15.90	36.7 ± 4.0 (1.0)	34.0 ± 3.6 (1.1)	126.7 ± 7.6 (1.2)	98.7 ± 4.2 (1.0)	154.0 ± 8.5 (1.4)	154.0 ± 10.5 (1.0)	214.0 ± 7.1 (0.7)	313.0 ± 13.5 (1.2)
MeOH								
DMSO	37.0 ± 3.61	28.7 ± 4.5	106.3 ± 19.5	97.7 ± 4.7	112.5 ± 7.8	156.0 ± 11.5	291.3 ± 6.7	266.3 ± 31.6
3.65	41.0 ± 5.2 (1.1)	32.7 ± 2.5 (1.1)	128.7 ± 7.6 (1.2)	92.3 ± 6.7 (0.9)	194.0 ± 1.4 (1.7)*	217.3 ± 20.0 (1.4)	293.7 ± 10.3 (1.0)	285.0 ± 12.8 (1.1)
7.30	28.3 ± 2.89 (0.8)	29.7 ± 4.0 (1.0)	122.0 ± 6.2 (1.5)*	104.7 ± 5.9 (1.1)	167.0 ± 5.7 (1.5)*	267.0 ± 10.9 (1.7)*	242.7 ± 11.1 (0.8)	293.0 ± 8.5 (1.1)
14.60	44.0 ± 19.2 (1.0)	30.7 ± 5.9 (1.1)	125.7 ± 12.9 (1.2)	Cytotoxic	100.5 ± 4.9 (0.9)	255.0 ± 6.0 (1.6)*	233.7 ± 31.2 (0.8)	285.3 ± 39.3 (1.1)
21.90	39.0 ± 9.54 (1.0)	33.0 ± 3.6 (1.1)	177.0 ± 11.8 (1.7)*	Cytotoxic	Cytotoxic	138.7 ± 6.5 (0.9)	219.0 ± 28.3 (0.7)	292.3 ± 13.6 (1.1)
29.20	48.3 ± 3.06 (1.3)	33.0 ± 5.6 (1.1)	191.3 ± 26.0 (1.8)*	Cytotoxic	Cytotoxic	140.3 ± 8.6 (0.9)	224.3 ± 9.1 (0.8)	291.7 ± 21.6 (1.1)
Positive control	766.3 ± 76.3[a]	1,314.3 ± 143.1[b]	1,213.9 ± 107.8[c]	1,534.9 ± 176.1[b]	1,456.7 ± 122.9[a]	1,316.7 ± 197.9[b]	1,857.6 ± 121.2[b]	1,973.3 ± 231.1[b]

Data are mean ± SD values (mutagenic index in parentheses). DMSO (at 100 μL per plate) was the negative control.
[a]NPD, 10.0 μg per plate.
[b]2-Aminoanthracene, 1.25 μg per plate.
[c]SAZ, 1.25 μg per plate.
[d]Daunomycin, 3.0 μg per plate.
[e]$P < .05$.

TABLE 2. ANTIMUTAGENIC ACTIVITY EXPRESSED BY THE NUMBER OF REVERTANTS AND PERCENTAGE OF INHIBITION OF THE MEOH EXTRACT OF *B. BASILOBA* WITH DIRECT MUTAGENIC COMPOUNDS, USING STRAINS TA97A, TA98, TA100, AND TA102 OF *S. TYPHIMURIUM* IN THE ABSENCE OF S9

		Extract concentration (mg per plate)				
Strain	*Mutagen*	*1.82*	*3.65*	*7.30*	*14.60*	*21.90*
TA97a	810 ± 30[a]	318 ± 80 (Tox)	335 ± 9 (Tox)	94 ± 30 (Tox)	45 ± 8 (Tox)	22 ± 3 (Tox)
TA98	504 ± 9[a]	341 ± 31 (32)	340 ± 39 (32)	327 ± 17 (35)	286 ± 37 (43)	96 ± 20 (81)
TA100	2,373 ± 90[b]	1,770 ± 130 (25)	2,016 ± 194 (15)	2,074 ± 12 (12)	1,366 ± 145 (Tox)	495 ± 130 (Tox)
TA102	1,737 ± 77[c]	1,507 ± 7 (13)	1,558 ± 85 (10)	1,265 ± 24 (27)	605 ± 88 (65)	620 ± 167 (64)
	503 ± 11[d]	332 ± 15 (34)	235 ± 75 (53)	242 ± 52 (52)	78 ± 3 (Tox)	67 ± 2 (Tox)

Data are mean ± SD values (percentage of inhibition). Tox, viability <60%.
[a]NPD, 10.0 μg per plate.
[b]SAZ, 1.25 μg per plate.
[c]MMC, 0.5 μg per plate.
[d]H_2O_2, 0.034 mg per plate.

matography quantitative data were expressed as milligrams of flavonoid derivative per gram of solid extract (see Table 6).

RESULTS

Mutagenic activity

In the mutagenicity tests with the MeOH and $CHCl_3$ extracts of the *B. basiloba* species, no positive mutagenicity was observed (Table 1). The results obtained for the $CHCl_3$ extract presented an increase in the frequency of revertants in the strain TA100 (-S9), but the mutagenic index was not higher than 2. In the same strain, the MeOH extract became cytotoxic after metabolic activation at the highest three doses tested. In strain TA97a, cytotoxicity was also evident without metabolic activation.

Antimutagenic activity

The results obtained from studies on the antimutagenic potential of *B. basiloba* extracts are presented in Tables 2–5.

The MeOH extract was evaluated in strains TA97a, TA98, TA100, and TA102 using direct (NPD, SAZ, MMC, and H_2O_2) and indirect (AFL, B[*a*]P, and H_2O_2) mutagens. When the strain TA97a was used, this extract presented no reduction in the number of revertant colonies. However, in strain TA98, levels of reduction were obtained when tested with NPD (32–81%; Table 2), AFL (68–81%), and B[*a*]P (66–82%) (Table 3). For strain TA100, the MeOH extract showed no protective effects against the action of SAZ (Table 2) but showed a strong protective effect when associated with AFL (84–89%; Table 3). The protective action of the extract against the H_2O_2 mutagenic effect was evaluated in strain TA102, and it was concluded that the muta-

TABLE 3. ANTIMUTAGENIC ACTIVITY EXPRESSED BY THE NUMBER OF REVERTANTS AND PERCENTAGE OF INHIBITION OF THE MEOH EXTRACT OF *B. BASILOBA* WITH INDIRECT MUTAGENIC COMPOUNDS USING STRAINS TA97A, TA98, TA100, AND TA102 OF *S. TYPHIMURIUM* IN THE PRESENCE OF S9

		Extract concentration (mg per plate)				
Strain	*Mutagen*	*0.46*	*0.91*	*1.83*	*3.65*	*7.30*
TA97a	1,182 ± 45[a]	276 ± 12 (Tox)	217 ± 10 (Tox)	245 ± 12 (Tox)	209 ± 1.5 (Tox)	199 ± 25 (Tox)
	749 ± 6[b]	187 ± 63 (Tox)	210 ± 13 (Tox)	193 ± 16 (Tox)	191 ± 15 (Tox)	144 ± 42 (Tox)
		1.82	*3.65*	*7.30*	*14.60*	*21.90*
TA98	613 ± 16[a]	199 ± 24 (68)	164 ± 14 (73)	194 ± 13 (68)	145 ± 16 (76)	119 ± 17 (81)
	595 ± 1[b]	201 ± 8 (66)	169 ± 12 (72)	163 ± 8 (73)	163 ± 10 (73)	110 ± 9 (82)
TA100	1,627 ± 77[a]	254 ± 16 (84)	228 ± 12 (86)	179 ± 8 (89)	128 ± 16 (Tox)	117 ± 17 (Tox)
	710 ± 22[b]	95 ± 3 (Tox)	96 ± 11 (Tox)	86 ± 7 (Tox)	53 ± 3 (Tox)	13 ± 2 (Tox)
TA102	614 ± 18[c]	200 ± 8 (68)	222 ± 11 (64)	188 ± 32 (Tox)	133 ± 32 (Tox)	61 ± 28 (Tox)

Data are mean ± SD values (percentage of inhibition). Tox, viability <60%.
[a]AFL, 0.5 μg per plate.
[b]B[*a*]P, 1 μg per plate.
[c]H_2O_2, 0.034 mg per plate.

Table 4. Antimutagenic Activity Expressed by the Number of Revertants and Percentage of Inhibition of $CHCl_3$ Extract of *B. basiloba* with Direct Mutagenic Compounds Using Strains TA97a, TA98, TA100, and TA102 of *S. typhimurium* in the Absence of S9

		Extract concentration (mg per plate)				
Strain	*Mutagen*	*0.99*	*1.99*	*3.97*	*7.95*	*15.90*
TA97a	810 ± 30[a]	203 ± 46 (75)	197 ± 20 (76)	178 ± 17 (78)	179 ± 16 (78)	193 ± 10 (76)
TA98	504 ± 9[a]	341 ± 31 (32)	340 ± 39 (33)	327 ± 17 (35)	286 ± 37 (43)	196 ± 20 (61)
TA100	2,373 ± 90[b]	1,770 ± 130 (25)	2,016 ± 194 (15)	2,074 ± 12 (13)	1,366 ± 145 (44)	495 ± 130 (79)
TA102	1,731 ± 77[c]	1,724 ± 182 (0.4)	1,182 ± 122 (32)	783 ± 61 (55)	578 ± 16 (67)	585 ± 84 (66)
	503 ± 11[d]	418 ± 29 (17)	493 ± 56 (2)	418 ± 26 (17)	392 ± 13 (22)	270 ± 28 (46)

Data are mean ± SD values (percentage of inhibition). Tox, viability <60%.
[a]NPD, 10.0 μg per plate.
[b]SAZ, 1.25 μg per plate.
[c]MMC, 0.5 μg per plate.
[d]H_2O_2, 0.034 mg per plate.

genic potential was significantly reduced independent of the presence or absence of metabolization. The levels of inhibition of the mutagenicity observed varied from 34% to 52% (Table 2) when tested in the absence of metabolic activation and around 60% when tested in the presence of metabolization (Table 3). Besides H_2O_2, a significant reduction on the number of revertant colonies also occurred in the same strain when the mutagen used was MMC (13–65%; Table 2).

As for the $CHC1_3$ extract, a general reduction in the mutagenic potential of the mutagens was observed. The levels of reduction of the mutagenicity were from 75% to 78% for NPD (Table 4) and 10% to 78% for B[*a*]P in strain TA97a (Table 5). For strain TA98, reductions varied from 32% to 61% for NDP (Table 4), 56% to 66% for B[*a*]P, and 66% to 74% for AFL (Table 5). In the case of TA100, the levels of reduction of the mutagenicity obtained with SAZ were 25–79% (Table 4), whereas for B[*a*]P and AFL, toxicity was observed at all concentrations (Table 5). For strain TA102, when H_2O_2 was used without metabolization, the values obtained varied from 2% to 46% (Table 4), and in the presence of metabolization, values were around 60% (Table 5). When the mutagenic agent was MMC, the percentages of reduction were 0.4–67% (Table 4).

Phytochemical analysis

The HF of the $CHCl_3$ extract from *B. basiloba* was analyzed by gas chromatography under the same condition as the aforementioned hydrocarbon standard, in order to obtain chromatograms and retention times. The results of hydrocarbon analyses showed the presence of a mixture of *n*-alkanes (C_{30}–C_{34}). These consisted of only saturated straight-chain hydrocarbons with triacontane (C_{30}) and dotriacontane (C_{32}) as the major components.

Fractionation of the DF resulted in the isolation of lupeol, and the MF provided ursolic and oleanolic acid.

The initial study of the MeOH extract of *B. basiloba* showed the presence of (+)-catechin and quercetin-3-*O*-α-L-arabinopyranoside.[6] In this work, the isolation or identifi-

Table 5. Antimutagenic Activity Expressed by the Number of Revertants and Percentage of Inhibition of the $CHCl_3$ Extract of *B. basiloba* with Indirect Mutagenic Compounds, Using Strains TA97a, TA98, TA100, and TA102 of *S. typhimurium* in the Presence of S9

		Extract concentration (mg per plate)				
Strain	*Mutagen*	*0.99*	*1.99*	*3.97*	*7.95*	*15.90*
TA97a	749 ± 6[a]	67 ± 10 (10)	289 ± 14 (61)	164 ± 14 (78)	178 ± 9 (76)	165 ± 13 (78)
	1,128 ± 45[b]	276 ± 12 (Tox)	217 ± 10 (Tox)	245 ± 12 (Tox)	209 ± 1.5 (Tox)	199 ± 2.5 (Tox)
TA98	595 ± 1[a]	259 ± 11 (56)	212 ± 4 (64)	208 ± 13 (65)	258 ± 13 (56)	197 ± 9 (66)
	613 ± 16[b]	211 ± 2 (66)	183 ± 18 (70)	186 ± 7 (70)	179 ± 4 (70)	158 ± 4 (74)
TA100	710 ± 22[a]	350 ± 15 (Tox)	253 ± 16 (Tox)	85 ± 16 (Tox)	74 ± 11 (Tox)	23 ± 2.3 (Tox)
	1,627 ± 75[b]	223 ± 16 (Tox)	169 ± 16 (Tox)	121 ± 19 (Tox)	194 ± 84 (Tox)	134 ± 9 (Tox)
TA102	614 ± 18[c]	254 ± 8 (59)	269 ± 31 (56)	267 ± 6 (57)	234 ± 13 (62)	261 ± 24 (57)

Data are mean ± SD values (percentage of inhibition). Tox, viability <60%.
[a]B[*a*]P, 1 μg per plate.
[b]AFL, 0.5 μg per plate.
[c]H_2O_2, 0.034 mg per plate.

cation of gallic acid, methyl gallate, the biflavonoid amentoflavone, quercetin-3-*O*-(2″-*O*-galloyl)-β-D-galactopyranoside, quercetin-3-*O*-(2″-*O*-galloyl)-α-L-arabinopyranoside, and quercetin is described and is shown in Table 6.

DISCUSSION

Scientific studies that involve medicinal plants are being developed each day. They are important not only on an academic level, but are also economically and socially important. People have been using alternative medicinal treatments and plants for centuries, and the pharmaceutical and cosmetics industries are looking for natural sources of raw materials and active principles for their products.

Nevertheless, in many cases, people do not realize that treatments based on medicinal plants can cause serious risks to their health. Diagnoses are often inaccurate, and the symptoms of numerous serious diseases could be hidden by these types of treatment.[21]

Plants are composed of many chemical compounds, which can be cyto- or genotoxic and could be related to the development of tumors.[22] Reid *et al.*[23] evaluated the mutagenic effect of dichloromethane and 90% MeOH extracts of 42 South African plants and observed that methanol extracts from whole plants of *Helichrysum simillimum*, *Helichrysum herbaceum*, and *Helichrysum rugulosum* demonstrated mutagenicity. Thus, the performance of assays for mutagenicity evaluation, as well as of other risks, is essential, given the consumption of natural products by the population.[24]

In this study, the mutagenicity and antimutagenicity of two different botanical extracts obtained from the leaves of *B. basiloba* were evaluated, since it is commonly used as a medicinal plant in Brazil. Using the Ames test, five different concentrations of a polar extract (MeOH) and an apolar extract ($CHCl_3$) were evaluated.

As shown in Table 1, the $CHCl_3$ and MeOH extracts presented no mutagenicity in any of the strains used. The absence of a mutagenic response by plant extracts against *S. typhimurium* bacterial strains in the Ames assay is a positive step forward in determining the safe use of plants used in traditional medicine. Considering the popular use of this plant and the reduction in gastrointestinal motility,[6] an effect that has been related to increased cancer risk because of longer exposure time to mutagens in the intestine, the negative mutagenic effect in bacterial systems is relevant.

Table 6. Content of the Main Flavonoids from *B. basiloba*

Compound	*Content (mg/g of methanol extract)*[a]
NI	2.20
Quercetin-3-*O*-β-galactopyranoside	3.82
NI	1.83
Quercetin-3-*O*-(2″-*O*-galloyl)-β-D-galactopyranoside	0.17
Quercetin-3-*O*-(2″-*O*-galloyl)-α-D-galactopyranoside	2.97
Quercetin	0.53
Amentoflavone	1.79
Total flavonoids	13.31

NI, not identified.

[a]Flavonoid contents determined by high-performance liquid chromatography-ultraviolet-photodiode array.

Assays were then performed to evaluate the antimutagenic activity against direct and indirect mutagens. B[*a*]P and AFL (indirect), SAZ, NPD, and MMC (direct), and H_2O_2 (direct and indirect) were included among these mutagens.

The extracts showed positive results when tested against the mutagenic agents, *i.e.*, they presented antimutagenic potential for more than one strain and were efficient against different mutational mechanisms (frameshift, TA98 and TA97a; base pair substitution, TA100 and TA102; and antioxidative action, TA102). The highest value for the percentage of inhibition of the mutagenicity (89%) was obtained with the MeOH extract, using the strain TA100 in the presence of AFL.

Phytochemical analysis of the $CHCl_3$ extract in this work showed the presence of a mixture of *n*-alkanes (C_{30}–C_{34}), of which triacontane (C_{30}) and dotriacontane (C_{32}) were the major components; in addition to these, lupeol, ursolic acid, and oleanolic acid were isolated.

The mutagenic or antimutagenic effects of *n*-alkanes have not been described in the literature, but lupeol, ursolic acid, and oleanolic acid are known for their antimutagenic activity.[25–28]

Ursolic acid isolated from the ethanolic fraction of *Eriobotrya japonica* markedly and significantly decreased the numbers of *S. typhimurium* TA100 revertants per plate, thus showing antimutagenic activity.[29]

In addition, lupeol, a triterpene that has had its pharmacological properties approved, was present. According to the literature, lupeol presents anti-inflammatory, anti-arthritis, antimutagenic, antioxidative, and anticarcinogenic properties,[30] as well as hepatoprotective properties against degenerative lesions caused by AFL.[31]

Phytochemical studies of the MeOH extract demonstrated the presence of (+)-catechin, quercetin-3-*O*-α-L-arabinopyranoside, quercetin, gallic acid, methyl gallate, and the amentoflavone. In previous studies by our group, using another species of *Byrsonima*, no mutagenic activity was observed for (+)-catechin, quercetin-3-*O*-α-L-arabinopyranoside, and methyl gallate; however, amentoflavone was positive for mutagenic activity.[32] Despite the fact that this biflavonoid presented mutagenic activity, the amount of this compound found in the MeOH extract of *B. basiloba* (1.79 mg/g of methanolic extract; Table 6) is much smaller than that found in *Byrsonima crassa* (17.04 mg/g of methanolic extract) and *Byrsonima intermedia* (13.70 mg/g of methanolic extract) extracts.[33]

Another aspect that should be considered is that quercetin also presents mutagenic activity[34]; however, in agreement with the present results the amount found in the MeOH extract is small (0.53 mg/g of methanol extract) in relation to

its glucoside derivatives (Table 6). The literature states that the glucoside quercetin presents significantly diminished pro-oxidant activity when compared to the pro-oxidant effect of quercetin aglycone.[35]

Methyl gallate presents no mutagenic potential.[36] In addition, according to Dauer *et al.*,[37] catechin caused minimal damage to the genetic material of hepatocytes only at high concentrations. Martínez *et al.*[36] also reported these data and showed that catechin was not capable of causing oxidative damage in specific strains of *Escherichia coli*. The same authors stated that gallic acid, also found in this vegetal extract, presented the capacity to cause oxidative damage, but that after metabolic activation, this property was no longer present.

Proanthocyanidins, condensed tannins, were isolated from the methanol extract of *B. basiloba*, and, according to Yamakoshi *et al.*,[38] an enriched fraction of these compounds obtained from grape seeds showed no mutagenic effects in the Ames test or on chromosome aberration *in vitro* or in the micronucleus of mouse cells. Dauer *et al*.[37] reported that a fraction of proanthocyanidins obtained from *Hamamelis virginiana* caused breaks in hepatocyte DNA, which were detected using the comet assay; although, as stated in the same study, this tannin fraction also presented antigenotoxic activity *in vitro.*

Botanical extracts are used as medicine for several types of illness and may be used as chemopreventives in different parts of the world. Recently, Bunkova *et al.*[39] demonstrated that green tea contains certain antioxidant substances, such as epigallocatechin, epicatechin, and catechin, among others, that are capable of reducing the level of mutagenicity to 70%. Using green tea extract, which is rich in polyphenols (catechin, epicatechin), Geetha *et al.*[40] showed antioxidative activity when the extract was tested against the oxidant potential of hydrogen peroxide in *S. typhimurium* TA102. In this study the antimutagenic activity of the *B. basiloba* extracts was observed in the presence of H_2O_2 for the TA102 strain that detects the effect of oxidative compounds.

Park *et al.*[41] analyzed flavonoids in *Rhus verniciflus* extract and discovered efficiency in the antimutagenic test, with a considerable reduction in the level of mutagenicity promoted by aflatoxin. In general, this study showed that extracts of *Byrsonima* presented positive results for antimutagenicity and that this occurs in the presence of flavonoids.

In the present study, both MeOH and $CHCl_3$ *B. basiloba* extracts were efficient at protecting bacterial genetic material against different types of damage caused by several mutagenic agents. A number of these agents, like AFL, B[*a*]P, and H_2O_2, are often found in the human daily diet and the environment.

All of these findings highlight that this plant extract could be useful for chemoprevention against damage produced by a variety of mutagenic compounds.

The occurrence rate of cancer is increasing worldwide, and the determination of chemopreventive or chemoprophylaxis compounds is important regarding efforts to reduce the risk of cancer.[42] An effect *in vitro* does not imply an effect *in vivo*, for example, because the responsible components may undergo mammalian metabolism modifying their activity and/or may not be bioavailable. A plant extract indicating antimutagenicity is not necessarily an anticarcinogen; however, it is a strong indication of possible anticarcinogenic properties that should stimulate further investigation. Antimutagenicity determination of plant extracts is also important in the discovery of new effective treatments for preventing the induction of cancer.

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