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Antimicrobial, cytotoxic and mutagenic activities of *Bauhinia holophylla* hydroalcoholic extract

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Abstract
The hydroethanolic leaves extract of *Bauhinia holophylla*, from Fabaceae family, a Brazilian medicinal plant, have been studied as an alternative therapy for the diabetes treatment. In line with the effort to discover novel therapeutic applications, the present study aimed to investigate the antimicrobial activity of *B. holophylla* against microorganisms of clinical relevance. Also, we have sought to determine the cyto-genotoxicity potential to evaluate if the extract exhibit beneficial or harmful effect. The antimicrobial activity of *B. holophylla* was measured by the determination of the minimum inhibitory concentration (MIC; microdilution technique), and the cytotoxicity index (IC_{50}) against the GM 07492, HeLa and HepG2 cell lines was used to calculate the selectivity index (SI). The mutagenicity was determined by the Ames test on *Salmonella* Typhimurium strains TA98, TA97a, TA100 and TA102. *B. holophylla* displayed weak activity against *Staphylococcus aureus* and *Escherichia coli* (MIC = 500 and 2000 µg mL^{-1}, respectively). On the other hand, this extract was active against fungi of the *Candida* species. *B. holophylla* showed MIC values 15.6 µg mL^{-1} against *C. glabrata*, 62.5 µg mL^{-1} against *C. krusei* and *C. tropicalis*, and 125 µg mL^{-1} against *C. parapsilosis and C. albicans*. At these concentrations, the extract showed an absence of cytotoxicity in the human cells. However, the extract induces, at a much higher rate, frame shift mutations (TA98) in the Ames test. These results contribute to valuable data on the antimicrobial potential of *B. hoplophylla* but suggest caution in their use.

Keywords mutagenicity; yeasts; cytotoxicity; *Bauhinia holophylla*

1. Introduction
The genus *Bauhinia* belongs to the Leguminosae family, also known as Fabaceae and comprises more than 300 species. Popularly known as ‘‘paw of cow’’ or ‘‘cow’s hoof’ due to the bilobed aspects of its leaves [1,2] these species are found in tropical regions such as Asia, Africa and South America [3], demonstrating antidiabetic, anti-inflammatory and antioxidant activities [4] and a broad spectrum of in vitro antimicrobial activity against Gram-positive and Gram-negative bacteria, yeast and mold fungi [4–7].
According to Silva and Cechinel Filho [8], several studies confirm their therapeutic properties, due the presence mainly of high contents of flavonoids, as quercetin-3-*O*-α-(2″-galloyl)-rhamnoside and kaempferol-3-*O*-α-(2″-galloyl)-rhamnoside [9,10]. However, other classes of organic compounds of medicinal interest have already been detected, such as glycosides, saponins, phenolic compounds, tannins, fixed oils, fats, and phytosterols [11]. *Bauhinia holophylla* is a species of flowering plant with great medicinal value as antidiabetic [3]. Moreover, Rozza *et al*. [12], demonstrated the antiulcerogenic effect of the hydroalcoholic leaves extract of *B. holophylla* by decreasing oxidative stress and attenuating the inflammatory response. The extract also induced an antidiarrheal effect and there were no signs of toxicity. At the same time, others studies report on their inconvenient effects, suggesting caution in their indiscriminate consumption [2,3]. Ribeiro *et al*. [2] confirmed the presence mainly of flavonol-*O*-glycosides derivatives of quercetin and isorhamnetin by HPLC-PAD and FIA-ESI-IT-MS as previously described by Rozza *et al* [12]. Considering the ethnopharmacological use of the *Bauhinia* species and the risk of using medicinal extracts without detailed investigation, the aim of this study was to evaluate the antimicrobial activity of hydroalcoholic leaves extract of *B. holophylla* against microorganisms of clinical relevance, and their cytotoxic and mutagenic potential, an important approach for safe use of the medicinal plants.

2. Material and Methods

2.1 Plant material and extraction

Samples of *B. holophylla* leaves were collected in the Botanical Garden of Bauru, São Paulo State, Brazil (22º20′30″S and 49°00′30″W), in November 2010. Specimens were identified by Professor Vaz, A.M.S.F and a voucher was deposited at the Herbarium of the Botanical Garden of Rio de Janeiro, Rio de Janeiro State, Brazil with record number RB 507.043.

The leaves were separated, hot air dried at 40° C to constant mass and then pulverized and stored in the dark, under cool, dry conditions, until used. Hydroalcoholic extract was prepared using 7:3 (v/v) EtOH/ H_2O, in a stainless-steel percolator (20 L). The percolation was performed at a moderate flow rate of 2 mL/ min/ kg. The solvent was reduced under reduced pressure from the extract in a rotary evaporator (HeidolphLaborota 4001) at temperature <40° C. The obtained hydroalcoholic extract was suspended in MeOH/H_2O (8:2, v/v), then partitioned with *n*-hexanes to remove lipophilic compounds. The dried extract was powdered and stored in amber bottles at 4°C.

2.2 Chromatographic profiling and identification of metabolite classes

The chemical fingerprint of the hydroalcoholic leaves extractof *B. holophylla* was obtained via High Performance Liquid Chromatography coupled to a Photodiode Array Detector (HPLC-PAD), using a Jasco (Tokyo, Japan) HPLC system equipped with a PU-2089 quaternary solvent pump, MD-2010 PAD and AS-2055 autosampler. The chromatographic separations were performed using a Phenomenex Luna C-18 (250 mm x 4.6 mm Hxi.d.; 5µm) maintained at 40°C. The gradient of separation was performed using water (eluent A) and acetonitrile (eluent B) as mobile phase, both containing 0.1% of formic acid (FA). The gradient program was: 5-100% of B in A (60 min), 100% of B isocratic (70 min). The flow rate was 1.0 mL/min and the total run time was 70 min. EZChrom Elite Data System software (Chromatec, Idstein, Germany) was used for detector operation and data processing. The chromatogram was recorded at 254 nm to detect the phenolic compounds already identified in the leaves of *B. holophylla* [2, 12, 13].

2.3 Antibacterial evaluation - MIC and MBC determinations

The strains of *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 were obtained from American type culture collection (ATCC). The microdilution technique (96-wells plate) was employed to determine the minimum inhibitory concentration (MIC) using a solution of hydroalcoholic extract of *B. holophylla* in different concentrations (from 2000 to 7.8 µg mL^{-1}) by serial dilutions in Mueller Hinton Broth (MHB). The experiment was performed according to the Clinical and Laboratory Standards Institute guidelines [14], with some modifications.

At the end of the serial dilutions, a suspension of each bacteria (10^7 cells/mL) was placed in each well containing different concentrations of *B. holophylla* in the respective wells plate. An ampicillin solution (Sigma-Aldrich®) was used as positive inhibition control (2.5 to 0.019 µg mL^{-1}) and as negative/ solvent control was used 20% of DMSO in MHB. Also, bacterial growth and sterility control of the medium and of extract were carried out. The microplates were incubated at 37°C for 24 h.

At the end of the incubation time, an aliquot of each well was subcultured on MH agar plate surface, to observe the presence or absence of bacterial growth. The determination of the minimum bactericidal concentration (MBC) aims to evaluate the capacity of the reestablishment of the bacterial growth after incubation of 24 h of contact with the vegetal samples.

The bacterial growth was determined by visual readings due the reduction of the medium with resazurin (Sigma-Aldrich®) [15,16]. MIC was defined as the lowest sample concentration that prevented the color changeand exhibited inhibition of microorganism growth. All tests were done in triplicate wells.

2.4 Antifungal evaluation - MIC and MFC determinations

The fungal strains were also obtained from ATCC: *Candida albicans* ATCC-10231, *C. glabrata* ATCC-2001, *C. krusei* ATCC-6258, *C. parapsilosis* ATCC-22019 e *C. tropicalis* ATCC-750. The MIC determination was also determined using the above-mentioned serial dilution assay according to [17], with some modifications.

Two-fold serial dilution was performed to obtain concentrations of the extract ranging from 1000 to 1.95 µg mL^{-1} in RPMI-1640 (Roswell Park Memorial Institute) culture medium. At the end of the serial dilutions a suspension of each yeast (10^3 cells/mL) was placed in each well containing different concentrations of *B. holophylla* in the respective wells plate. The positive controls were the reference standard drugs amphotericin B (8 to 0.06 µg mL^{-1}) and fluconazole (64 to 0.5 µg mL^{-1}), and 20% DMSO was used as negative/ solvent control. The microplates were incubated at 37 °C (48 h).

As an indicator of fungal growth, 20 µL of 2,3,5-triphenyltetrazolium chloride (TTC) dissolved in water was added to the wells and incubated at 37ºC for 2 h. Where fungal growth was inhibited, the solution in the well remained clear after incubation with TTC.

The minimum fungicidal concentration (MFC) was evaluated employing the same procedures in the MBC experiments, however, the Sabouraud Dextrose agar plates (ASD) was used the culture medium. The MFC was considered as the lowest concentration of extract at which there was no microbial growth after 48 h of incubation.

2.5 Cytotoxic activity

The effect of *B. holophylla* extract on the growth of the normal fibroblasts (GM-07492), human hepatocellular carcinoma cells (HepG2 ATCC HB-8065 ™) and human cervical adenocarcinoma (HeLa ATCC CCI-2 ™) was measured with the resazurin cell viability assay. The cells (1.5 x 10^4 cells/ well) were allowed to adhere to the plate surface for 24 h before being exposed to various concentrations of the extract (62.5 to 1000 µg mL^{-1}) for 24 h. The extract was diluted in complete media to reach the final concentrations and 1% of DMSO (final concentration) was used as control. As positive control was used DMSO 20%.

Resazurin solution (0.01%, 50 µL) was added to each well and incubated for 2 h. Fluorescence was evaluated in a microplate reader Synergy H1 (BioTek®) using excitation and emission filters at wavelengths of 530 and 590 nm, respectively.

All data were statistically analysed by GraphPad Prism 5 software, using One-way ANOVA to determine statistical significance, followed by Dunnet's test considering $p < 0.05$. The negative control was designated as 100%, and the results were expressed as a percentage of the negative control. The potency of cell growth inhibition also was expressed as an IC_{50} value that represents the concentration required to reduce

the viability of cells at 50%. IC_{50} was calculated from GraphPad Prism 5.0 program (GraphPad Software, San Diego, CA, USA) by plotting cell survival against the respective concentrations of the *B. holophylla* extract.
The selectivity index (SI) can be calculated by dividing the IC_{50} value by the MIC value. An SI greater than or equal to 10 indicates that the test compound can be applied at a concentration that is ten-fold higher than the MIC value without exhibiting cytotoxicity [18].

2.6 Mutagenic activity

For the evaluation of mutagenicity, the Ames test was performed according to the preincubation methodology developed by Maron and Ames [19]. Five different concentrations of extract were evaluated. The upper limit of the dose range tested was either the highest non-toxic dose or the lowest toxic dose determined in preliminary toxicity test; toxicity was evidenced by a reduction in the number of His + revertants or as background growth on the minimal agar test plates. The *S.* Typhimurium tester strains, TA98, TA97a, TA100, and TA102 were kindly provided by Dr. B.N. Ames (Berkeley, CA, USA) and the experiments were performed with and without metabolic activation.
The metabolic activation mixture (S9 fraction), prepared from livers of Sprague–Dawley rats treated with the polychlorinated biphenyl mixture Aroclor 1254 (500 mg/kg), was purchased from Molecular Toxicology Inc. (Boone, NC, USA) and freshly prepared before each test. The metabolic activation system consisted of 4% S9 fraction, 1% 0.4 M magnesium chloride ($MgCl_2$), 1% 1.65 M potassium chloride (KCl), 0.5% 1 M D-glucose-6-phosphate disodium and 4% 0.1 M nicotinamide adenine dinucleotide phosphate sodium salt (NADP), 50% 0.2 M phosphate buffer and 39.5% sterile distilled water. After 14 h of incubation of bacterial strains in 'Oxoid' nutrient broth No. 2, three independent series of experiments with three plates/treatment for each were carried out. After a 48 h incubation with the extract histidine-independent colonies of *S.* Typhimurium strains were counted manually. The extract is recognized as mutagenic if it causes at least a two-fold increase in the number of histidine-dependent revertant colonies in comparison with control, in at least one of the test strains, while the same result observed only after metabolic activation is indicative of an indirect mechanism of action. A dose-response effect observed in one or more tester strains confirms mutagenicity of a compound. The results are presented as a mean number of histidine-independent revertant colonies with standard deviation for each dose of the test substance [20]. The standard mutagens used as positive controls in experiments without the S9 mix were 4-nitro-*o*-phenylenediamine (10 µg/plate) for TA98, sodium azide (1.25 µg/plate) for TA100 and mithomycin C (0.5 µg/plate) for TA102. 2-aminoanthracene (1.25 µg/plate) was used with TA98 and TA100 and 2-aminofluorene (1.25 µg/plate) with TA102 in the experiments with metabolic activation. DMSO (100 µL/plate) was used as solvent control. The results were analyzed with the Salanal statistical software package (U.S. Environmental Protection Agency, Monitoring Systems Laboratory, Las Vegas, NV, version 1.0, from Research Triangle Institute, RTP, North Carolina, USA), adopting the Bernstein *et al.* [21] model. The data (revertants/plate) were assessed by analysis of variance (ANOVA), followed by linear regression.

3. Results and discussion

This is the first report on the antimicrobial activity of *B. holophylla*, that demonstrated various degrees of growth inhibition against bacteria and yeast.
Phytochemical studies on species belonging to the genus *Bauhinia* have demonstrated flavonoids as one of the most predominant classes of secondary metabolites [8] with flavonols as major subclasse followed by flavones, flavans and flavanones [13]. Chromatographic analysis of the *B. holophylla* leaf extracts confirm the presence of flavonoids (Figure 1). The hydroalcoholic extract of the leaves of *B. holophylla* is known to possess mainly flavonoid-*O*-monoglycosides derivatives of quercetin, myricetin, luteolin, kaempferol and isorhamnetin [22], which possibly contribute to inhibitory activity of the extract, with synergistic multi-target effects.

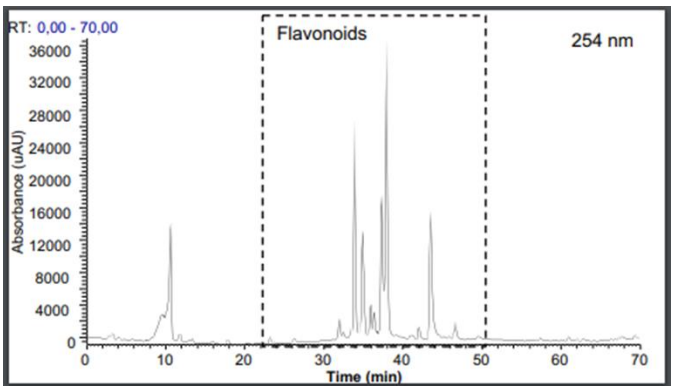

Fig 1: Analytical chromatogram obtained via HPLC-PAD of the hydroalcaholic extract of the leaves of *Bauhinia holophylla* indicating peaks identified as flavonoid derivatives. For conditions see Material and Methods section.

The MIC values of *B. holophylla* extract are show in the Table 1. The extract was active against *S. aureus* (MIC: 500 µg mL^{-1}) and *E. coli* (MIC: 2000 µg mL^{-1}), however, a possible bacteriostatic behavior was found.
It is interesting to observe that yeasts were more susceptible than bacteria, in which, the most promising MIC values were found on *C. glabrata* with 15.6 µg mL^{-1}, besides the highest SI values, followed by 62.5 µg $mL^{-1}$for *C. krusei* and *C. tropicalis* and 125 µg $mL^{-1}$for *C. albicans* and *C. parapsilosis*, with fungicide behaviour only for *C. tropicalis* (125 µg mL^{-1}).

Table 1 - The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) or minimum fungicidal concentration (MFC) for *Bauhinia holophylla* against the evaluated microorganisms

	MIC*/MBC*		MIC*/MFC*				
Treatments	***S. aureus***	***E. coli***	***C. glabrata***	***C. krusei***	***C. parapsilosis***	***C. tropicalis***	***C. albicans***
DMSO 20%	-	-	-	-	-	-	-
B. holophylla	500/ >2000	2000/ >2000	15.6/ >125	62.5/ >125	125/ >125	62.5/ 125	125/ >125
Ampicillin	0.04	2.5	-	-	-	-	-
Amphotericin B	-	-	0.25	0.5	0.25	0.05	0.25
Fluconazole	-	-	R	15.8	7.9	64	R

* µg mL^{-1}; R: resistant; (-): withoutinhibition

The antimicrobial activity of *Bauhinia* species is not consolidating in the scientific literature, and unlike synthetic drugs standardized for antimicrobial therapy, the scientific community and publications of the microbiological field do not present a consensual classification in relation to MIC values obtained in research related to the inhibitory potential of vegetal derivatives. Aligiannis *et al*. [23] it was considered as potent inhibitors samples with MIC values equal or less than 500 µg/mL; values between 600 and 1500 µg/mL as moderate inhibitors and above 1600 µg/mL as weak inhibitors.

Webster *et al*. [24] established MIC values equal to or less than 1000 µg/mL as being satisfactory. In sequence, the study developed by Gallucci *et al*. [25] also considered as promising inhibitory compounds concentrations of MICs below 1000 µg/mL. In this sense, the inhibitory effects of *B. holophylla* can be promising and offer a new applicability in studies about new antimicrobials.

Ahmed *et al*. [4] evaluated among other activities, the antimicrobial potential of polyphenolic-rich extracts and fractions of *B. bowkeri*, *B. galpinii*, *B. petersiana* and *B. variegate*. According to the authors, the mechanism of their antimicrobial activity may be related to their ability to form complexes with protein and polysaccharides, inactivating microbial adhesions, enzymes, and cell envelope transport protein.

Alves *et al*. [6] showed antimicrobial activity of *B. forficata* against *C. albicans* with MIC of 15.62 µg/mL, inhibited biofilm adhesion and caused alterations in cell morphology.

Rashed and Butnariu [26] showed that *B. racemosa* extract inhibited the growth of *Bacillus subtilis* and also it was highly active against *C. albicans* suggesting that it can be used in the treatment of fungal infections. Phytochemical analysis has shown that it has interesting phytochemical bioconstituents, include methylgallate, gallic, kaempferol, quercetin, querection 3-*O*-α-rhamnoside, kaempferol 3-*O*-β-glucoside, myricetin-3-*O*-β- glucoside, querection-3-*O*-rutinoside (rutin).

Correia *et al*. [5] evaluated the in vitro antifungal activity of six Brazilian Cerrado medicinal plant species against clinically relevant *Candida* species, and *B. Rufa* showed significant inhibitory activity against *C. parapsilosis* and *C. glabrata.*

C. glabrata has been identified as the second most common cause of invasive fungal infections in North America and fourth in rank order in Latin America [5], representing an urgent need to evaluate novel compounds with antifungal activity. Natural compounds as sources for anti-*Candida* therapeutics from botanical sources are attractive candidates to counteract the emergence of *Candida* drug resistances [27] and flavonoids are the most studied groups of phenolic compounds, comprising molecules with established antifungal properties [5,28].

Numerous research groups have sought to elucidate the antimicrobial mechanisms of action of selected flavonoids. The activity of quercetin, for example, has been at least partially attributed to inhibition of DNA gyrase [29].

Aside from interest in the efficacy of medicinal plant extract against various diseases, safety is also an important factor in traditional medicine. Therefore, we report herein the cytotoxic and mutagenic effect of *B. holophylla* extract.

GM 07492 cells were chosen because they are normal human fibroblast cell line, HepG2 cells are human metabolizing cells, and HeLa was chosen based on their importance as a host site for infections of *Candida* spp. Data obtained from the resazurin assay showed a decrease in cell viability after treatment for 24 h the at highest concentrations tested in this study. IC_{50} values found were much higher than the MIC of the extract against microorganisms, mainly against *C. glabrata*. This selectivity represents significant advances for therapy (Table 2).

Table 2: Results of IC_{50} and selectivity index (SI) for *Bauhinia holophylla* extract

		SI						
	IC_{50} (µg mL^{-1})	***S. aureus***	***E. coli***	***C. glabrata***	***C. krusei***	***C. parapsilosis***	***C. tropicalis***	***C. albicans***
GM 07492	411.3 ± 28.6	0.82	0.21	26.37	6.58	3.29	6.58	3.29
HepG2	>1000	2.0	0.5	64.1	16.0	8.0	16.0	8.0
HeLa	549.7 ± 20.8	1.1	0.27	35.24	8.8	4.4	8.8	4.4

However, *B. holophylla* showed mutagenic activity, in a dose-dependent manner, in the *Salmonella* strain TA98, with mutagenic indices of 5.9 and 3.7, without and with S9 mix, respectively, at the highest concentration. In the *Salmonella* strain TA97a, the mutagenic indices were 2.0 only in the presence of metabolic activation (Table 3). The *S.* Typhimurium test strain TA97a detects frame shift mutations in C-C-C-C-C-C-C;+1 cytosine and TA98 frame shift in DNA target-C-G-C-G-C-G-C-G [20]. Thus, according to the strains involved, the *B. holophylla* extract mainly induces, at a much higher rate, frame shift mutations (TA98 and TA97a). These results are worrying and show that *B. holophylla* should be used with caution by the population.

Table 3: Mutagenicity activity expressed as mean and standard deviation of number of revertents/ plate and mutagenicity index (IM - value in parentheses) in *S.* Typhimurium TA98, TA97a, TA100 and TA102 strains after treatment with the hydroalcoholic leaves extractof *Bauhinia holophylla*, at diferente concentrations, with (+ S9) and without (-S9) metabolic activation

Treatments	**Number of revertents (M ± SD)/ plate and MI**							
	TA 98		**TA 100**		**TA 102**		**TA 97a**	
mg/plate	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
0.0[a]	16 ± 1	23 ± 2	146 ± 10	122 ± 13	262 ± 63	243 ± 31	157 ± 5	101 ± 5
0.62	28 ± 5* (1.7)	40 ± 8* (1.7)	169 ± 6 (1.2)	165 ± 16 (1.3)	303 ± 41 (1.2)	319 ± 14 (1.3)	171 ± 17 (1.1)	212 ± 20** (2.1)
1.25	37 ± 4** (2.3)	45 ± 9* (1.9)	166 ± 13 (1.1)	176 ± 14 (1.4)	297 ± 37 (1.1)	321 ± 64 (1.3)	187 ± 24 (1.2)	237 ± 31** (2.3)
2.50	61 ± 5** (3.8)	58 ± 2** (2.5)	190 ± 26 (1.3)	157 ± 29 (1.3)	284 ± 64 (1.1)	331 ± 36 (1.4)	181 ± 23 (1.2)	263 ± 34** (2.6)
3.75	93 ± 9** (5.8)	64 ± 6**	197 ± 36	179 ± 26	226 ± 30	329 ± 51	182 ± 12	214 ± 24**

		(2.8)	(1.3)	(1.5)	(0.9)	(1.4)	(1.2)	(2.1)
5.00	94 ± 15** (5.9)	84 ± 2** (3.7)	205 ± 16 (1.4)	160 ± 27 (1.3)	239 ± 68 (0.9)	269 ± 23 (1.1)	200 ± 14 (1.3)	163 ± 26* (1.6)
C +	1105 ± 123[b]	1206 ± 97[e]	1412 ± 74[c]	1428 ± 105[e]	2056 ± 197[d]	1885 ± 156[f]	1043 ± 134[b]	1410 ± 69[e]

* P <0.05 (ANOVA); ** P <0.01 (ANOVA), M ± SD = mean and standard deviation; [a] Negative control: DMSO, dimethylsulfoxide: 100 µL/ plate; Positive control (C +): [b] 4-nitro-*o*-phenylenediamine (10.0 µg/ plate - TA98); [c] Sodium azide (1.25 µg/ plate - TA100); [d] Mithomycin C (0.5 µg/ plate - TA102), in the absence of S9 and [e] 2-aminoanthracene (1.25 µg/ plate - TA98, TA100); [f] 2-aminofluorene (10 µg/ plate - TA102) in the presence of S9.

The *Salmonella*, or Ames test is used worldwide for initial screening of the mutagenic potential of new drugs and a positive response in any single bacterial strain either with or without metabolic activation is sufficient to designate a substance as a mutagen, the mutagenic response has high predictive value for carcinogenicity [20, 30].

Regarding the genotoxic/ mutagenic potential, studies with species of the genus *Bauhinia* show divergent results. Reid *et al*. [31], Aderogba *et al*. [32] and Verschaeve and Van Staden [33] reported the antimutagenic potential of *B. galpinii* and Agrawal and Pandey [34] showed anticarcinogenic and antimutagenic activities of *B. variegata*, while *B. forficata* extract was able to induce mutagenesis in strain TA102 [35]. *B. monandra* leaf extract did not show an increase in the frequency of reverse mutations in *S*. Typhimurium TA97a, TA98, TA100 and TA102 strains, with and without metabolic activation [1]. However, *B. platypetala* extract induced DNA damage in V79 cells in the Comet assay [36].

On *B. holophylla*, Ribeiro *et al*. [2] demonstrated that at concentrations higher than 7.5 µg/mL (between 10 and 50 µg/mL), the extract was cytotoxic, induced apoptosis, and caused anti-proliferative effects. However, it did not induce micronucleus and a reduction of apoptotic and micronucleated cells was observed in treatments that included the extract and benzo [*a*] pyrene. Already, Rozza *et al*. [12] demonstrated the gastroprotective effect of *B. holophylla* and absence of acute toxicity. Considering that medicinal herbs contain complex mixtures of components, generally the synergistic properties of these constituents are responsible for the beneficial or harmful effects found.

4. Conclusions

B. holophylla showed good antifungal potential against several *Candida* strains and low cytotoxicity. Considering the increasing number of infections by non-*albicans Candida* species, it is clear that the data obtained are of great importance, since our results may contribute to the search of natural products with antifungal activity. However, while many beneficial properties are confirmed, as shown here, some medicinal plants may pose risks to users, such as *B. holophylla* because it is mutagenic. Therefore, it is necessary to clarify the mechanisms and conditions that mediate the biological effects of natural products.

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6. Author contribution

Conceived and designed the experiments: GDM FAR. Performed the experiments: GDM MASR RAG. Analysed the data: GDM. Produced, characterized and supplied *B. holophylla* extract: LLS ALD. Contributed reagents/materials/analysis tools: TMB FRP. Wrote the paper: GDM. Critical revision and final approval of manuscript: FAR

7. Conflict of Interest

The authors declare no conflict of interest.

8. References

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