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ORIGINAL ARTICLE

Chromatographic Profiles, Anti-inflammatory, and Cytotoxicity Potential of Extracts of *Banisteriopsis pubipetala* (A. Juss)

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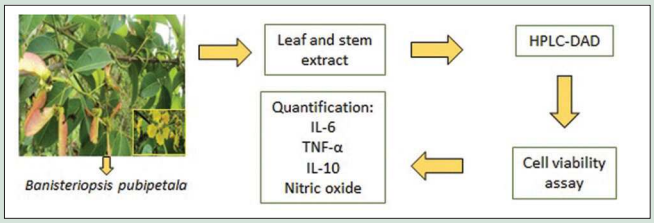
ABSTRACT

Background: *Banisteriopsis pubipetala* is a little-studied species belonging to the same genus of *B. caapi*, which stands out for the bioactive compounds known to be important in the degenerative diseases treatment. **Objective:** The objective is to analyze the anti-inflammatory and cytotoxic potential and chromatographic profile of the extracts of *B. pubipetala*. **Materials and Methods:** The investigation of the chromatographic profile was performed through high-performance liquid chromatography diode-array detector. Cell viability was determined by a quantitative colorimetric assay with 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide. In the pharmacological tests, interleukin (IL)-6, tumor necrosis factor-alpha (TNF-α), IL-10, and nitric oxide (NO) levels in cell culture supernatants were performed. **Results:** In the leaf were observed nine major compounds and in the stem five major compounds. The extracts of *B. pubipetala* demonstrated concentration-dependent behavior regarding cytotoxicity. The fraction in dichloromethane had inhibitory concentration of 50% (IC_{50}) = 67.39 μg/mL whereas the extract in ethyl acetate had IC_{50} = 103.37 μg/mL. The results showed that extracts significantly reduced the production of IL-6 and TNF-α by cells 3T3 cells, however increased the production of IL-10 and NO. **Conclusion:** The results of the tests indicate that the extracts of *B. pubipetala* evaluated have potential anti-inflammatory properties and may promote the regulation of inflammation levels.

Key words: *Banisteriopsis pubipetala,* interleukin-10, interleukin-6, *Malpighiaceae*, nitric oxide, tumor necrosis factor-alpha

SUMMARY

- In this study, to analyze the *Banisteriopsis pubipetala* extracts to known its pharmacological actions through specific tests for anti-inflammatory and cytotoxic potential and chromatographic profile. The results of the tests indicate that the extracts of *B. pubipetala* evaluated have potential anti-inflammatory properties, and may promote the regulation of inflammation levels.



Abbreviations Used: LEBPD: Leaf extract of *Banisteriopsis pubipetala* in dichloromethane, LEBPA: Leaf extract of *Banisteriopsis pubipetala* in ethyl acetate, NO: Nitric oxide, MTT: 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide, SEBPD: Stem extract of *Banisteriopsis pubipetala* in dichloromethane, SEBPA: Stem extract of *Banisteriopsis pubipetala* in ethyl acetate.

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INTRODUCTION

The family *Malpighiaceae* is one of the most common in the Brazilian Cerrado and comprises 75 genera and about 3000 species distributed in the tropical, subtropical and savanna forests, being well represented in number of species in Brazil.[1-4] The genus *Banisteriopsis* is one of the largest of this family,[5] and it has species that count on the presence of numerous compounds with biological activity.[6-10]

The biological activities peculiar to some species are due to the presence of secondary metabolites, natural products produced by the plants and originating from the primary metabolism, whose synthesis is related to the defense processes that the plants develop in the environment in which they are found, being classified mainly in alkaloids, phenolic compounds, and terpenoids.[11-13]

Flavonoids are phenolic compounds that can be classified into six diversified subgroups (flavonols, flavones, isoflavones, flavanones, anthocyanidins, and flavanols), in which variations in the number and position of hydroxyl grouping and degree of methylation and glycosylation will differentiate them.[14] They are found in several foods, conferring them color, flavor, prevention of the oxidation of fats, and protection of vitamins and enzymes. The anti-inflammatory capacity of flavonoids has been used in Chinese medicine in the form of crude plant extracts, and many papers have proved that the various flavonoid molecules have anti-inflammatory activity, demonstrating even inhibition of chronic inflammation in various *in vivo* experiments.[15,16] In this context, they present anti-inflammatory and immunomodulatory actions; flavonoids constitute a potential alternative as therapeutic agents against the inflammatory processes.[17,18]

Added to that, the need to find new anti-inflammatory agents stimulates the analytical study of natural products, since several plants may present

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bioactive substances capable of causing this effect. Little is known about the *Banisteriopsis pubipetala* species in relation to biological activities, especially on the anti-inflammatory potential. Therefore, in the present work, it was proposed to investigate the chromatographic profile of extracts of *B. pubipetala*, its cytotoxicity, and its anti-inflammatory potential from the quantifying of cytokines and nitric oxide (NO).

MATERIALS AND METHODS

Cell lines and chemicals

The 3T3 cells (mouse embryonic fibroblast cells – passages 20–30), were obtained from the Cell Bank at Universidade Federal do Rio de Janeiro_UFRJ, Brazil. Methanol, dichloromethane, ethyl acetate, and trifluoroacetic acid were purchased from Vetec. The chromatographic grade acetonitrile was obtained from Carlo Erba.

Plant material

Leaves and stems of healthy individuals (with no visible attack evidence of galling insects and herbivores) from *B. pubipetala* (A. Juss.) were collected in September 2016, between 8 and 10 O' clock in the morning in the district of Nova Esperança, municipality of Montes Claros, North of the State of Minas Gerais, Brazil. Exsiccates of the plant material are deposited in the herbarium of the State University of Montes Claros (UNIMONTES) under the number 4033.

Preparation of plant material

The plant material was washed in running water and dried in a circulating air oven circulate (Nova Ética, model 400-4ND, Brazil) at 35°C–40°C for 7 days. The material was pulverized in a knife mill Willey type (model SL30, Solab, Brazil) and the powder obtained was stored in paper bags and kept under refrigeration at 4°C.

Liquid-liquid partitions for flavonoids

It was weighed 3.00 g of pulverized plant material in a beaker; it was adding 30 mL of methanol: water solution (90:10) and it was maintained in ultrasonic (model USC-2850A, Unique, Brazil) for 20 min. The mixture was filtered, and the obtained filtrate was transferred to separator funnel. They were realized in sequence, 4-fold partitions of 50 mL were with the solvents: dichloromethane and ethyl acetate, respectively. Next, the extracts were concentrated at a temperature of 35°C in a circulating air oven and stored in microtubes at a temperature of approximately 4°C. The extracts yields were calculated.

Chromatographic profile of the secondary compounds by high-performance liquid chromatography - diode-array detector

Preparation of the extract samples in ethyl acetate

It was weighed a mass of 10.01 mg of leaf extract of *B. pubipetala* in ethyl acetate (LEBPA) leaf extract and 10.00 mg of stem extract of *B. pubipetala* in ethyl acetate stem extract. It was solubilized in a 0.1% trifluoroacetic acid solution in water: acetonitrile (90:10), and they were subjected to the ultrasonic bath for 10 min for complete solubilization. In this way, stem and leaf were analyzed separately, and at the end, the working solutions presented concentration of 1 mg/mL. Caffeine, quercetin, and rutin patterns were prepared.

High-performance liquid chromatography – diode-array detector

The investigation of the chromatographic profile was performed through high-performance liquid chromatography. The equipment used was the liquid chromatograph (Waters, USA), equipped with a binary pump (model 1525), automatic injector (model 717), automatic fraction collector (model III), photodiode array detector (model 2996), and software Empower Pro. It was used a C_{18}, 250 mm × 4.6 mm column and 5 μm particle (Spherisorb, Waters, USA) in the separation of the compounds. The mobile phase employed was a mixture on 5:95 ratio of chromatographic grade acetonitrile and 0.1% trifluoroacetic acid in ultrapure water, being pumped in the isocratic mode, and the reading was performed at the wavelength of 220 nm. The peaks with good resolution were collected for the next stages of the study, which involved the identification of the chemical compounds of the species and investigation of the anti-inflammatory potential.

Biological assays

They were used the fractions of *B. pubipetala* leaves partitioned in dichloromethane and ethyl acetate, respectively.

Cell culture

The 3T3 cells were cultivated in an RPMI-1640 culture medium (Sigma Aldrich, USA) and supplemented with 10% (v/v) bovine fetal serum (Invitrogen Co Ltd, USA), 100 IU penicillin/mL, and 100 μg streptomycin/mL (Sigma Aldrich, USA). Cells were cultivated in 75 cm^2 bottles and incubated at 37°C in a humidified with 5% CO_2.

Cell viability analysis

Stock solutions of 2000 μg/ml of leaf extract of *B. pubipetala* in dichloromethane (LEBPD) and LEBPA were prepared in sterile culture medium (RPMI). In the assays the concentrations used were: 6.25; 12.5; 25; 50; 100; 150; 200 and 400 μg/mL.

Cell viability was determined by a quantitative colorimetric assay with 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide (MTT).[19] The cells were placed in 96-well plates in a concentration of 1.0 × 10^6 cells/well containing 180 μL of medium and were then incubated with 20 μL of eight different concentrations of the extracts for 24 h at 37°C in humidified air supplemented with 5% CO_2. Having completed these exposure times, the medium containing the extracts was removed, 20 μL of MTT solution (5.0 mg/mL) was added, and the plates were incubated for 1 h at 37°C in humidified air supplemented with 5% CO_2. The MTT solution was then removed, and 100 μL of dimethyl sulfoxide was added to each well. The absorbance was read at 570 nm (Thermo Plate model TP-READER, China) and the results were expressed as a percentage of the viability present in treated cells compared to control cells.

Quantification of cytokines interleukin-6, tumor necrosis factor-alpha and interleukin-10

Interleukin (IL)-6, tumor necrosis factor-alpha (TNF-α) and IL-10 levels in cell culture supernatants were performed in triplicate using commercially available high-sensitivity enzyme-linked immunosorbent assay kit (Enzo Life Sciences, USA) according to the manufacturer's instructions. 3T3 cells were plated at 5.0 × 10^5 cells/well into 24-well Plates. 24 h after cells were treated for 30 min with 50 μg/mL of dichloromethane extract and 100 μg/mL of ethyl acetate extract, respectively. After 24 h, supernatants cells were obtained by centrifugation 413 g, 10 min, and were stored at −80°C.

Quantification of nitric oxide

3T3 cells were plated at 5.0 × 10^5 cells/well into 24-well Plates. Twenty-four hours after cells were treated for 30 min with 50 μg/mL of dichloromethane extract and 100 μg/mL of ethyl acetate extract, respectively. After 24 h supernatants were obtained by centrifugation 413 g, 10 min and NO production was measured by means the Griess reaction. This involved comparing 100 μL aliquots of culture supernatant with serial dilutions $NaNO_2$ (from 7.81 mM to 1000 mM). To this, an

equal volume of Griess reagent (N-1-naphthylethylenediamine 0.1% in H_2O + sulfanilamide 1% in 2.5% H_3PO_4) was added and then incubated at room temperature then incubated at room temperature for 10 min and read at 540 nm.[20]

Statistical analysis

All results were analyzed by ANOVA and Tukey post-test using GraphPad Prism version 5.00 for Windows (USA). $P < 0.05$ was considered as statistical significance.

RESULTS AND DISCUSSION

Chromatographic profile of the partitions extracted in ethyl acetate in high-performance liquid chromatography-diode-array detector

They were calculated the yields of the stem and leaf vegetable powder after extraction with ethyl acetate and with dichloromethane [Table 1]. The amount of material extracted from the leaf was higher when compared to the stem, in both solvents used: the LEBPA was the extract with the highest yield (19.50%), whereas in the stem partition stem extract of *B. pubipetala* in dichloromethane (SEBPD) the yield was only 0.52%, being the lowest observed. The age and stage of the development of the plant, as well as the different plant organs, are of considerable importance and may impact not only the concentration of natural products produced but also the relative proportions of the components.[21]

Table 1: Yield of extracts of stem and leaf of *Banisteriopsis pubipetala* partitioned with dichloromethane and ethyl acetate

Extract coding	Part of the plant	Solvent used in partitioning	Yield (%)
SEBPA	Stem	Ethyl acetate	8,40
SEBPD	Stem	Dichloromethane	0,52
LEBPA	Leaf	Ethyl acetate	19,50
LEBPD	Leaf	Dichloromethane	2,03

LEBPD: Leaf extract of *Banisteriopsis pubipetala* in dichloromethane; LEBPA: Leaf extract of *Banisteriopsis pubipetala* in ethyl acetate; SEBPD: Stem extract of *Banisteriopsis pubipetala* in dichloromethane; SEBPA: Stem extract of *Banisteriopsis pubipetala* in ethyl acetate

The chromatographic profile of the leaf and stem of the species *B. pubipetala* [Figure 1] allowed to determine the number of major compounds that occur in each structure of the species and to make a relation between them. In the samples and analytical conditions adopted, in the leaf were observed 12 peaks and in the stem 6 peaks, being that all peaks observed in the stem are prevalent in the leaf, with variation in concentration. In the stem are visualized five major compounds (1, 2, 3, 4, and 7) and peak 1 represents the compound with the largest area in this part of the plant, being produced in similar concentrations in the leaf and stem. Already in the leaf were observed nine major compounds (peaks 1, 2, 3, 4, 8, 9, 10, 11, and 12), with highlight to peak 9, major compound with a notable concentration in relation to the others with the largest area when compared the two structures, and it is synthesized only on the leaf. Thus, as in the leaf, there is a higher concentration of natural products when compared to the stem, as well as all the compounds present in the stem are prevalent in the leaf, only the leaf extracts were chosen to be used in the biological assays. The low yield observed in the extraction with the solvent dichloromethane [Table 1] made the chromatographic profile tests impossible.

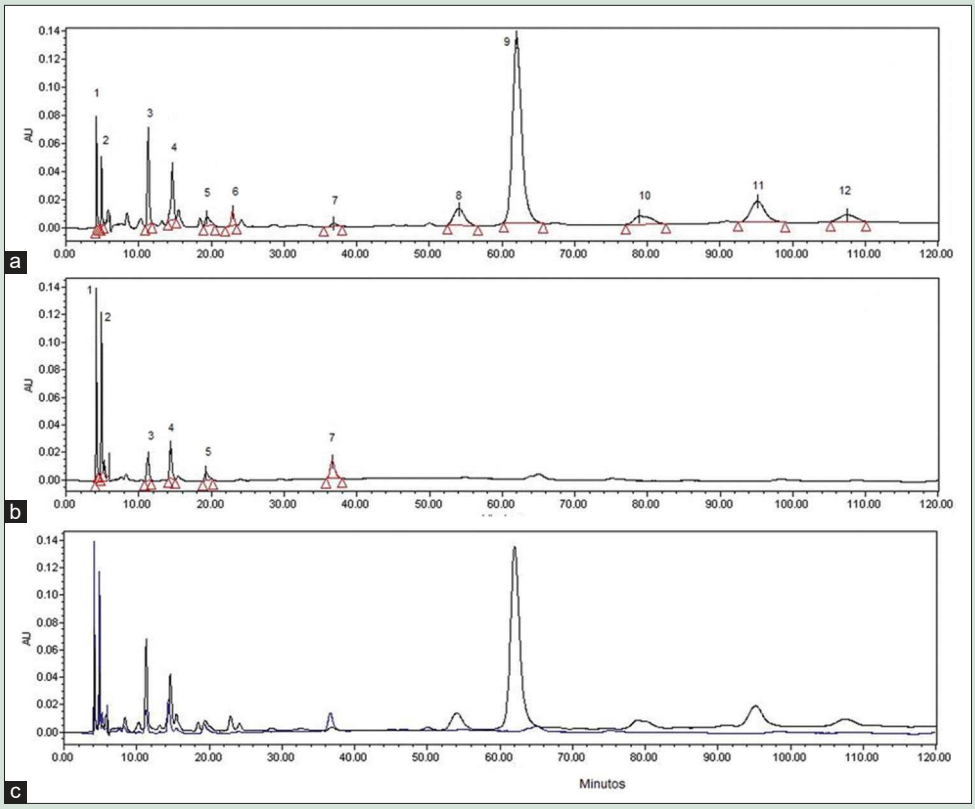

Figure 1: Chromatographic profile of *Banisteriopsis pubipetala* extracts. (a) Chromatogram of *Banisteriopsis pubipetala* leaf ethyl acetate extract; (b) chromatogram of *Banisteriopsis pubipetala* stem ethyl acetate extract; (c) overlapping of leaf and stem chromatograms of *Banisteriopsis pubipetala*

The compounds corresponding to peaks 1, 2, and 7 are in higher concentration in the stem-derived partition while all other compounds are produced in greater quantity in the leaf [Table 2]. Already the peaks 6, 8, 9, 10, 11, and 12, under the established analysis conditions, were found in concentration detectable only in the leaf extract.

In the chromatograms, no correlation was observed in the retention time of the patterns injected with the compounds present in the plant extract, however when we evaluated the absorption spectra of the major compounds found in *B. pubipetala* we can infer that compounds 8, 9, and 11 appear to belong to the same group of secondary metabolites because of the observed similarity in relation to ultraviolet light absorption. The flavone and flavonols groups exhibit two main absorption bands: a band with absorption between 320 and 385 nm, representing the absorption of the benzene ring B, and a second band with absorption between 250 and 285 nm, representing the absorption of the benzene ring A.[22] Compounds 3, 4, 8, 9, 11, and 12 presented similar spectral characteristics to the behavior of these classes of flavonoids; however, other analytical techniques of detection should be employed to discover the chemical identity of the compounds.

In front of the results obtained in the analysis of the chromatographic profile of the species *B. pubipetala,* it was evidenced that in the leaf there is a higher concentration of natural products when compared to the stem, as well as all the compounds present in the stem are prevalent in the leaf, and such compounds have spectral characteristics similar to the behavior of flavonoids.

Effect of extracts of *Banisteriopsis pubipetala* on the viability of 3T3 cells

Cell viability was determined by establishing of a ratio between the absorbance values obtained, converted to percentage, in the untreated (control) and treated groups, as shown in Figure 2 (panels a and b). The extracts from *B. pubipetala* were cytotoxic to 3T3 cells in a concentration-dependent manner. There was a significant difference ($P < 0.05$) from the 12.5 µg/mL concentration when compared to the results from the control group (untreated group). According to the partitioning solvent, there was a difference in cell viability, being that the fraction in dichloromethane presented an inhibitory concentration of 50% (IC_{50}) equal to 67.39 µg/mL, while the extract in ethyl acetate presented IC_{50} = 103, 37 µg/mL.

3T3 cells were placed at the density of 1.0×10^6 cells/well in a 96-well plate and were treated with eight concentrations of dichloromethane partitioned leaf extract (a) and with ethyl acetate partitioned leaf extract, (b) (6.25, 12.5, 25.0, 50.0, 100, 150, 200 e 400 µg/mL) in sextuplicates. Cell viability was analyzed using the MTT assay. The media of absorbance of each concentration was compared with the control group (cells not exposed to the extracts), and for this 100% viability was considered. Results represent the mean ± standard deviation (SD) of sextuplicates from three independent experiments. *$P < 0.05$ when compared with the control group (untreated cells).

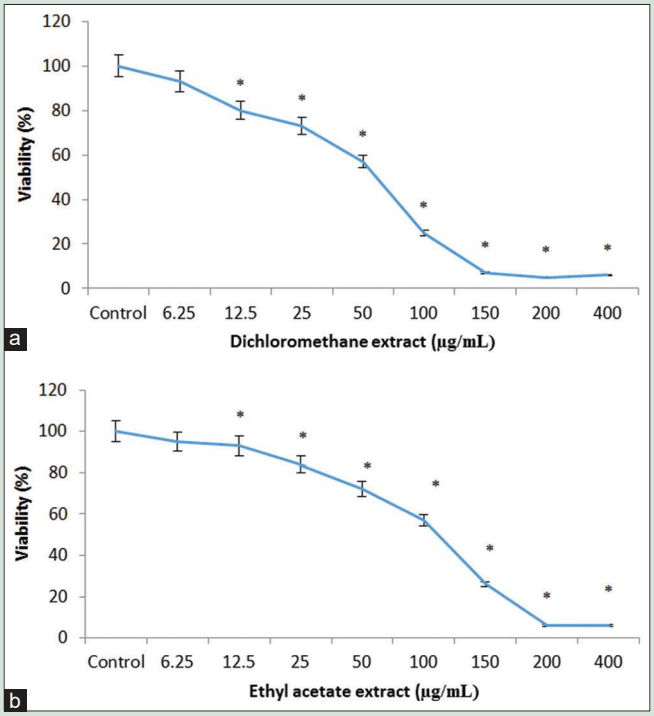

Figure 2: Extracts of *Banisteriopsis pubipetala* decreases cell viability in 3T3 cells. 3T3 cells were placed at the density of 1.0×10^6 cells/well in a 96-well plate and were treated with eight concentrations of dichloromethane partitioned leaf extract (a) and with ethyl acetate partitioned leaf extract (b) (6.25, 12.5, 25.0, 50.0, 100, 150, 200 e 400 µg/mL) in sextuplicates. Cell viability was analyzed using the 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide assay. The media of absorbance of each concentration was compared with the control group (cells not exposed to the extracts) and for this 100% viability was considered. Results represent the mean ± standard deviation of sextuplicates from three independent experiments. *$P < 0.05$ when compared with the control group (untreated cells)

Table 2: Comparison of retention time, area and percentage area (percentage area) in the compounds isolated from the leaf and stem of *Banisteriopsis pubipetala*, partitioned with the solvent ethyl acetate

Compound	LEBPA			SEBPA		
	Tr (min)	Area	Percentage area	Tr (min)	Area	Percentage area
1	4.18	632875	3.11	4.14	1171720	30.20
2	4.88	384327	1.89	4.85	928048	23.92
3	11.29	1170889	5.75	11.27	413443	10.66
4	14.62	904567	4.44	14.38	551956	14.23
5	19.39	295308	1.45	19.27	229784	5.92
6	22.91	317559	1.56	-	-	-
7	36.82	107721	0.53	36.62	584936	15.08
8	54.06	1292928	6.35	-	-	-
9	62.01	11550174	56.72	-	-	-
10	78.93	1039111	5.10	-	-	-
11	95.13	1962548	9.64	-	-	-
12	107.45	704693	3.46	-	-	-

LEBPA: Leaf extract of *Banisteriopsis pubipetala* partitioned with ethyl acetate; SEBPA: *Banisteriopsis pubipetala* stem extract partitioned with ethyl acetate

Thus, the results of the present study demonstrated that 3T3 cells present different sensitivity to the cytotoxic effect of *B. pubipetala*, being this behavior a dependent concentration and the partitioning solvent dichloromethane appears to carry more cytotoxic compounds when compared to the extract in ethyl acetate. Other work showed differences of cytotoxicity according to the solvents used. Extracts from the *B. laevifolia* leaf extracted with dichloromethane presented IC_{50} >512 μg/mL while the extract in ethyl acetate presented IC_{50} = 361 + 29 μg/mL tested in Vero cells.[23] Antitumor cytotoxic activity was found in compounds isolated from the ethyl acetate fraction of *B. anisandra*.[24]

The *in vitro* assays make possible a better control over experimental conditions and serve as screening for biological activity analysis of natural and synthetic compounds. The MTT cytotoxicity assay is a widely used *in vitro* test being one of the most commonly used in detecting the viability of cells when exposed to different substances. It is mainly based on the enzymatic conversion of this substance to a compound formazan, in mitochondria.[25,26] In this way, it can be inferred that the compounds present in the leaves of *B. pubipetala* promote some type of alteration in the mitochondrial metabolism since the MTT evaluates the integrity of this organelle.[27]

Effect of the extracts of *Banisteriopsis pubipetala* on the production of cytokines interleukin-6, tumor necrosis factor-alpha and interleukin-10

The results demonstrated that extracts partitioned with dichloromethane (LEBPD = 50 μg/mL) and with ethyl acetate (LEBPA = 100 μg/mL) significantly decreased the production of IL-6 pro-inflammatory cytokines (LEBPD = reduction of 45,1% and LEBPA = reduction of 40,4%) and TNF-α (LEBPD = reduction of 35,7% e LEBPA = reduction of 32,9%) by 3T3 cells after 24 h incubation in comparison to those that did not receive treatment and it was not observed difference in relation to the partitioning solvent of the fraction [Figure 3, panels a and b]. It should be emphasized that the used concentrations of the respective extracts are lower than the IC_{50} determined in the cytotoxicity tests. Regarding the produced levels of the anti-inflammatory cytokine IL-10, cells treated with the LEBPD and LEBPA presented a significant increase (LEBPD = increase of 86.4% and LEBPA = increase of 78.4%) when compared to the control group and there was no difference in behavior between the extracts of *B. pubipetala* in relation to the partitioning solvent [Figure 3, panel c].

Cytokine quantification was determined by ELISA after 24 h. Cells were treated with 50 μg/mL dichloromethane partitioned leaf extract and with 100 μg/mL ethyl acetate partitioned leaf extract. Each experiment was performed in triplicate. *$P < 0.05$ when compared with negative control group (untreated cells).

In this context, it is probable that the immunomodulatory capacity of pro-inflammatory and anti-inflammatory cytokines production demonstrated by *B. pubipetala* extracts is related to the flavonoids present in the species, as detected by our results.

Flavonoids are a group of natural products that act on the anti-inflammatory response, modulating cells involved with the inflammation (e.g., inhibiting proliferation of T lymphocytes), inhibiting the production of proinflammatory cytokines (e.g., TNF-α), modulating the activity of the enzymes of the arachidonic acid pathway, such as cyclooxygenase and lipoxygenase, in addition to modulating the inductive NO synthase.[15]

Other studies have demonstrated a significant decrease of the production of IL-6 and TNF-α in brain tissues of mice treated with chrysin (5–7, dihydroxyflavone), confirming the anti-inflammatory properties of this flavonoid.[16] The flavones velutine, apigenin, luteolin, and chrysoberyl also decreased the production of these

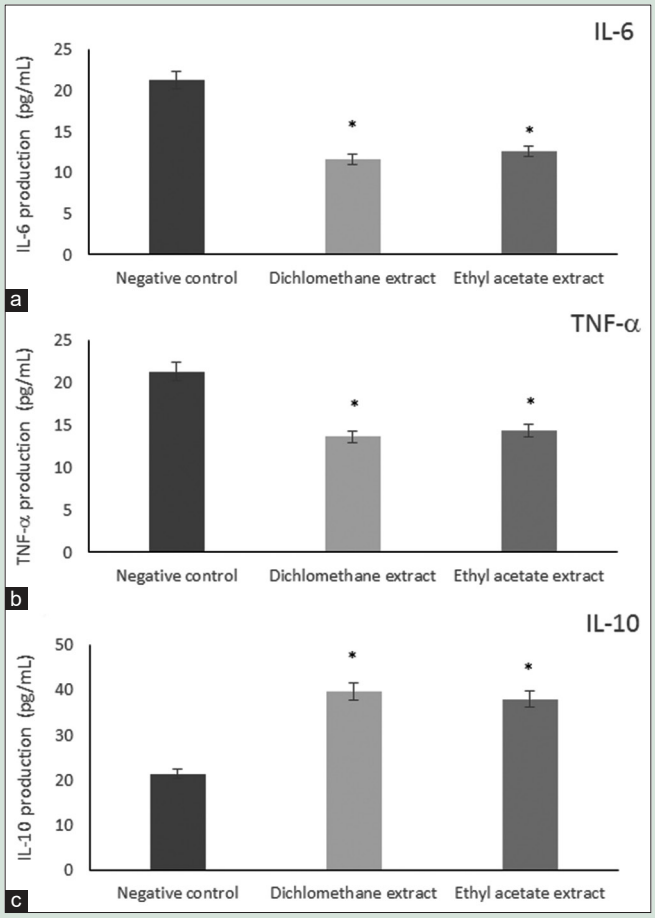

Figure 3: Effect of extracts of *Banisteriopsis pubipetala* on cytokines production in 3T3 cells. interleukin-6 cytokine production (a), tumor necrosis factor-alpha cytokine production (b) and IL-10 cytokine production in cell supernatants (c). Cytokine quantification was determined by ELISA after 24 h. Cells were treated with 50 μg/mL dichloromethane partitioned leaf extract and with 100 μg/mL ethyl acetate partitioned leaf extract. Each experiment was performed in triplicate. *$P < 0.05$ when compared with negative control group (untreated cells)

cytokines.[28] However, epicatechins had no significant effect on IL-6 and TNF-α production in human leukocytes *in vitro*, but they significantly reduced IL-10 production by these cells.[29]

IL-6 is one of the earliest and important mediators of the acute phase of pain stimuli such as trauma, infection, burns, among others. TNF-α is a cytokine that acts on the induction of muscle metabolism, stimulation to lipolysis, inhibition of lipoprotein lipase, activation of coagulation, among other actions. IL-10 is an anti-inflammatory cytokine that inhibits pro-inflammatory cytokines, mainly TNF-α, IL-1 and IL-6, and stimulates the endogenous production of other anti-inflammatory cytokines. After severe lesions or infections, the exacerbated and persistent response of pro-inflammatory cytokines may contribute to lesions in target organ, leading to multiple organ failure and death. Anti-inflammatory cytokines may minimize some of these undesirable effects.[30]

The effect of the extracts of *Banisteriopsis pubipetala* on the production of nitric oxide

The results of the present study showed that in the 3T3 cells treated with extracts partitioned with dichloromethane (LEBPD) and with ethyl acetate (LEBPA) from *B. pubipetala* the NO production was significantly

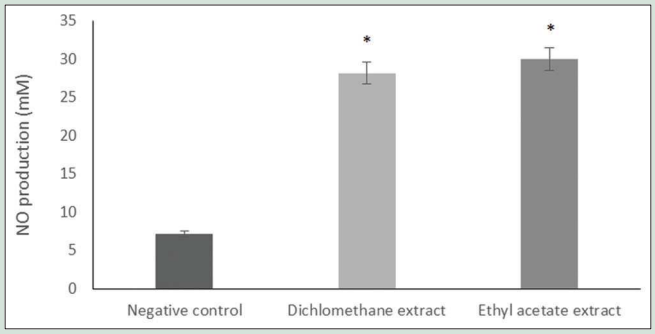

Figure 4: Effect of extracts of *Banisteriopsis pubipetala* on nitric oxide production in 3T3 cells. The production of nitric oxide in the 3T3 supernatants cultures was determined by Griess reaction after 24 h incubation with 50 μg/mL dichloromethane partitioned leaf extract and with 100 μg/mL ethyl acetate partitioned leaf extract. The results represent the mean ± standard deviation of the results of three independent experiments performed in sextuplicate. *$P < 0.05$ when compared with a negative control group (untreated cells)

higher in relation to the control group (untreated cells), being observed an increase of 291.7% in NO production in cells treated with LEBPD and 316.7% in cells treated with LEBPA, respectively. There was no statistical difference regarding the partitioning solvent used [Figure 4].

The production of NO in the 3T3 supernatants cultures was determined by Griess reaction after 24 h incubation with 50 μg/mL dichloromethane partitioned leaf extract and with 100 μg/mL ethyl acetate partitioned leaf extract. The results represent the mean ± SD of the results of three independent experiments performed in sextuplicate. *$P < 0.05$ when compared with negative control group (untreated cells).

In front of these results, we can suggest that the increase in NO production by 3T3 cells may be related to the presence of the flavonoids present in the species. Other authors have reported the ability of flavonoids to increase NO production. Two compounds, provisions of the ethyl acetate and methanol-chloroform extracts isolated from the stem bark of *Hiptage benghalensis* (*Malpighiaceae*), intensified NO production in RAW 264.7 macrophages[31] and in endothelial cells, the flavonoids seem to induce the release of NO.[32] Certain flavonoids, especially those derived from flavones, may exert anti-inflammatory activity, at least in part, through the modulating of the pro-inflammatory gene expression.[15]

NO is a molecular mediator that acts in many physiological processes[33] and high NO levels may be important in defense against cellular invaders, cell tumors and still in vascular lesions with endothelial loss.[34] When the inflammatory response occurs as part of an adaptive response (infection or sepsis), increased NO concentration has a protective effect, resulting in the inhibition of growth of microbial pathogens, protection of tissues against damage caused by systemic acute inflammation and vasodilation, maximizing tissue perfusion.[35]

CONCLUSION

The results of the present study showed that leaf extracts were cytotoxic to 3T3 cells in a concentration-dependent manner, decreased the production of the proinflammatory cytokines (TNF-α and IL-6) and increased IL-10 and NO production, what indicate that the extracts of *B. pubipetala* leaf evaluated have potential anti-inflammatory properties, and may exert an immunoregulatory activity. It is yet considered that, in the near future, can be developed *in vivo* investigations to understand the pharmacodynamics and pharmacokinetics of the natural products and to confirm the anti-inflammatory activity of the species *B. pubipetala*. Therefore, in front of the evidence of the present study, the identification of the detected compounds that cause the anti-inflammatory effects becomes essential and will depend on new steps of purification of the components of the fractions obtained.

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Conflicts of interest

There are no conflicts of interest.

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