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Original Article

Antioxidant and Anti-inflammatory Activities of *Bauhinia ungulata* L. (Fabaceae) on LPS-Stimulated RAW 264.7 Cells

Raphael de Oliveira Rodrigues[1,*], Juliana Navarro Ueda Yaochite[1], Milena Aguiar Braga[1], Amanda Ribeiro de Sousa[1], Greyce Luri Sasahara[1], Said Gonçalves da Cruz Fonseca[2], Thiago Dias de Vasconcelos Araújo[2], Gilvandete Maria Pinheiro Santiago[2], Leôncio Mesquita de Sousa[3], Jarbas Lima de Carvalho[3], Francisca Bruna Stefany Aires do Nascimento[1], Hélio Vitoriano Nobre Júnior[1], Aparecida Tiemi Nagao-Dias[1]

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[1]*Department of Clinical Analysis and Toxicology, Faculty of Pharmacy, Universidade Federal do Ceara (UFC), Rua Capitao Francisco Pedro 1210, 60430-370 Fortaleza, CE, BRAZIL.*
[2]*Department of Pharmacy, Faculty of Pharmacy, Universidade Federal do Ceara (UFC), Rua Capitao Francisco Pedro 1210, 60430-370 Fortaleza, CE, BRAZIL.*
[3]*Department of Organic and Inorganic Chemistry, Sciences Center, Universidade Federal do Ceara (UFC), Campus do Pici 12.200, 60021-940, Fortaleza, CE, BRAZIL.*

Correspondence

Dr. Raphael de Oliveira Rodrigues

Laboratory of Immunology, Department Clinical Analysis and Toxicology, Faculty of Pharmacy, Universidade Federal do Ceará, Rua Capitão Francisco Pedro, 1210, CEP 60430-370 Fortaleza, Ceará, BRAZIL.

Phone no : 55-85-33668270

E-mail: raphaelolrodrigues@gmail.com

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ABSTRACT

Objective: The present study aimed to investigate the antioxidant, immunomodulatory and antimicrobial activities of *Bauhinia ungulata* L. **Method:** A flavonoid-rich fraction was obtained from the *Bauhinia ungulata* L stem, called the ethyl acetate fraction of *Bauhinia ungulata* (FABU). The total antioxidant capacity of the FABU was determined through the phosphomolibdenium reduction method. For the evaluation of its antioxidant activity on a cell culture model, LPS-stimulated RAW 264.7 cells were treated with different concentrations of FABU and the reactive oxygen species (ROS), nitric oxide (NO), hydrogen peroxide (H_2O_2) and thiobarbituric acid reactive substances (TBARS) production levels were measured. For the analysis of its immunomodulatory capacity, TNF-α, TGF-β and IL-10 levels were determined in the culture supernatant. In order to determinate the antimicrobial activity of FABU, antifungal and antibacterial susceptibility testing was performed against *Candida albicans*, methicillin-sensitive *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa* strains. **Result:** The FABU demonstrated neither significant antimicrobial activity nor immunodulatory capacity; on the other hand, its potential antioxidant activity was demonstrated by the phosphomolibdenium reduction assay. Also, FABU treatment inhibited the ROS, NO, H_2O_2 and TBARS levels in the supernatant of LPS-stimulated cells. **Conclusion:** A significant reduction in the amount of reactive oxygen and nitrogen species (RONS) was observed, in addition to lipid peroxidation inhibition. Our data suggest that the FABU is a natural antioxidant complex that may interfere in the cascade of cell damage caused by free radicals and a promising potential drug in chronic disease models in which immunopathogenicity involves high levels of RONS.

Key words: Bauhinia ungulata; RAW 264.7 cells; Lipopolysaccharide; Free radicals; Antioxidant activity.

INTRODUCTION

The genus *Bauhinia* (family Fabaceae) is widely distributed worldwide and is frequently used in folk medicine-related practices. For instance, leaves, stems and roots are employed for the treatment of diabetes *mellitus*, inflammatory processes, infections and pain.[1] *In vitro* and *in vivo* studies have suggested that the therapeutic properties of the plant are mostly due to the presence of flavonoids.[2]

Bauhinia ungulata L, a Brazilian native species, is popularly known as "pata-de-vaca",[3] and can be found in various regions of the country. The popular use of this plant in Brazil originates mainly from its hypoglycemic properties.[4]

Phytochemical analysis of leaves of *B. ungulata* L. revealed the presence of quercetin, quercetin arabinofuranoside, quercitrin and the alkaloids, harmane and eleagnine.[5] Several compounds of *B. ungulata* L were isolated and identified, among them, bibenzyls, flavonoids, triterpenoids and oxepinic derivatives.[6]

Macrophages play an important role in inflammation, particularly through the phagocytosis of pathogens and production of inflammatory mediators.[7,8] The cells interact through their pattern recognition receptors with pathogen-associated molecular patterns such as lipopolysaccharide (LPS) to initiate an inflammatory response.

After binding to the toll-like receptor 4, LPS induces the cell to produce several inflammatory mediators, including tumor necrosis (TNF)-α, interleukin (IL)-1β and -6, prostaglandin (PG)E_2 in addition to free oxygen and nitrogen radicals (ROS and RNS, respectively).[9] LPS-stimulated macrophages constitute

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an *in vitro* experimental model frequently used to evaluate anti-inflammatory activities of natural products.[10]

Oxidative stress may play an important role in the immunopathogenicity of some chronic inflammatory diseases, including neurodegenerative and cardiovascular diseases and cancer.[11] For this reason, polyphenolic substances such as flavonoids are gaining attention in research because of their antioxidant properties that occur via some mechanisms of action, including inhibition of pro-oxidant enzymes, ROS and RNS scavenging and increase in the production of antioxidant defenses.[12] The present study aimed to evaluate antioxidant, immunomodulatory and antimicrobial activities of *B. ungulata* L on LPS-stimulated RAW 264.7 macrophages.

MATERIALS AND METHODS

Plant material

Stem wood of *B. ungulata* was collected in Caucaia city, Ceará, Brazil. The identification was performed by Professor Edson P. Nunes, Department of Biology, Universidade Federal do Ceará. A voucher specimen has been deposited at the Herbarium Prisco Bezerra, Department of Biology (UFC) under number 54609.

Obtaining of fraction rich in flavonoids

The flavonoid-rich fraction was obtained as described elsewhere.[6] Briefly, air-dried and finely powdered stem wood of *B. ungulata* (3.4 kg) was successively extracted with hexane and ethanol (4 x 10 L for each solvent) at room temperature for 48 h. The ethanol solution was concentrated under reduced pressure to yield the ethanol extract of *B. ungulata* (EEBU, 51.7 g). A portion of the EEBU (20.0 g) was suspended in H_2O and extracted with EtOAc (4 x 200 mL) to yield a dark residue (16 g), which was the called ethyl acetate fraction of *B. ungulata* (FABU).

For the phytochemical analysis of FABU, part of this fraction (4.78 g) was fractionated over silica gel (79.9g) by elution with CH_2Cl_2/MeOH (95/5, 9/1) and methanol to yield 75 fractions (30 mL each) that were subject to thin layer chromatography (TLC) analysis and then were pooled into 8 fractions (F1–F8). F3 (0.1404 g, CH_2Cl_2/MeOH (95/5) and F4 (0.2677 g, CH_2Cl_2) yielded liquiritigenin (**1**; 6.6 mg) and guibourtinidol (**2**; 24.5 mg), respectively, by silica gel chromatography using CH_2Cl_2/MeOH (95/5) as eluent. F6 (2.0880 g, CH_2Cl_2/MeOH (9/1)) was subject to silica gel column chromatography and eluted with CH_2Cl_2/MeOH (9/1) to yield fisetinidol (**3**; 1.62 g). The molecular structures of the isolated compounds are presented in Figure 1 and were elucidated by spectroscopic analysis, using mainly 1D and 2D 1H- and 13C-NMR spectra and comparison with literature values.

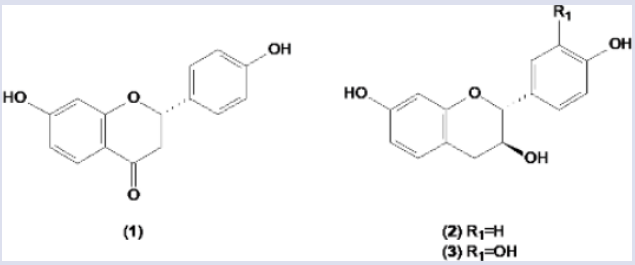

Figure 1: Structures of isolated compounds from ethyl acetate fraction of *Bauhinia ungulata*. (1): liquiritigenin, (2): guibourtinidol and (3): fisetinidol.

Total antioxidant capacity

The total antioxidant capacity was evaluated based on molybdemium reduction,[13] with modifications. The technique analyzed the reduction of Mo (VI) to Mo (V) by antioxidant substances. This reaction resulted in the formation of a green colored compound at acidic pH. A 100µL aliquot of the test sample solution, dissolved in ethanol, was added to a polypropylene tube containing 1 mL of the reagent solution, which consisted of 0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate. The reaction mixture was incubated for 90 min at 95°C. After cooling at room temperature, optical densities were measured at 700 nm in a spectrophotometer. A calibration curve was obtained with the ethanolic solution of butylated hydroxytoluene (BHT) at 12.5 to 50.0 µg/mL. The following equation was used:

$$A = 0.01C - 0.0118$$

$$r^2 = 0.998$$

Where, **A** is the absorbance obtained with the test sample and **C** is the concentration of BHT equivalents

The total antioxidant capacity of the fraction was expressed as BHT equivalents (mg of BHT equivalent per mg of fraction).

Antifungal Susceptibility Testing

The determination of the minimum inhibitory concentration (MIC) of FABU against *Candida albicans* strain ATCC® 10231 was performed using the broth micro dilution method.[14] Various concentrations of FABU were tested (0.9765–500 µg/mL). The yeast inoculum was adjusted according to the 0.5 McFarland scale and diluted with RPMI 1640 culture medium (pH 7.0 ± 0.1) and buffered with 0.165M morpholinopropane sulfonic acid (Sigma, USA) in order to obtain the final concentration of 2.5×10^3 CFU/mL. The microplates were incubated for 24 h at 35°C. The MIC was determined as the lowest concentration of the drug capable of inhibiting 50% growth of the microorganism compared to the control well.[14]

Antibacterial susceptibility testing

The MICs of the FABU against methicillin-sensitive *S. aureus* (MSSA) ATCC® 6538P, methicillin-resistant *S. aureus* ATCC 65398 and *Pseudomonas aeruginosa* ATCC® 9027 were determined by using the broth micro dilution method, according to the protocol M07-A10.[15] Various concentrations of FABU were tested (0.9765 – 500 µg/mL). The bacterial inoculum was adjusted according to the 0.5 McFarland scale and diluted with calcium-adjusted Mueller-Hinton broth in order to obtain the final concentration of 5×10^5 CFU/mL. The microplates were incubated for 20 h for *P. aeruginosa* and 24 h for MSSA and MRSA at 35°C. The MIC was determined as the lowest concentration of the drug capable of completely inhibiting the growth of the microorganism compared to the control well by visual reading.[15]

Cell culture

RAW 264.7 cells obtained from the Rio de Janeiro Cell Bank (BCRJ, Brazil) were cultured in cultured culture flasks containing high-glucose Dubecco's Modified Eagle's Medium (Gibco®, USA), supplemented with 10% fetal bovine serum and gentamicin at 5 µg/mL; and kept at 37°C and 5 % CO_2.

Cell viability assay (MTT)

RAW 264.7 cells (1×10^5 cells/well) were plated in 96 well tissue culture plates and incubated at 37°C and 5% CO_2 overnight. The cells were treated with various concentrations of the FABU (7.81–500 µg/mL) and incubated for 24 h. Afterward, the supernatant was discarded and the adhering cells were washed twice with phosphate-buffered saline (PBS). Afterwards, DMEM medium supplemented with 500 µg/mL of 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT,

Sigma, USA) was added to the wells. After incubation for 4 h at 37 °C and 5% CO_2, the supernatant was discarded and 100µL of 100% dimethylsulfoxide (DMSO, Sigma, USA) were added in the wells in order to solubilize the formazan salt products. The plates were shaken vigorously for 15 min and the final solution was measured at 570 nm using a plate reader. The assays were performed in triplicate.

Detection of ROS using H_2DCFDA

RAW 264.7 cells were plated at 5 x 10^5 cells/mL (200 µL/well) in 96-well plates and incubated overnight at 37°C and 5% CO_2. The cells were pretreated with various concentrations of the FABU (15.63–62.5 µg/mL) or controls (0.06% DMSO or 4µM dexamethasone) for 1 h and then stimulated with 1 µg/mL of LPS for 24 h. Afterward, the culture supernatant was discarded and DMEM medium containing 2',7'-dichlorodihydrofluorescein diacetate (H_2DCFDA) was added at a concentration of 10 µM. After cleavage by intracellular esterases and ROS oxidation, the non-fluorescent form, H_2DCFDA, was converted to the highly fluorescent, 2',7'-dichlorofluorescein (DCF) molecule. The cells were again incubated at 37°C and 5% CO_2 for 1 h and thereafter, the supernatant was discarded and cells washed twice with PBS. Fluorescence was measured at 485 and 535 nm excitation and emission, respectively, using a Synergy ™ H1 plate reader (BioTek, USA).

Determination of hydrogen peroxide levels

For determination of hydrogen peroxide (H_2O_2),[16] RAW 264.7 cells were plated at a density of 2 x 10^6cells/mL in 96-well plates and incubated at 37°C for 24 h. The medium was removed and cells were then pretreated with the FABU (15.63–62.5 µg/mL) or with the control agents for 1 h and then stimulated with 1 µg/mL LPS for 24 h. The cells were then washed twice with PBS and 100µL of the phenol red reagent solution (140 mM NaCl, 10 mM potassium phosphate, pH 7.0, 5.5 mM dextrose, 0.56 mM phenol red and 0.01 mg/mL type II horseradish peroxidase [Sigma, USA]) was added and incubated for 1 h at 37°C. Afterward, 10µL of the 1 N NaOH solution was added and the absorbance was read using a plate reader at 600 nm.

Activity of the ethyl acetate fraction on LPS- stimulated RAW 264.7 cells

RAW 264.7 cells plated at 5 x 10^5 cells/well in 24-well plates were incubated at 37°C and 5% CO_2 overnight. Subsequently, the cells were pretreated with various concentrations of FABU (15.63–62.5 µg/mL) or controls (0.06% DMSO or 4 µM dexamethasone) for 1 h and then stimulated with 1 µg/mL LPS for 24 h. After this time, the culture supernatant or cells were collected and kept at –80ºC for nitrite and cytokine determination. The FABU at 62.5 µg/mL contained about 0.086 µg/mL of liquiritigenin, 0.32µg/mL of guibourtinidol and 21.2 µg/mL of fisetinidol.

Nitrite determination

The nitrite dosage indicates macrophage-associated nitric oxide (NO) production. After stimulation of the RAW 264.7 cells with LPS, nitrite concentration in the supernatant was estimated.[17] A volume of 100 µL of the supernatant was added to 150 µL Griess's reagent (1% sulfanilic acid in 5% H_3PO_4 and 0.1% N (1-naphthyl) ethylenediaminedihydrochloride). A standard curve was obtained using different concentrations of sodium nitrite (Sigma-Aldrich, USA).

Cytokine levels

An aliquot of 100 µL of the culture supernatant was collected for the measurement of TNF-α, TGF-β and IL-10 levels using a sandwich-enzyme ELISA according to the manufacturer's recommendations (Novex®, Invitrogen, USA).

Lipid peroxidation determination

The action of free radicals on cell membrane unsaturated lipids results in the formation of lipid peroxidation products such as malondialdehyde (MDA). The determination of MDA was done according to the following protocol[18] in which RAW 264.7 cells were plated at a density of 5 × 10^5 cells/well in 24-well plates and incubated. Cells pretreated with FABU (15.63–62.5 µg/mL) for 1 h were stimulated with LPS (1 µg/mL) for 12 h. Cells were re-suspended with the aid of a cell scraper and lysed with 3 cycles of freezing and ultrasonic bath treatment. After centrifugation at 13,000 x g for 10 min, 60 µL of perchloric acid was added to the supernatant and proteins were removed by centrifugation. A volume of 600 µL thiobarbituric acid (TBA) (in 0.5% acetic acid) was added into a reaction tube containing 200 µL of the final supernatant and incubated at 95°C for 60 min. Volumes corresponding to 300 µL of 1-butanol and 100 µL of 5M NaCl were added to the reaction tubes and centrifuged for 3 min at 16,000 x g. After centrifugation, the upper layer was incubated in a dry bath at 55°C until 1-butanol was totally evaporated. The resulting precipitate was re-suspended with 200 µL of ultra-pure water and transferred to a 96-well plate for spectrophotometric reading at 532 nm (Asys UVM 340, Biochrom, USA).[19] The assay was performed in triplicate and the results were expressed in µM/mg protein. For the standard curve, MDA was obtained from the acid hydrolysis of 1,1,3,3-tetramethoxypropane (Sigma-Aldrich, Saint Quentin Falavier, France).

Statistical analysis

The results were expressed as mean ± standard error of the mean. For the analysis of the results, analysis of variance (ANOVA) followed by Tukey's post-test was used as the method for multiple comparisons. All statistical analyzes were performed using GraphPad Prism version 5.00 for Windows (GraphPad Prism, USA) and p <0.05 was considered as significant.

RESULTS

Antimicrobial activity

The MICs of FABU against MSSA, MRSA, *P. aeruginosa* and *C. albicans* were > 500 µg/mL (Tables 1 and 2), which represented low effectiveness of the fraction against these pathogens.

Total antioxidant capacity

The total antioxidant activity of the FABU was 1.70 mg equivalent of BHT per mg FABU, indicating that the antioxidant capacity performed per 1mg of FABU corresponded to the same activity as presented by 1.70mg BHT. The data demonstrated that the FABU presented more antioxidant capacity than the control.

Table 1: Evaluation of antibacterial activity of FABU against methicillin-sensitive *Staphylococcus aureus* (MSSA), methicillin-resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa* strains.

	MIC[b]		
	MIC values (500 to 0.9765 µg/mL)[c]		
Strains[a]	ATCC® *P. aeruginosa*	ATCC® MSSA	ATCC® MRSA
Sample	#9027	#6538P	#65398
FABU	>500	>500	>500
Tween 80	>1,5%	>1,5%	>1,5%

[a]ATCC bacterial strain collection. [b]MIC was defined as the lowest concentration that completely inhibited bacterial growth after 24 h of incubation. [c]The procedure was performed according to the CLSI protocol M07-A10, 2015. The range of compounds tested ranged from 500 to 0.9765 µg/mL of the fraction and from 1.5 to 0.0029% of Tween 80.

Table 2: Evaluation of antifungal effect of FABU against *Candida albicans*.

	MIC[b]
	MIC values (500 to 0.9765 µg/mL)[c]
Strains[a]	ATCC® *C. albicans*
Sample	#22019
FABU	>500
Tween 80	>1.5%

[a]ATCC yeast strain collection. [b]MIC was defined as the lowest concentration which reduced 50% the yeast growth after 24h incubation. [c]The procedure was performed according to the CLSI protocol M27-A3, 2008. The concentration of the compounds ranged from 500 to 0.9765 µg/mL of the fraction and from 1.5 to 0.0029% of Tween 80.

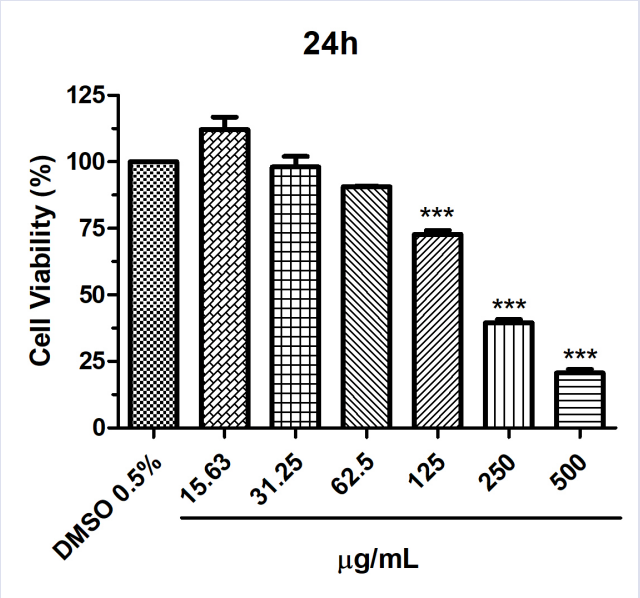

Figure 2: Cell viability of RAW 264.7 cells after incubation in the presence of various concentrations of FABU for 24 h. The citotoxicity was evaluated using MTT method. ***p<0.001 vs control group (Tukey's Test).

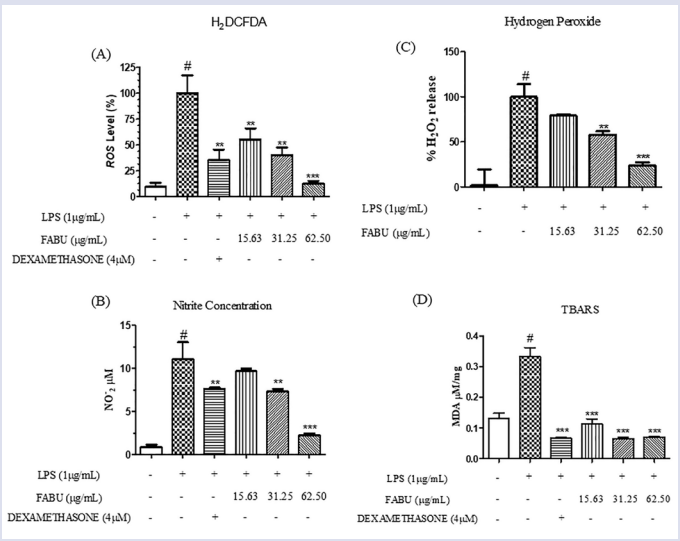

Figure 3: Antioxidant activity of *Bauhinia ungulata* L on LPS-stimulated RAW 264.7 macrophages. (A) Inhibition of ROS generation. ***p<0.001 and **p< 0.01 *vs* LPS-stimulated cells group (Tukey's test); # p<0.001 *vs* Control. (B) Inhibition of NO production. ***p<0.001 and **p< 0.01 *vs* LPS-stimulated cells group; # p<0.001 *vs* Control. (C) Inhibition of hydrogen peroxide production. ***p<0.001 and **p< 0.01 *vs* LPS-stimulated cells group; # p<0.001 *vs* Control. (D) Inhibition of TBARS generation. ***p<0.001 *vs* LPS-stimulated cells group; # p<0.001 *vs* Control.

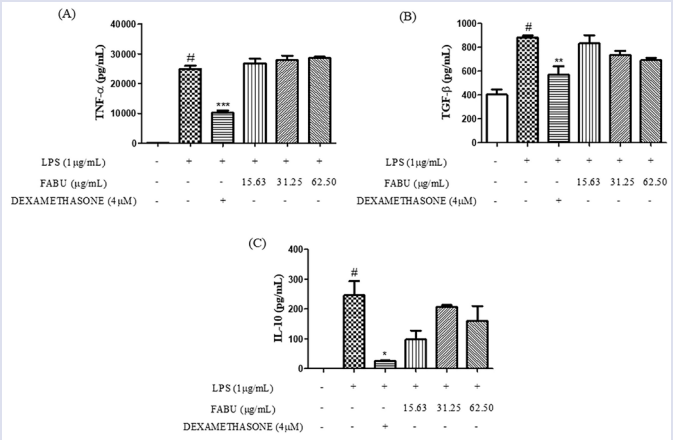

Figure 4: Immunomodulatory activity of *Bauhinia ungulata* L on LPS-stimulated RAW 264.7 macrophages. (A) Inhibition of TNF-α secretion. ***p<0.001 *vs* LPS-stimulated cells group (Tukey's test); # p<0.001 *vs* Control. (B) Inhibition of TGF-β secretion. **p< 0.01 vs LPS-stimulated cells group; # p<0.001 *vs* Control. (C) Inhibition of IL-10 secretion. *p<0.05 *vs* LPS-stimulated cells group; # p<0.01 *vs* Control.

ROS and RNS cascades in RAW 264.7 macrophages

As the FABU was shown to be cytotoxic at levels ≥ 125 µg/mL (Figure 2), doses below this concentration were used in the assays. As observed in Figure 3A, FABU at 15.63, 31.25 and 62.5 µg/mL could significantly decrease ROS levels via the H_2DCFDA assay when compared to the LPS-stimulated group (p <0.01 to 15.63 and 31.25 µg/mL, p <0.001 for 62.5 µg/mL). Figure 3B shows that FABU doses of 31.25 and 62.5 µg/mL were able to inhibit NO production (p <0.01 and p <0.001, respectively) in comparison to the untreated and LPS-stimulated group. Finally, the production of H_2O_2 by macrophages was decreased in a dose-dependent manner after treatment of LPS-stimulated cells with FABU at 31.25 and 62.5 µg /mL (Figure 3C, p <0.01 and p <0.001, respectively). The data clearly demonstrate that FABU presents an effective antioxidant capacity by interfering with ROS and RNS cascades.

Lipid peroxidation

As seen in Figure 3D, MDA production was significantly reduced after cell treatment with the FABU at all tested concentrations (p <0.001). The result indicates that FABU could effectively reduce cellular lipid peroxidation based on the MDA assay.

Cytokine quantification

Secretion of TNF-α, TGF-β and IL-10 levels were significantly increased in LPS-stimulated cells compared to unstimulated cells (Figure 4). However, FABU was not able to alter these cytokine levels at any tested concentration, suggesting that the fraction presented no significant immunomodulatory activity (Figure 4).

DISCUSSION

Several studies have shown that high concentrations of ROS and RNS may be responsible for causing damage to DNA, proteins and lipids during an oxidative stress-related process such as lipid peroxidation of membrane phospholipids. Moreover, high amounts of free radicals may overload the endogenous protection system, affecting the anti-oxidant activities exerted by glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT), among others. As an alternative, external source of antioxidants can delay or inhibit the oxidative damage.[20,21]

Various authors have demonstrated that some medicinal plants contain high concentrations of free radical scavenging molecules. Among these molecules, phenolic compounds such as flavonoids and tannins, present potential antioxidant activity and act directly on ROS and RNS by neutralizing them or inhibiting their production.[21,22]

In the present study, it was demonstrated that FABU presented anti-oxidant activity, acting more efficiently in reducing Mo (VI) to Mo (V) than the BHT control. In addition, Paula *et al.*[3] demonstrated that the ethanol extract and hexane, chloroform and ethyl acetate fractions from *B. ungulata* leaves also presented antioxidant activity. Flavonoids are recognized as antioxidant agents and the phytochemical investigation of FABU led to the isolation of the compounds identified as liquiritigenin (1), guibourtinidol (2) and fisetinidol (3).[6] These flavonoids presented antioxidant activity, according to Gai *et al.*[23] Sobeh *et al.*[24] and Imai *et al.*[25]

In the model of oxidative stress evaluation by the method of H_2DCFDA, FABU was shown to inhibit ROS production by LPS-stimulated cells. Although some studies use H_2DCFDA to specifically measure H_2O_2, oxidation of H_2DCF to DCF can be induced by various ROS, including hydroxyl and nitrogen dioxide. In addition, superoxide anions can be converted to hydrogen peroxide by the action of superoxide dismutase. Also, H_2O_2 can contribute to the amplification of the DCF signal.[26] H_2O_2 concentrations were also measured using the method described by Pick and Mizel.[16] We also demonstrated lower H_2O_2 levels in supernatants from LPS-stimulated RAW 264.7 cells after treatment with FABU.

LPS-stimulated macrophages also activate inducible enzyme nitric oxide synthase (iNOS), which converts L-arginine to nitric oxide and L-citruline and contributes to formation of another free radical, nitric oxide, which reacts with superoxide anion producing a potent biological oxidizing agent, peroxynitrite. High concentrations of peroxynitrite can lead to tissue damage, mainly by lipid peroxidation.[27] In our study, NO levels in the culture supernatant from LPS-stimulated cells was significantly reduced after treatment with FABU at 31.25 and 62.5 µg/mL. We have also tested the antioxidant activity of fisetinidol, a major constituent found in FABU (33.89%). We observed that fisetinidol at 3.125, 6.25 and 12.50 µg/mL was not as effective as FABU in reducing NO levels (data not shown). Concentrations ≥25.0 µg/mL of fisetinidol were not tested because they were cytotoxic to macrophages. Finally, our data suggest that the antioxidant activity exerted by FABU may be attributed to the synergist effect promoted by the various components of the fraction.

Sayago *et al.*[28] demonstrated that extracts from dried and fresh *B. variegate* leaves were capable of signifcantly inhibiting Fe^{2+}-induced TBARS production in a model using egg yolk homogenate as a phospholipid-rich substrate. However, only the fresh leaf extract was able to significantly inhibit lipid peroxidation in a model using mouse brain homogenates. In our study, the results demonstrated that thiobarbituric acid reactive substance formation by LPS-stimulated cells was significantly inhibited after treatment with FABU. Since lipid peroxidation is involved in various diseases such as atherosclerosis, rheumatoid arthritis, cancer, Alzheimer's disease in addition to several other immunological disorders, the search for compounds that inhibit oxidative stress is crucial. *In vivo* studies are necessary to confirm the antioxidant capacity of *B. ungulata*.

In respect to the antimicrobial activity, the MICs of the FABU against MSSA ATCC® 6538P, MRSA ATCC® 65398 and *P. aeruginosa* ATCC® 9027 and *C. albicans* strain ATCC® 22019 were >500 µg/mL. Paula *et al.*[29] previously demonstrated that the MICs of the crude extract of leaves of the same plant species against strains of *S. aureus* ATCC 25923, *Escherichia coli* ATCC 5922, *P. aeruginosa* ATCC 27853 and *Enteroccocus faecalis* ATCC 29212 were >1000 µg/mL and of hexane, chloroform, ethyl acetate and residual hydroalcohol fractions were >200 µg/mL. The data suggest that the species probably does not present good antimicrobial activity.

CONCLUSION

Our results demonstrated that the ethyl acetate fraction of the *B. ungulata* stem wood presented antioxidant activity, demonstrated in the phosphomolybdenum model in addition to a cell culture model using LPS-stimulated macrophages. In such a model, a significant decrease in the amount of reactive oxygen and nitrogen species was observed in addition to lipid peroxidation inhibition. Based on the measurement of TNF-α , TGF-β and IL-10 levels in supernatants from LPS-stimulated cells after treatment with FABU, the extract did not present any significant immunomodulatory activity. In conclusion, FABU is a natural antioxidant complex that may interfere in the cascade of cell damage caused by free radicals. Further studies are needed to demonstrate its beneficial effects using *in vivo* models.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ABBREVIATIONS

FABU: Ethyl acetate fraction of *Bauhinia ungulata*; **LPS:** Lipopolysaccharide; **ROS**: Reactive oxygen species; **TBARS:** Thiobarbituric acid reactive substances.

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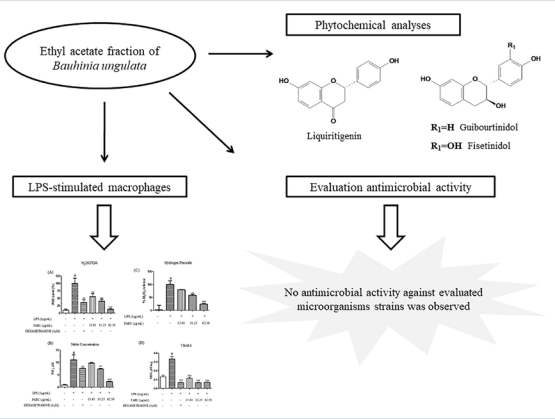
GRAPHICAL ABSTRACT



SUMMARY

- The minimal inhibitory concentration of the FABU against MSSA, MRSA, *P. aeruginosa* and *C. albicans* was ≥ 500 µg/mL.
- The total antioxidant activity of the FABU was 1.70 mg equivalent of BHT per mg FABU.
- The study demonstrated that FABU could significantly inhibit ROS levels and also decrease NO, H_2O_2 and TBARS levels when compared to the LPS-stimulated group.
- FABU did not alter the levels of TNF-α, TGF-β and IL-10 in the culture supernatant after treatment of LPS-stimulated cells.
- Our results demonstrated that FABU presents significant antioxidant activity.

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