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ORIGINAL ARTICLE

Antioxidant, Anti-inflammatory, and Antiproliferative Activity of Extracts Obtained from *Tabebuia Rosea* (Bertol.) DC

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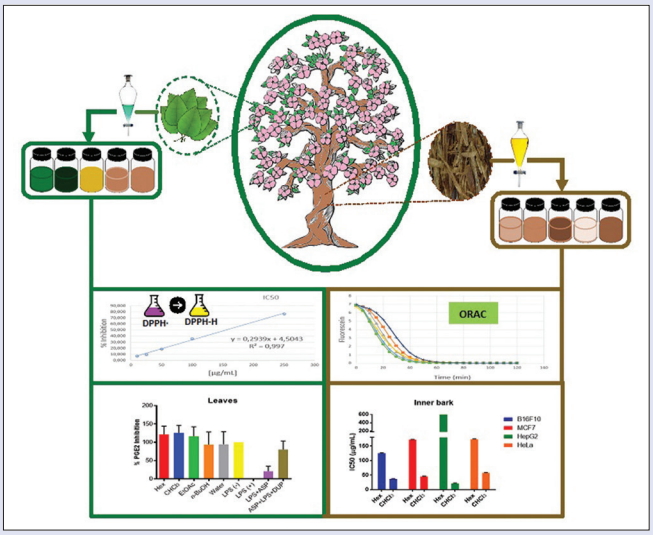
ABSTRACT

Background: *Tabebuia rosea* (Bertol.) DC. is a neotropical tree used in traditional medicine in the Northern coast of Colombia as well as Latin America for infectious diseases treatment. Few studies have evaluated the biological activity of this species. **Objective:** The objective of this study is to determine the antioxidant, anti-inflammatory, and antiproliferative potential of leaf and inner bark extracts from *T. rosea*. **Materials and Methods:** The antioxidant activity was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and oxygen radical absorbance capacity (ORAC) methods. The anti-inflammatory activity was evaluated in lipopolysaccharide-stimulated murine macrophages. *In vitro* antiproliferative effect was determined in HepG2, HeLa, MCF-7, and B16F10 cell lines. **Results:** The highest DPPH radical scavenging activity was observed for *T. rosea* ethyl acetate leaf extract (IC_{50} of 157.5 ± 2.4 µg/mL). This extract also induced the best antioxidant activity as determined by ORAC (11,112.2 ± 1,255.3 µmol TE/g of extract). Moreover, *T. rosea* leaf *n*-hexane, chloroform, and aqueous extracts, in addition to inner bark aqueous extract did inhibit nitric oxide production by over 90%. In addition, inner bark extracts markedly inhibited prostaglandins E2 and tumor necrosis factor alpha (>90%). The best antiproliferative activity was displayed by the inner bark chloroform extract against HepG2 (selectivity index [SI] = 5.50) and B16F10 (SI = 3.18) cell lines. **Conclusion:** These results demonstrate the potential biological activity of *T. rosea* extracts.

Key words: Anti-inflammatory agents, antineoplastic agents, antioxidant agents, bignoniaceae, *Tabebuia rosea*

SUMMARY

- *Tabebuia rosea* extracts have a promising antioxidant, anti-inflammatory, and anti-proliferative activity. Important antioxidant activity was observed in the ethyl acetate extract obtained from leaves. We report for the first time the antiproliferative effect of the inner bark extracts and the potential of *Tabebuia rosea* extracts to inhibit the production of key inflammatory mediators such as nitric oxide, prostaglandins E2, and tumor necrosis factor alpha.



Abbreviations used: DPPH: 2,2-diphenyl-1-picrylhydrazyl; ORAC: Oxygen radical absorbance capacity; LPS: Lipopolysaccharide; NO: Nitric oxide; TNF-α: Tumor Necrosis Factor Alpha; PGE_2: Prostaglandin E2; TAC: Total antioxidant content; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide.

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INTRODUCTION

The isolation and characterization of secondary metabolites with biological value from natural products are still an area of great relevance and interest to identify molecules with potential antimicrobial,[1] antioxidant,[2,3] anti-inflammatory,[4-6] and antitumoral[7-9] activities. Several studies have focused on the anti-inflammatory effect of different extracts obtained from leaves, stems, roots, and bark of different plant species, contributing to the discovery of a considerable number of molecules in the past decades. These molecules have the capacity to inhibit mediators of the inflammatory response in a specific manner. During the inflammatory process, macrophages play an important role as cells of the innate response with the capacity to produce pro-inflammatory cytokines (tumor necrosis factor alpha [TNF- α], interleukin [IL]-1β, and IL-6), prostaglandins E2 (PGE_2), reactive oxygen species (ROS) and nitric oxide (NO), and among others. These molecules are directly associated with acute inflammatory processes and the development

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of chronic inflammatory diseases such as atherosclerosis, obesity, rheumatoid arthritis, intestinal inflammatory disease, neurodegenerative disease, and cancer.[10-12]

Chronic inflammatory diseases are also associated with the accumulation of free radicals or other ROS that can elicit direct or indirect damage to the body. All biological systems have innate antioxidant defense mechanisms that remove damaged molecules; however, these mechanisms can be inefficient. Therefore, antioxidant molecules are important to protect cells from damage caused by free radicals.

In the search for products derived from plants with potential biological activity, it has been described that some species belonging to the Bignoniaceae family have anti-inflammatory, antimicrobial, and antitumoral potential, due to its empirical use in rural areas in Colombia, Bolivia, Brazil, and other Latin American countries.[13,14] Within this family, *Tabebuia rosea* (Bertol.) DC. has been used in traditional medicine in the Northern Coast of Colombia for the treatment of skin conditions such as pruritic diseases and infections with fungi and yeast.[15] *T. rosea* is a tree that reaches 30 m height and has a 1 m diameter trunk, its bark is flaky and is recognized by bell-shaped ornate purple and pink flowers. In Colombia, it is commonly known as "apamate," "ocobo rosado," "guayacán rosado," and "roble morado."

Few studies have evaluated the biological activity of *T. rosea* extracts; therefore, the aim of this work was to determine the antioxidant, anti-inflammatory, and antiproliferative potential of the leaf and inner bark extracts obtained from *T. rosea*.

MATERIALS AND METHODS

Chemicals and reagents

Analytical grade organic solvents were purchased from Mallinckrodt Baker (San Diego, CA, USA) and JT Baker (Phillipsburg, NJ, USA). Folin-Ciocalteu's reactive was obtained from Merck (Darmstadt, Germany). Gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), dimethyl sulfoxide molecular grade (DMSO 99.9%), Griess reagent (modified), and *Escherichia coli* lipopolysaccharide (LPS) serotype O111:B4 were purchased from Sigma-Aldrich (Deutschland, Germany). DUP-697 and Prostaglandin E_2 EIA-monoclonal kit were purchased from Cayman Chemical (Ann Arbor, MI, USA). ELISA kits for IL-10 and TNF-α were acquired from BD Biosciences (Palo Alto, CA, USA). Dulbecco's Modified Eagle's Medium (DMEM) + Glutamax II and fetal bovine serum (FBS) were purchased from Gibco (Gaithersburg, MD, USA). Acetylsalicylic Acid (Aspirin, ≥99.0%) and Limulus Amebocyte Lysate Test, E-Toxate Kit were acquired from Sigma Chemical Co, Saint Louis, MO, USA.

Plant material and extract preparation

Leaves and inner bark from *T. rosea* (Bertol.) DC. were collected at Universidad Tecnológica de Pereira Campus in April 2011. The plant was identified at the Colombian National Herbarium (Voucher No. COL 582577). The collection and processing of the material were covered by the collection permission number 1133/2014 issued by the National Environmental Licensing Authority-ANLA-of Colombia.

Plant material was dried and macerated in methanol for 48 h followed by homogenization, filtration, and concentration under vacuum using a vacuum rotary evaporator to obtain the crude extract. This procedure was repeated three times. Crude extract was dissolved in distilled water and underwent liquid–liquid extraction with increasing polarity solvents: *N*-hexane, chloroform, ethyl acetate, and *n*-butanol. Each extract was vacuum dried by vacuum rotary evaporator until its mass was attained. Endotoxin levels in the extracts (that can induce the release of inflammatory mediators) were assayed using the Limulus Amebocyte Lysate Test, E-Toxate Kit (Sigma Chemical Co, Saint Louis, MO, USA). All samples were negative for the presence of endotoxins (detection limit 0.05–0.1 EU/mL).

Preliminary phytochemical screening

The preliminary phytochemical screening was performed using selective derivatization reactions for the characterization of secondary metabolites present in the *n*-hexane, chloroform, ethyl acetate, and *n*-butanol extracts obtained from leaves and inner bark of *T. rosea*.[16] The extracts were evaluated under different elution systems using normal and reverse phase thin layer chromatography. Chromatographic plates were revealed with aluminum chloride and ferric chloride for detection of flavonoids, phenols, and phenolic acids; potassium hydroxide in ethanol for detection of anthrones, quinones, and coumarins; oleum for detection of sesquiterpenic lactones and the Liebermann–Burchard reagent for detection of terpenes and steroids.

Cell culture

Mouse macrophage cell line RAW264.7 (ATCC; MD, USA), human embryonic kidney (HEK-293, ATCC, CRL-1573), human hepatocarcinoma (HepG2; ATCC; CRL-11997), human cervix adenocarcinoma (HeLa; ATCC; CCL-2), human mammary gland adenocarcinoma (MCF-7; ATCC; HTB-22), and mouse skin melanoma (B16F10; ATCC; CRL-6475) were purchased from American Type Culture Collection (ATCC, Rockville, MD, USA) and cultured with DMEM supplemented with Glutamax and 10% heat inactivated FBS, 200 μg/mL penicillin, 200 μg/mL streptomycin, 400 μg/mL neomycin, 5 μg/mL amphotericin, 0.05 mM 2-β-mercaptoethanol, and 1 mM sodium pyruvate. Cells were maintained at 37°C with 5% CO_2.

Antioxidant content determination

Total antioxidant content in the extracts obtained from *T. rosea* was evaluated by a colorimetric assay previously described,[17] with modifications. Fifty microliters of the samples were mixed with 2 mL distilled water and 250 μL Folin-Ciocalteu's-water reagent (1:1). After three min, 750 μL of a Na_2CO_3 saturated solution were added and was brought to 5 mL with distilled water. The reaction was maintained in the dark for 30 min at room temperature (RT), and absorbance was quantified at 760 nm in a Shimadzu UV-1700 spectrophotometer. Gallic acid (0.25–5 mg/mL) was used to generate a standard curve ($y = 0.101x + 0.086$; $R^2 = 0.996$). Results are presented as mg gallic acid equivalents per g of extract (mg GAE/g extract). All experiments were performed in triplicate.

2,2-diphenyl-1-picrylhydrazyl radical scavenging activity

Antioxidant capacity was determined by measuring DPPH radical scavenging through the Brand-Williams methodology with some modifications.[18] Thirty microliters of *T. rosea* extract prepared at 10, 25, 50, 100, 500, and 1.000 μg/mL were mixed with 2 mL of a methanol solution of DPPH (20 μg/mL DPPH, 5×10^{-5} mol/L); this mix was agitated and kept in the dark for 30 min at RT. Absorbance was measured at 517 nm in a Shimadzu UV-1700 spectrophotometer. Ascorbic acid, gallic acid, and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox®) at 5, 25, 50, 100, and 250 μg/mL were used for the standard curves. Each experiment was repeated three times.

Oxygen radical absorbance capacity

Oxygen radical absorbance capacity (ORAC) was determined following the method described by Ou, with some modifications.[19] 2,2'-Azobis (2-amidinopropane) dihydrochloride (AAPH) and sodium fluorescein stock solutions were prepared in phosphate buffer (75 mM, pH 7.4).

Samples (31 µL) were diluted in 187 µL of fluorescein (120 nM) and incubated at 37°C for 10 min. The reaction was started by the addition of 31 µL of AAPH (143 mM) to reach a final volume of 249 µL per well. Extracts were evaluated in the following concentrations ranges: 5, 10, 15, and 20 µg/mL. A Trolox® standard curve was prepared (10, 20, 40, and 60 µM). Changes in fluorescence were measured with a Varian Cary Eclipse 1.1 fluorescence spectrophotometer at 2 min intervals for 120 min with emission and excitation wavelengths of 515 and 493 nm, respectively. The antioxidant capacity was calculated as the area under the curve[20] and expressed as µmol Trolox® equivalents per g of extract (µmol TE/g of extract).

Cell viability assay

T. rosea leaf and inner bark extracts effect on RAW264.7 cells line were determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) method.[21] Cells were initially seeded at 5×10^4 cells/well and incubated for 24 h, time at which extracts at 0.5, 1.0, and 2.0 µg/mL diluted in DMSO (final concentration 0.1%) were added to the cells. Then, 200 µL MTT (0.5 mg/mL) were added to each well and incubated for 4 h at 37°C and 5% CO_2. After removing culture media, formazan crystals were dissolved in 100 µL DMSO. Absorbance was measured in an ELISA plate reader at 492 nm (ELx800; Bio Tek Instruments Inc., USA). Viability percentage was calculated based on nontreated control. Results were expressed in µg/mL as half-maximal inhibitory concentration (IC_{50}) of cell proliferation. Three independent assays were performed each in triplicate.

Inhibition in nitric oxide, interleukin-10, prostaglandins E2, and tumor necrosis factor alpha production

RAW264.7 cells were seeded at 1×10^6 cells/well in 24 well plates and incubated for 24 h. At 22 h of culture, cells were treated with aspirin (50 µM, Sigma Chemical Co, Saint Louis, MO, USA) to inhibit cyclooxygenase-1 (COX-1) activity. At 24 h wells were washed with DMEM without supplementation and extracts were added at 2 µg/mL in addition to 10 µg/mL of LPS (*E. coli* serotype O111:B4) to determine NO and IL-10 production. For TNF-α and PGE_2 production, LPS was added at 5 µg/mL. For NO production, cells were incubated during 18 h, for TNF-α and PGE_2 production cells were incubated for 12 h, and for IL-10, production cells were incubated for 6 h. Incubation times were previously determined by kinetic assays. The COX-2-specific inhibitor (DuP 697) was used at a concentration of 9.0 µM. Supernatants were collected and stored at −80°C until evaluated. NO was determined by the Griess test. PGE_2 production was determined by ELISA following manufacturer's instructions (Prostaglandin E2 EIA Kit-monoclonal, Cayman Chemical Ann Arbor MI, USA). Furthermore, IL-10 and TNF-α were quantified by ELISA following manufacturer's instructions (BD Biosciences Palo Alto CA, USA). Three independent assays were performed in triplicate.

Cytotoxic activity

Cytotoxic activity was assayed with the MTT assay.[21] HEK-293 cells were seeded at 1×10^4 cells/well in 96 well plates and incubated for 24 h at 37°C and 5% CO_2. Media were removed and 100 µL of extract were added at 10, 50, and 400 µg/mL. As a control, 100 µL tetracycline (200 µM) were used. Cells were incubated for 72 h at 37°C and 5% CO_2. Media were removed and 20 µL MTT (5 mg/mL) were added and incubated for 4 h. After incubation, supernatant was removed and 100 µL DMSO were added. Absorbance values were determined at 570 nm using an ELISA plate reader (ELx800; Bio Tek Instruments Inc., USA). Cytotoxic activity was expressed as half-maximal cytotoxic concentration (CC_{50}).

Antiproliferative activity

Antiproliferative activity was also determined with the MTT assay.[21] Cells were seeded as follows: HepG2 (1.5×10^4 cells/well), HeLa (1.5×10^4 cells/well), MCF-7 (1.5×10^4 cells/well), and B16F10 (1×10^4 cells/well), in 96 well plates. 100 µL of extract were added at 1, 10, 50, and 200 µg/mL. Cells were incubated for 72 h at 37°C and 5% CO_2. Media were removed and 20 µL MTT (5 mg/mL) were added and incubated for 4 h. After incubation, supernatant was removed and 100 µL DMSO were added. Absorbance values were determined at 570 nm using an ELISA plate reader (EL × 800; Bio Tek Instruments Inc., USA). Antiproliferative activity was expressed as IC_{50}. In addition, the selectivity index (SI) was calculated ($SI = CC_{50}/IC_{50}$). A SI >1 indicates that the extract is more toxic for the tumoral cell, whereas a SI <1 indicates that the extract is more toxic for normal cells.

Statistical analysis

Results are presented as mean ± SEM All tests were performed as three independent assays, each with triplicates. IC_{50} values for antioxidant and antiproliferative activities were determined by linear regression analysis. Data analysis was performed using a Mann–Whitney test, and P value < 0.05 was considered statistically significant. The statistical tests were carried out using GraphPad Prism, version 6.0 (GraphPad Software, San Diego, CA, USA).

RESULTS AND DISCUSSION

Preliminary phytochemical screening

The preliminary phytochemical screening did show the presence of anthrones, quinones and coumarins in all the extracts evaluated [Table 1]. The presence of terpenes, steroids, and sesquiterpenic lactones was evidenced in the *n*-hexane, chloroform, and ethyl acetate extracts obtained from both inner bark and leaves of *T. rosea*. However, the presence of flavonoids and phenolic acids was observed only in the ethyl acetate extracts. These results are in agreement with those previously reported for the ethanolic and methanolic leaf extract of *T. rosea*.[22,23] Only one study reports the presence of carbohydrates, quinones, terpenes, glycosides, saponins, flavonoids, and phenolic compounds in extracts obtained from the flowers of *T. rosea*.[24] Although there are few reports concerning the phytochemical analysis of *T. rosea*, several compounds have been isolated such as 6-O-(p-coumaroyl)-catalpol (specioside), an iridoid glycoside isolated from the methanolic extract obtained from the inner bark.[25,26] In addition, lapachol, dehydrotectol, dehydro-α-lapachone, dehydro-iso-α-lapachone, and β-sitosterol were isolated from both inner bark and roots.[27,28] It is important to

Table 1: Preliminary phytochemical screening in extracts obtained from the inner bark and leaves of *Tabebuia rosea*

Part of the plant	Extract	Reagent				
		$AlCl_3$	KOH/EtOH	Oleum	$FeCl_3$	Liebermann-Burchard
Inner bark	*n*-hexane	-	+	+	-	+
	Chloroform	-	+	+	-	+
	Ethyl acetate	-	+	+	+	+
	n-butanol	-	+	-	-	-
Leaves	*n*-hexane	-	+	+	-	+
	Chloroform	-	+	+	-	+
	Ethyl acetate	+	+	+	+	+
	n-butanol	-	+	-	-	+

+: Presence of compounds; -: Absence of compounds; KOH: Potassium hydroxide; $AlCl_3$: Aluminum chloride; $FeCl_3$: Ferric chloride

mention that several compounds have been isolated from the roots of *T. rosea*, such as Tabebuialdehydes A–C, 3,4-dimethoxybenzoic acid, 4-methoxybenzoic acid, 4-hydroxycinnamic acid, lapachol, 5-hydroxy-dehydro-iso-α-lapachone, and isopaulownin.[29]

Total antioxidant activity and antioxidant content

In vitro antioxidant activity of *T. rosea* extracts as well as mass yield expressed as weight/weight percentage (w/w %) are presented in Table 2. The highest DPPH radical scavenging activity was observed for *T. rosea* leaf ethyl acetate extract with an IC_{50} of 157.5 ± 2.4 μg/mL, lower than the Trolox control (251.6 ± 0.8 μg/mL) and higher than the gallic acid control (55.9 ± 0.7 μg/mL). The activity was only comparable to that obtained with ascorbic acid (146.3 ± 0.8 μg/mL). The remaining extracts had IC_{50} much higher than 250 μg/mL. Our results indicate that compounds present in *T. rosea* ethyl acetate leaf extract have the highest antioxidant activity. This was not the case for the inner bark extract. In addition, these results are in agreement with the phytochemical analysis [Table 1], suggesting that the presence of phenolic hydroxyl groups and flavonoids in the extract are responsible for this activity. The absence of an important antioxidant activity in the extracts obtained from the inner bark of *T. rosea* is in concordance with a previous report showing a low DPPH radical scavenging activity in extracts obtained from the inner bark of *T. rosea* collected in the Northern Coast of Colombia (IC_{50} values were higher than 489 μg/mL, using ascorbic acid as control).[30]

Regarding ORAC activity, the results are like those obtained with the DPPH assay [Table 2]. *T. rosea* leaf ethyl acetate extract had the highest activity (11,112.2 ± 1,255.3 μmol TE/g extract), followed by the inner bark ethyl acetate extract (8,245.6 ± 703.8 μmol TE/g of extract), and *n*-butanol leaf extract (6,139.5 ± 769.6 μmol TE/g of extract). The ethyl acetate leaf extract displayed a powerful ORAC activity, similar to the activity of the reference flavonoid used as control, quercetin 3-β-D-glucoside (5,780.9 ± 982.3 μmol TE/g of extract).

The antioxidant activity measured by the DPPH and ORAC methods did correlate with the antioxidant total content of the extracts (R^2 = 0.9146). The ethyl acetate extracts obtained from leaves and inner bark as well as the *n*-butanol extract obtained from leaves did show values of 3.5, 2.2 and 2.1 mg GAE/g extract, respectively. However, the *n*-hexane and aqueous extracts obtained from both inner bark and leaves were not evaluated in the study due to its low solubility in methanol. The presence of phenolic metabolites such as flavonoids and phenolic acids are in agreement with the antioxidant effect observed. In the phytochemical analysis reported by Suo, the ethyl acetate extract obtained from the inner bark of *T. avellanedae* was separated, and phenylpropanoid glycosides with strong antioxidant activity in the DPPH assay were isolated.[31] In a previous study carried out with methanol extracts obtained from the flowers, leaves, stem bark and root bark of *Tabebuia pallida*, a considerable antioxidant potential was observed in the extract using the DPPH method.[32] In our study, a correlation between the antioxidant activity and the content of total antioxidants was observed. In this sense, low concentration of phenolic compounds decreases the antioxidant capacity.

Anti-inflammatory activity

Murine macrophages (RAW264.7) were selected for the *in vitro* model due to their critical role in both immune recognition and development of the inflammatory response.[33] After bacterial LPS stimulation, macrophages release a variety of mediators that have been implicated in the development of the inflammatory process such as IL-1β, IL-6, IL-10, TNF-α, NO, and prostaglandin E_2 (PGE_2).[34]

The effect of the extracts on LPS-induced NO production in macrophages was evaluated by measuring the accumulated nitrite in culture medium using the Griess reaction. The stimulation of macrophages with LPS in the presence of *T. rosea* extracts inhibited NO production. A complete inhibition was observed for the *n*-hexane and chloroform leaf extracts as well as for the water extract obtained from the inner bark, as shown in Figure 1a and b. A moderate inhibition was induced by the inner bark chloroform extract (73.3%) and the *n*-butanol and ethyl acetate extracts obtained from leaves (68.9% and 82.7%, respectively). The differences were significant ($P < 0.05$) when compared with LPS stimulation. It is important to note that the ethyl acetate extract obtained from the inner bark did not induce a strong inhibition in NO production (31%) in spite of having and important antioxidant activity.

The production of PGE_2 in culture supernatants of RAW264.7 macrophages was determined by ELISA to evaluate the inhibitory effects of *T. rosea* extracts on COX-2 activity. LPS stimulation induced the production of PGE_2 whereas very low amounts of PGE_2 were observed in unstimulated cells. All of the extracts obtained from the leaves displayed inhibitory effects on PGE_2 production higher than 90%. In contrast, inner bark extracts inhibited PGE_2 production in a range between 50% and 100% [Figure 1c and d]. The inhibitory effects of the samples were statistically significant when compared with LPS stimulated cells ($P < 0.01$). The COX-2 specific inhibitor, DuP 697(IC_{50} 9.0 μM), inhibited PGE_2 production by 80%. It is important to mention that the extracts obtained from the leaves induced an inhibitory effect on PGE_2 strongest that the inhibition obtained with DuP 697. This suggest that extracts contain compounds with a possible inhibitory effect on the catalytic activity of COX-2.

Table 2: Antioxidant effect in extracts obtained from the inner bark and leaves of *Tabebuia rosea*

Part of the plant	Extract	Yield (w/w %)	Antioxidant activity (mean±SEM)		
			DPPH (IC_{50}; μg/mL)	ORAC (μmol TE/g extract)	TAC (mg GAE/g extract)
Inner bark	*n*-hexane	0.7	>250	1284.9±233.3	ND
	Chloroform	2.3	>250	4181.0±198.2	0.6
	Ethyl acetate	7.3	>250	8245.6±703.8	2.2
	n-butanol	24.9	>250	5286.4±524.0	0.9
	Water	43.4	>250	736.5±170.8	ND
Leaves	*n*-hexane	13.0	>250	1194.8±71.2	ND
	Chloroform	11.8	>250	ND	0.1
	Ethyl acetate	7.5	157.5±2.4	11,112.2±1255.3	3.5
	n-butanol	26.0	>250	6139.5±769.6	2.1
	Water	26.9	>250	831.2±57.0	ND

TE: Trolox equivalents; GAE: Gallic acid equivalents; ND: Not determined; TAC: Total antioxidant content; ORAC: Oxygen radical absorbance capacity; DPPH: 2,2-diphenyl-1-picrylhydrazyl; SEM: Standard error of mean

Although all the extracts evaluated inhibited both NO and PGE_2 production, the *n*-hexane and chloroform extracts obtained from leaves, exhibited the highest inhibitory percentage (>90%). Studies are in progress to isolate and to identify the molecules responsible of this activity.

With regard to the inhibitory effect of *T. rosea* extracts on TNF-α production, both inner bark and leaf extracts did inhibit the production of this cytokine [Figure 1e and f]. The inner bark chloroform and *n*-hexane extracts induced a strong inhibition on TNF-α production (>90%), whereas slightly lower inhibition percentages were induced by the leaf extracts (75%–83%). The differences were statistically significant when compared with the LPS-stimulated cells ($P < 0.01$), except for the ethyl acetate extract obtained from leaves ($P = 0.142$). In unstimulated macrophages, only small amounts of TNF-α were secreted into the medium. Stimulation with LPS induced the production of TNF-α.

In general, the evaluation of the anti-inflammatory activity indicates that the chloroform and *n*-hexane extracts obtained from both leaves and inner bark displayed the best activity. However, a correlation between antioxidant and anti-inflammatory activity was not found. The results from this study agree with a recent work reporting the *in vivo* anti-inflammatory activity of the methanolic leaf extract obtained from *T. rosea*, using the carrageenan-induced paw edema model in rats.[35]

To the best of our knowledge, this is the first study showing the potential of *T. rosea* extracts to inhibit the production of key inflammatory mediators such as NO, PGE_2, and TNF-α. These results provide information to suggest that *T. rosea* extracts are promising in the search of new anti-inflammatory molecules. It is important to evaluate the molecular mechanisms responsible of this activity. Recent studies report the anti-inflammatory activity *in vivo* of the methanol extract obtained

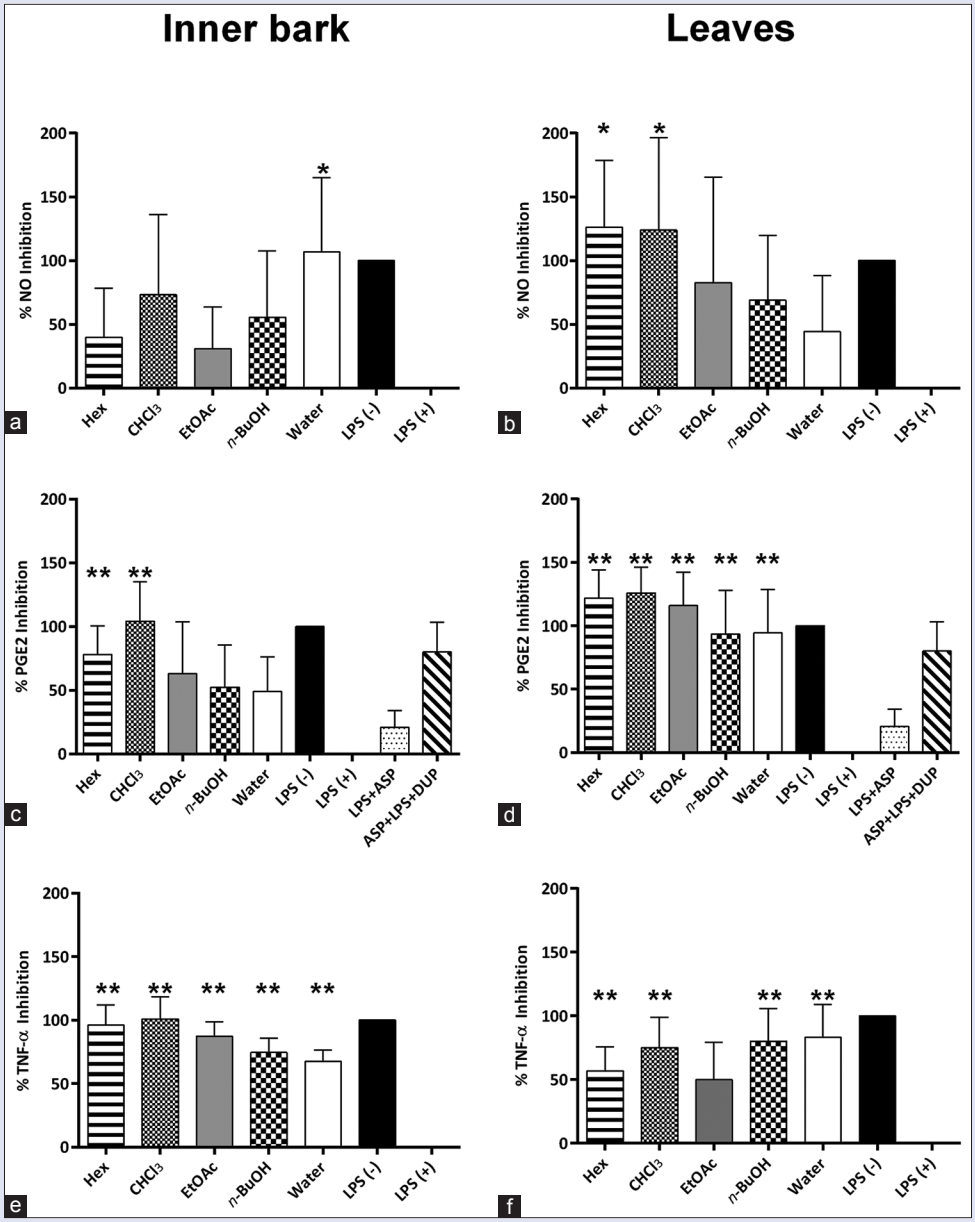

Figure 1: Inhibitory effects of *Tabebuia rosea* extracts on nitric oxide (a,b), Prostaglandin E2 (c,d) and tumor necrosis factor alpha (e,f) production in RAW 264.7 macrophages stimulated with LPS. Inner bark extracts (left), leaves extracts (right). Values are expressed as the mean ± standard error of mean from three independent experiments (*$P < 0.05$, **$P < 0.01$, compared with LPS). Hex: *n*-hexane, CHCl3: chloroform, EtOAc: ethyl acetate, *n*-BuOH: *n*-butanol, ASP: Aspirin, DUP: DuP 697, LPS: Lipopolysaccharide

Table 3: Antiproliferative effect of extracts obtained from the inner bark and leaves of *Tabebuia rosea*

Part of the plant	Extract	CC_{50}±SEM (μg/mL)	IC_{50}±SEM (μg/mL)			
		HEK-293	B16F10	MCF7	HepG2	HeLa
Inner bark	*n*-hexane	178.4±1.4	125.6±1.6	172.2±1.5	>200	173.2±1.2
	Chloroform	115.9±1.3	36.4±1.7 (SI=3.18)	45.5±1.2 (SI=2.55)	21.1±1.4 (SI=5.50)	57.6±1.2 (SI=2.01)
	Ethyl acetate	137.1±1.4	>200	155.08±1.3	>200	>200
	n-butanol	>400	>200	>200	>200	>200
	Water	>400	>200	>200	>200	>200
Leaves	*n*-hexane	164.5±1.3	182.0±1.8	114.4±1.2	>200	119.1±1.3
	Chloroform	1.1±1.2	17.6±1.3 (SI=0.06)	5.0±1.2 (SI=0.22)	17.3±1.3 (SI=0.06)	24.7±1.4 (SI=0.05)
	Ethyl acetate	24.9±1.2	187.8±1.7	112.4±1.3	175.2±1.4	>200
	n-butanol	49.0±1.4	>200	>200	>200	>200
	Water	>400	>200	>200	>200	>200

CC_{50}: 50% cytotoxic concentration; IC_{50}: Half maximal inhibitory concentration; SEM: Standard error of the mean; SI: Selectivity Index; HEK-293: Human embryonic kidney; HepG2: Human hepatocarcinoma; HeLa: Human cervix adenocarcinoma; B16F10: Mouse skin melanoma; MCF7: Human mammary gland adenocarcinoma

from the stems of *Tabebuia hypoleuca* and the isolation of new iridoid esters (Avelladoids A–H) from the inner bark of *Tabebuia avellanedae* with anti-inflammatory activity *in vitro*.[36,37] The study of different species from the genus *Tabebuia* is important to evaluate new natural sources of biologically active molecules that could be used for drug development.

Antiproliferative activity

The results concerning the *in vitro* antiproliferative activity are summarized in Table 3. The most important activity was displayed by the *T. rosea* leaf chloroform extract against the MCF-7 cell line, with an IC_{50} of 5.0 ± 1.2 μg/mL, followed by HepG2, B16F10, and HeLa cell lines with IC_{50} values of 17.3 ± 1.3, 17.6 ± 1.3, and 24.7 ± 1.4 μg/mL, respectively. However, regarding SI, the most promising activity was observed for the inner bark chloroform extract against HepG2 (21.1 ± 1.4 μg/mL SI = 5.50), B16F10 (36.4 ± 1.7 μg/mL, SI = 3.18), MCF7 (45.5 ± 1.2 μg/mL, SI = 2.55), and HeLa (57.6 ± 1.2 μg/mL, SI = 2.01) cell lines. None of the remaining extracts evidenced an important antiproliferative activity. To the best of our knowledge, this is the first report describing *T. rosea* inner bark extracts with antiproliferative effects on tumor cells. The previous studies have shown the presence of several naphthoquinones exhibiting cytotoxic effects against HeLa and KB cells lines in the roots of *T. rosea*.[29] The total alkaloid extract obtained from *T. rosea* leaves is preferentially cytotoxic to human T-cell leukemia (MOLT-4) cells *in vitro*.[9] On the other hand, the cytotoxic activity of synthetic furonaphthoquinones previously isolated from *Tabebuia* plants against U937 and HL-60 cells suggests that these compounds have an important antileukemic activity *in vitro*.[8] Further studies are required to validate the antitumor activity of *T. rosea* extracts and its constituents in animal models.

CONCLUSION

This study contributes to the knowledge of the biological activity of *T. rosea*. The results indicate that *T. rosea* extracts have a promising antioxidant, anti-inflammatory, and antiproliferative activity. Future studies are required to isolate molecules responsible of these activities and to elucidate their mechanisms of action.

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Conflicts of interest

There are no conflicts of interest.

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