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Original Article

Schinus terebinthifolius: phenolic constituents and *in vitro* antioxidant, antiproliferative and *in vivo* anti-inflammatory activities

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ABSTRACT

Schinus terebinthifolius Raddi, Anacardiaceae, native to Brazil, is referred to as "pimento-rosa" and is used to treat inflammatory disease in folk medicine. Studies have reported important pharmacological properties, but these effects have still not been fully exploited. This study reports that the crude extract and isolated compounds of *S. terebinthifolius* (leaves) have *in vitro* antioxidant, antiproliferative, and *in vivo* anti-inflammatory activities. The samples were evaluated for antioxidant activity using 2, 2-diphenyl-1-picrylhydrazyl, β-carotene/linoleic acid and 2,2′-azino-bis-(3-ethylbenzothiazoline)-6-sulphonic acid reagents. The anti-inflammatory effects were assayed against a carrageenan-induced paw oedema model in mice to test doses of 10, 100 and 300 mg/kg at different time points in addition to myeloperoxidase activity analysis. The antiproliferative activity was evaluated using ten human tumour cell lines. Two derivatives of gallic acid and four flavonoids were isolated and exhibited considerable antioxidant activity. The extract and its compounds showed selectivity towards ovarian cancer cells, with growth inhibitory activity values ranging from 1.9 to 6.5 μg/ml. Sample extracts and methyl gallate significantly inhibited carrageenan-induced oedema in the mice paw oedema experimental model. The calculated topological polar surface area for methyl gallate (86.98 Å^2) showed good intestinal absorption. The effects reported herein are be related to the presence of flavonoids and the galloyl phenolic derivative content.

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Introduction

Schinus terebinthifolius Raddi, Anacardiaceae, is an evergreen shrub that grows in South and Central America. In Brazil, it is popularly known as "pimenta-rosa", "aroeira-vermelha", "aroeira-pimenteira", "aroeira-da-praia", "aroeira-negra" and/or "aroeira-de-minas" and used in folk medicine for treatment of several health disorders as well anti-inflammatory processes (Morton, 1978; Gazzaneo et al., 2005). Its biological applications have been described since the first edition of the Brazilian Pharmacopoeia, published in 1926. A Brazilian gel-based aqueous bark extract of *S. terebinthifolius* has been marketed since 1999 for the treatment of vaginitis and cervical vaginitis (Leite et al., 2011). The Brazilian Pharmacopoeia recommends the decoction of *S. terebinthifolius* for use as a natural anti-inflammatory agent (Santos and Amorim, 2002). Pharmacological studies with extracts obtained from leaves have reported antioxidant, anti-allergic, antimicrobial, anti-inflammatory, antiulcer and antiadherent properties as well as wound-healing properties (Castelo Branco Neto de et al., 2006; Carvalher-Machado et al., 2008; Gomes et al., 2010; Johann et al., 2010; Carvalho et al., 2013; Barbieri et al., 2014; Uliana et al., 2016). Chemical studies showed that polyphenolic and flavonoid are major constituents of the extracts of *S. terebinthifolius* leaves (Farag, 2008; El-Massry et al., 2009; Santana et al., 2012).

Investigations by our research group show that the essential oil of *S. terebinthifolius* fruits contains a predominance of monoterpenes, with β-pinene as the major constituent. This oil was effective against persistent inflammation caused by Complete Freund Adjuvant (CFA) or acute inflammation induced by carrageenan in the paw or in pouches (Formagio et al., 2011). In another study,

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the extract of the leaves has anti-inflammatory, immunomodulatory, chemopreventive, antigenotoxic and antimutagenic effects owing to phenol and flavonoid compounds (Fedel-Miyasato et al., 2014a,b).

The oxidative damage induced in cells and tissues is related to the aetiology of various diseases including inflammatory and cancer diseases. Thus, the natural products are candidates for these tests because the insertion of food compounds or phytopharmaceuticals may be an important alternative to treat inflammation and the prevention of cancer. Although cancer is a specific disease, it has been only slightly defined in terms of traditional medicine. Recent contributions to the armament of chemotherapeutic agents, in alliance with natural products approved as drugs in this 30-year time frame, include paclitaxel (Taxol®), isolated from *Taxus brevifolia*; the alkaloids vincristine and vinblastine from *Catharanthus roseus*; camptothecin and derivatives from *Camptotheca acuminata*; combretastatin from *Combretum caffrum* (Newman et al., 2005; Newman and Gragg, 2012); and curcumin from the rhizome of *Curcuma longa* (Aggarwal and Bharti, 2003) in addition to synthetic derivatives or combinations of agents such as flavopiridol and roscovitine (Newman et al., 2002; Dancey and Sausville, 2003), justifying the importance of the search for cancer therapy. Even if *S. terebinthifolius* has been proposed as a folk remedy in the treatment of inflammation, more studies must be reported. Therefore, we evaluated the *in vitro* antioxidant, antiproliferative and *in vivo* anti-inflammatory activities of methanolic extracts and compounds isolated from *S. terebinthifolius* (leaves). As a complement, a computational study for predicting the ADME properties of compounds was performed by determining the lipophilicity, topological polar surface area (TPSA), absorption (% ABS) and simple molecular descriptors using Lipinski's rule.

Materials and methods

Plant material

The leaves of *Schinus terebinthifolius* Raddi, Anacardiaceae, were collected at the Medicinal Plants Garden of Federal University of Grande Dourados (22°11′43.7″S, 54°56′08.5″W and 430 m) in November 2014. A voucher specimen was deposited in the Herbarium of the UFGD under the number DDMS 4600 and was identified by Dr. Maria do Carmo Vieira. Authorization for accessing and studying samples from the Brazilian genetic heritage site was obtained from the Brazilian government through Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq authorization no. 010220/2015-1 – CNPq/CGEN/MMA).

Extraction, fractionation and identification procedures

Leaves (860 g) were dried and extracted by maceration with methanol, filtered, concentrated under reduced pressure and lyophilized to yield the methanolic extract (MEST) (42.7 g). The MEST (30 g) was partitioned with hexane, chloroform and ethyl acetate. The chloroform fraction (1.8 g) was submitted to column chromatography (CC) silica gel, yielding sitosterol-3-*O*-β-glucopyranoside (64 mg). Fractionation of part of the ethyl acetate fraction (6.2 g) by CC in silica gel was performed using a mixture of hexane/EtOAc and EtOAc/MeOH, in increasing polarity, to afford compounds **1** (25.4 mg), **2** (19.4 mg) and **3** (12.2 mg). The hydromethanolic fraction (13 g) was purified by successive CC on Sephadex LH-20 using H_2O, H_2O/MeOH 7:3–3:7, and MeOH as eluents, yielding compounds **4** (22.8 mg), **5** (17.8 mg) and **6** (26 mg). The ^{1}H and ^{13}CNMR spectra were collected using a Varian Mercury Plus BB spectrometer operating at 300 MHz and 75.5 MHz using CD_3OD as the solvent and tetramethylsilane (TMS) as the internal standard.

HPLC analysis

The LC data with MEST include caffeic acid (Rt = 14.78 min), *p*-coumaric acid (Rt = 25.82 min), luteolin (Rt = 62.08 min), quercetin (Rt = 64.62 min) and apigenin data (Rt = 66.79 min). These data were described in a previous report by our research group (Fedel-Miyasato et al., 2014a).

In vivo *anti-inflammatory activity*

Animals

Male *Swiss* mice (25–35 g) were used for *in vivo* anti-inflammatory evaluation and were provided by the Universidade Federal da Grande Dourados. The mice were kept under a 12 h light-dark cycle with controlled humidity (60–80%) and temperature (22 ± 1 °C). Two hours before the experiments, the animals were placed in the laboratory and were used only once for experiments (n = 5/group). All experimental procedures were performed in accordance with the U.S. National Institute of Health and were approved by the ethics committee for research on laboratory animals of the UFGD (Nbr. 005/2010).

Carrageenan-induced paw oedema

Five groups of Swiss mice were orally treated with MEST (100 and 300 mg/kg) and **2** (100 and 300 mg/kg) as well as a vehicle. Two groups were treated intraplantarly with **2** (10 and 100 mg/kg). One group of mice was treated subcutaneously with an anti-inflammatory positive control drug dexamethasone (1 mg/kg). After 1 h, the animals received an intraplantar injection (50 μl) of a solution of carrageenan (300 μg/paw, diluted in sterile 0.9% saline) into the right hind paw. The contralateral paw received only saline and was used as a control.

The oedema was the difference in thickness of both paws using a digital micrometre (DIGIMESS 110-284) at several time points (0.5, 1, 2, and 4 h) after carrageenan injection. The results were expressed in μm (Kassuya et al., 2009).

Myeloperoxidase activity

Myeloperoxidase (MPO) activity was measured in the paw after 6 h to evaluate indirect neutrophil migration to this tissue (De Young et al., 1989). The paw tissue was homogenized in 5% (w/v) 80 mM phosphate buffer at pH 5.4 containing 0.5% of hexadecyltrimethylammonium bromide. The homogenate was centrifuged at 3200 × *g* and 4 °C for 20 min. Thirty microliters of each supernatant was mixed with 100 μl of 80 mM phosphate buffer, 85 μl of 0.22 M phosphate buffer and 15 μl of 0.017% H_2O_2 in a 96-well plate. The reaction was initiated with 20 μl of 3,3,3-tetramethylbenzidine (dissolved in N,N-dimethylformamide). The plate was maintained at 37 °C for 3 min, after which the reaction was interrupted by adding 30 μl of 1.46 M sodium acetate (pH 3.0). The enzymatic activity was determined by measuring the optical density at 630 nm and was expressed as mOD/mg of protein.

In vitro *antiproliferative activity*

MEST and other compounds were assessed in the following ten human tumour cell lines from various tissues, kindly provided by the National Cancer Institute (Frederick, MA, USA): U251 (glioma, CNS), MCF-7 (breast), NCI-ADR/RES (ovarian expressing the multiple drug resistance phenotype), 786-0 (renal), NCI-H460 (lung, non-small cells), PC-3 (prostate), OVCAR-3 (ovarian), HT-29 (colon),

K-562 (leukaemia) and HaCaT (human keratinocytes, immortalized non-tumoural cell). The test results were measured using the colorimetric sulphorhodamine B method, according to the NCI standard protocol, and doxorubicin (0.025–25 μg/ml) was used as positive control (Monks et al., 1991). Assays were performed in a 96-well plate using four concentrations produced by a 10-fold dilution (0.25–250 μg/ml). The activity was deduced from the concentration response, and GI_{50} parameters (growth inhibitory activity) were calculated.

In vitro *antioxidant activity*

DPPH radical scavenging assay

Sample stock solutions of MEST (1.0 mg/ml) and compounds **1–6** (0.1 mg/ml) were diluted to final concentrations of 300, 200, 125, 50, 25, 10 and 5 μg/ml in methanol. Samples were added to 3 ml of methanolic DPPH (2, 2-diphenyl-1-picrylhydrazyl) (0.1 mM) and were prepared daily, shaken, and left at room temperature in the dark for 30 min. Absorbance was measured at 517 nm against a blank containing all reagents except the test samples (Brand-Williams et al., 1995). Assays were carried out in triplicate. The percentage of inhibition of DPPH (*I*%) was calculated using the following equation: $I\% = (A_{blank} - A_{sample}/A_{blank}) \times 100$; the IC_{50} concentration, indicating 50% inhibition of DPPH, was plotted in a graph of *I*% *versus* sample concentration.

β-Carotene/linoleic acid assay

A β-carotene-chloroform solution (1 ml) was mixed with 20 mg linoleic acid and 0.2 g Tween 40®, with subsequent evaporation of the chloroform. Distilled water (50 ml) was slowly added with vigorous agitation to form an emulsion. Emulsion aliquots (5 ml) were transferred with 0.2 ml of extracts (1 mg/ml) and isolated compounds (0.1 mg/ml) at different concentrations (10–200 μg/ml). Control samples contained all reagents except the test samples (Jayaprakasha et al., 2001). An emulsion was added to each tube, and the absorbance was read at 470 nm for zero time. Tubes were placed in a water bath at 50 °C, and oxidation was monitored by absorbance at 15-min intervals until the colour of β-carotene in the control sample disappeared (105 min). The analyses were performed in triplicate. Antioxidant activity (*AA*) was calculated as the % inhibition relative to the control: $\%AA = [1 - (A_{sampleT0} - A_{sampleT105})/(A_{controlT0} - A_{controlT105})] \times 100$.

ABTS radical scavenging assay

MEST and compound stock solutions (1 mg/ml) were diluted to final concentrations of 250–5 μg/ml. Briefly, 7 mM of 2, 2′-azino-bis-(3-ethylbenzothiazoline)-6-sulphonic acid (ABTS) and 140 mM potassium persulphate were mixed and kept in the dark for 16 h at ambient temperature. Thereafter, 3 ml of $ABTS^{\bullet+}$ solution was added to 30 μl samples with varying concentrations. After 5 min, the absorbance was measured at 734 nm using a spectrophotometer (Djeridane et al., 2006). The $ABTS^{\bullet+}$ scavenging activity was calculated using the following equation: ABTS radical scavenging activity (%) = $(A_{blank} - A_{sample}/A_{blank}) \times 100$.

In silico *study and Lipinski's rule of five*

An *in silico* computational study of the isolated compounds (**1–6**) was performed by the determination of Lipinski's parameters, topological polar surface area (TPSA) and percentage of absorption (% ABS) (Lipinski et al., 1997). Calculations were performed using the "Molinspiration online property calculation toolkit" (http://www.molinspiration.com) (Ertl, 2014). The percentage of absorption was estimated using the following equation: %ABS = 109 − [0.345 × TPSA] (Lipinski et al., 1997).

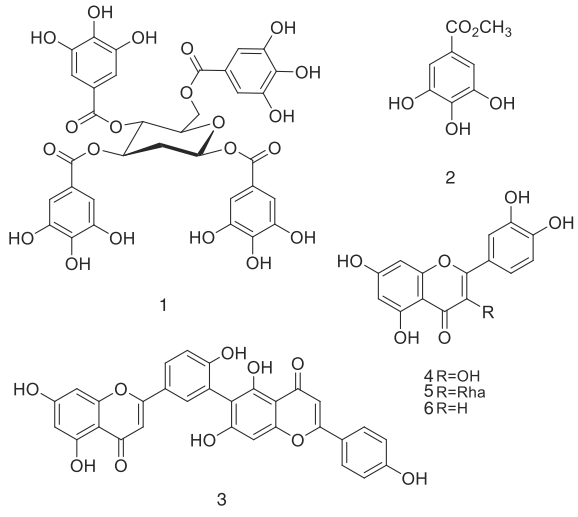
Lipinski's "rule of five" (Zhao et al., 2006) was used to evaluate the drug-like character of those compounds. Generally, orally bioavailable drugs follow these rules: they have fewer than five hydrogen bond donors, no more than ten hydrogen bond acceptors, a molecular weight below 500 Da, and an octanol–water partition coefficient log P of no more than 5.

Statistical analysis

All data are presented as the mean ± S.E.M. The difference between groups was evaluated by analyses of variance (one-way ANOVA) followed by the Tukey or Student–Newman–Keuls test. The number of animals per group is indicated in the legends. Statistical differences were considered significant at $p < 0.05$.

Results and discussion

In the present study, we characterized seven previously known compounds, including one steroid, sitosterol-3-*O*-β-glucopyranoside; two gallic acid derivatives, 1,2,3,4,6-penta-*O*-galloyl-β-glucopyranoside (**1**) and methyl gallate (**2**); and the four following flavonoids: robustaflavone (**3**), quercetin (**4**), quercetrin (**5**) and luteolin (**6**) from *S. terebinthifolius*. The structures of compounds were elucidated using 1D and 2D NMR spectral data and a comparison of ^{1}H and ^{13}C NMR reported data (Agrawal, 1989; Carvalher-Machado et al., 2008; Ceruks et al., 2007). The quercetin derivatives of the sugar unit and hydrogen, as well as the position of their linkage to the aglycone, were determined using a combination of 2D NMR experiments. To the best of our knowledge, this study is the third phytochemistry study of *S. terebinthifolius* leaves and the first report of luteolin from methanolic extract, conducted during the previous HPLC data study.



The antioxidant activity was initially evaluated for *S. terebinthifolius* MEST. The results showed that the sample presented potent antioxidant activity when tested against DPPH (IC_{50} 12.32 ± 1.50 μg/ml), β-carotene/linoleic acid (*AA* = 70.44 ± 0.88%) and ABTS (*AA* = 86.94 ± 2.04%) (Table 1). Fractionation of these extracts by solvent partition and purification by a chromatographic column provided six phenolic compounds. The results showed that phenolics (**2**), hydrolysed tannins (**1**) and flavonoids (**4** and **6**) were active in all assays, comparable to the natural antioxidant ascorbic acid (Table 1). Methyl gallate (**2**) and quercetin (**4**) are also considered natural antioxidants. Anacardiaceae species showed antioxidant potential, including *Anacardium occidentale* (Ajileye et al., 2015), *Lannea alata* (Okoth et al., 2013), *Sclerocarya birrea*

Table 1
Antioxidant assays of MEST and phenolic compounds (2–7) from *Schinus terebinthifolius*.

Sample	DPPH IC_{50}[a] (95% confidence limit)	β-Carotene/linoleic acid *AA* (%)	ABTS *AA* (%)
MEST	12.32 ± 1.50c (11.01–14.86)	70.44 ± 0.88b	86.94 ± 2.04ab
1	3.03 ± 0.89a (2.54–3.10)	87.57 ± 2.28a	90.41 ± 1.84a
2	5.54 ± 1.55b (5.30–5.78)	86.26 ± 2.84a	77.65 ± 1.06c
3	19.44 ± 1.89d (18.64–20.11)	68.92 ± 0.50b	65.72 ± 0.83d
4	4.03 ± 1.57ab (3.88–4.12)	84.40 ± 2.13a	83.07± 3.34b
5	20.43 ± 0.54d (18.10–21.66)	62.18 ± 0.64c	63.21 ± 1.55d
6	4.57 ± 2.43ab (3.35–5.08)	86.36 ± 2.18a	85.32 ± 1.32b
Ascorbic acid	3.99 ± 0.36ab (14.3–20.1)	88.37 ± 1.91a	87.32 ± 1.33ab

Values are expressed as the mean ± SD ($n = 3$); n.d. = not determined; [a]IC_{50} = concentration resulting in 50% inhibition of DPPH, derived from the graph of *I%* (inhibition percentage) *versus* concentration in μg/ml (MEST and ascorbic acid) and μM (2–7 compounds). *%AA* = antioxidant activity, evaluated using the β-carotene/linoleic acid and ABTS methods. Different superscript letters indicate statistically significant differences in each line ($p < 0.05$) using the Tukey test.

and *Harpephyllum caffrum* (Moyo et al., 2010). These compounds exhibited a similar scavenging activity as our extract, and this activity was attributed to various compounds found in these species, including phenolic acids, flavonoids and tannin compounds in their crude extracts.

Our data showed that the IC_{50} and *AA*% values indicated correlations with the presence of the sugar unit, hydroxyl or hydrogen of the aglycone linked at C-3, which were due to the steric hindrance between the C-3 substituent and the B ring of the flavonol, whose decrease significantly enhanced free radical-scavenging ability, as evidenced by compound **5**. The catechol, α, β-unsaturated carbonyl moiety and β-hydroxyketone groups conferred higher stability to the radical form and participated in electron delocalization (Pietta, 2000). Compound **3**, which belongs to the flavone subclass, did not present catechol groups in the structure, which likely explains the inactivity of the sample.

Lipinski's "rule of five" [molecular weight (MW) ≤ 500 Da, log $P \leq 5$, H-bond donors (HBD) ≤ 5 and H-bond acceptors (HBA) ≤ 10], topological polar surface area (TPSA) and per cent absorption (%ABS) were calculated and presented in Table 2. Molecules violating more than one of Lipinski's parameters may have problems with bioavailability and a high probability of failure to display drug-like character. From the data obtained, **2** and **4** were found to obey Lipinski's rule, showing good permeability in the cellular plasmatic membrane (78.79% and 50.33%, respectively). The computational TPSA for **3** (86.98 $Å^2$) showed good intestinal absorption, whereas **1**, **3** and **6** violated three parameters.

Free radical species are also responsible for activating several pro-inflammatory transcription factors involved in the promotion of inflammatory diseases. The anti-inflammatory activity (MEST and **2**) was evaluated using carrageenan-induced paw oedema and assessment of myeloperoxidase (MPO). As shown in the literature results, carrageenan injection-induced paw oedema was observed at all time points in the present work (Fig. 1A). MEST treatments inhibited oedema formation with 33 ± 9 (100 mg/kg)

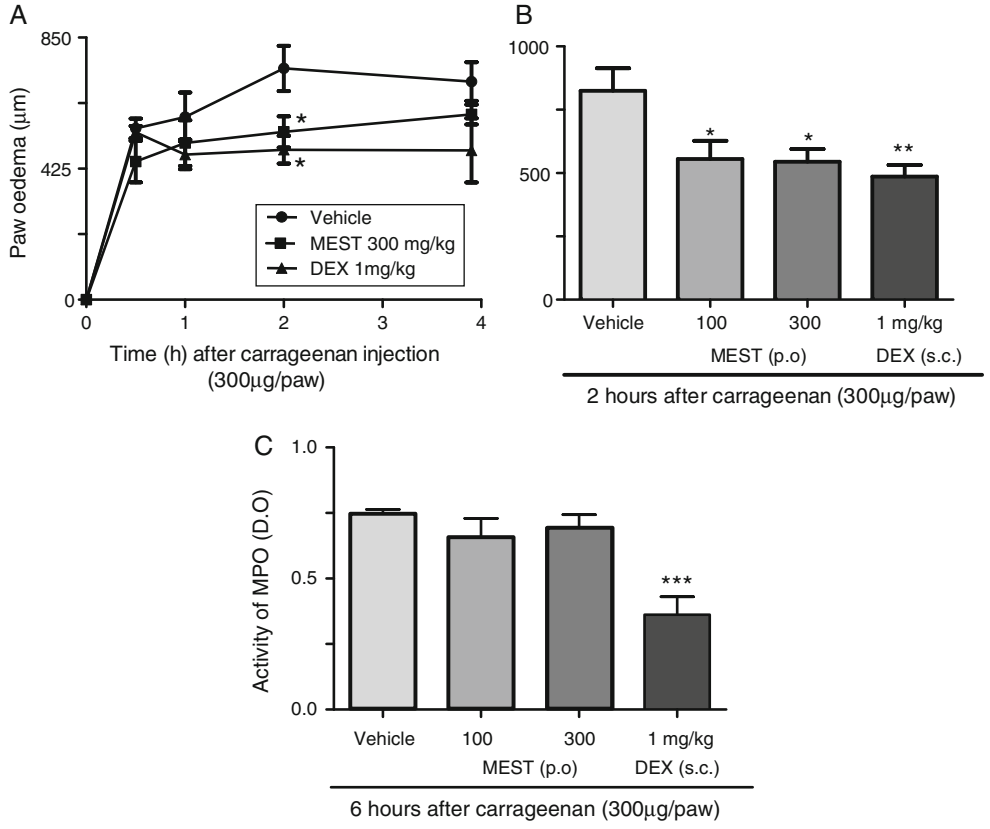


Fig. 1. Anti-inflammatory effect of MEST administration on carrageenan-induced paw oedema in mice. Animals received MEST (100 or 300 mg/kg, *p.o.*), dexamethasone (DEX, 1 mg/kg, *s.c.*) or a vehicle control. After 1 h, an intraplantar injection of carrageenan (300 μg/paw) was performed. In (A), the time course of inhibition, induced by 300 mg/kg MEST and DEX, is presented. In (B), the bars indicate the effect of various MEST and DEX doses in paw oedema (μm) 2 h after carrageenan injection. In (C), MEST did not alter the increase in myeloperoxidase (MPO) activity induced by local carrageenan injections. The bars express the mean ± SEM of five animals. Comparisons were made between vehicles and treated groups. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, one-way ANOVA followed by the Student–Newman–Keuls method.

Table 2
Lipinski's parameters, topological polar surface area (TPSA) and percentage of absorption (% ABS) of the compounds (2–7).

Compound	% ABS	Lipinski's parameters					
		TPSA[a] (Å^2)	*n*HBA[a] (*n*ON)	*n*HBD[a] (*n*OHNH)	log *P*[a]	MW[a]	*n* violations[a]
1	−37.26	423.95	25	14	3.65	938.70	3
2	78.99	86.98	5	3	0.84	184.14	0
3	46.28	181.79	10	6	5.16	538.46	3
4	63.68	131.35	7	5	1.68	302.23	0
5	50.33	170.04	10	6	1.13	432.38	1
6	18.12	263.42	16	10	−1.063	610.52	3

[a] www.molinspiration.com; %ABS = 109 − 0.345 × TPSA; number of hydrogen bond acceptors (NO) = *n*HBA ≤ 10; number of hydrogen bond donors (OHNH) = *n*HBD ≤ 5; MW ≤ 500; octanol–water partition coefficient = log *P* < 5.

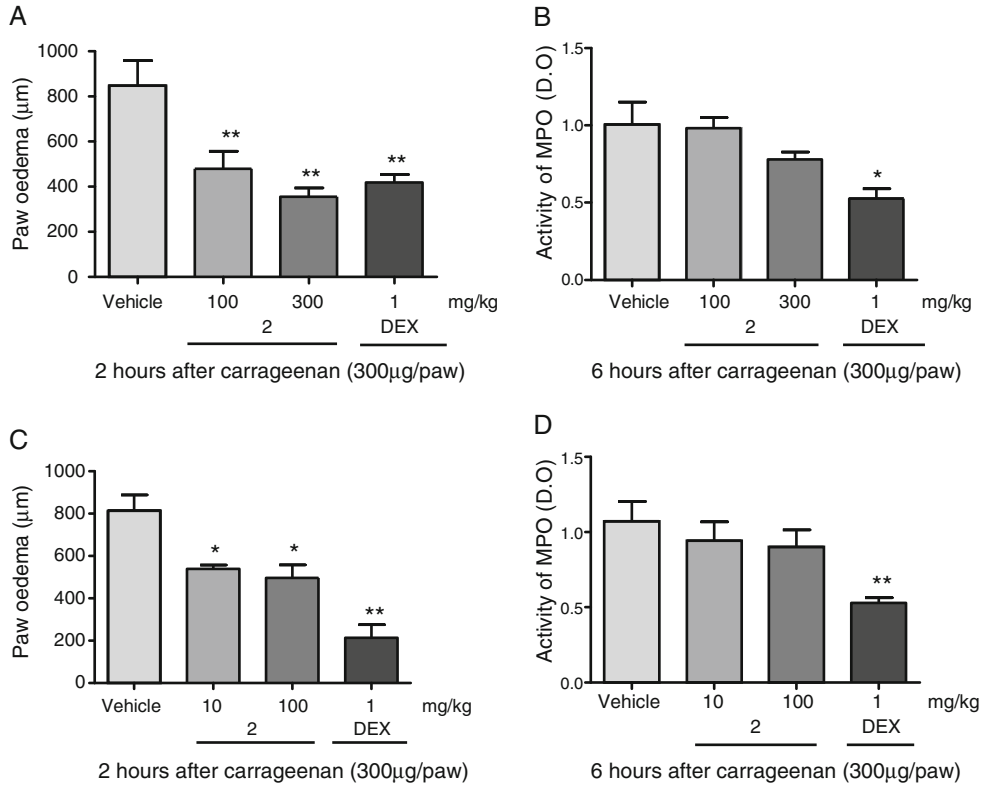


Fig. 2. Anti-inflammatory effect of methyl gallate (**2**) on carrageenan-induced paw oedema in mice. Animals received **2** orally (100 and 300 mg/kg, *p.o.*), **2** intraplantarly (10 and 100 mg/kg, *p.o.*), dexamethasone (DEX, 1 mg/kg, *s.c.*) or a vehicle control. After 1 h, the mice received an intraplantar injection of carrageenan (300 µg/paw). In (A), the bars indicate the effects of various doses of **2** and DEX in paw oedema (µm) 2 h after carrageenan injection. In (B), **2** did not inhibit a carrageenan-induced increase in myeloperoxidase (MPO) activity. In (C), bars demonstrate the effects of various doses of intraplantar injections of **2** (10 and 100 µg/paw) and DEX on paw oedema (mm) 2 h after carrageenan injection. In (D), **2** did not inhibit carrageenan-induced increase of MPO activity. The bars express the mean ± SEM of five animals. Comparisons were made between the vehicle and the treated groups. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, one-way ANOVA followed by the Student–Newman–Keuls method.

and 34 ± 6% (300 mg/kg) reductions (Fig. 1A and B). Moreover, our results indicated that 300 mg/kg of the MEST displayed significant inhibition 2 h after administration, as indicated by the time course analysis (Fig. 1A). In addition, 41 ± 5% inhibition was observed in the dexamethasone-treated group 2 h after carrageenan injection (Fig. 1A). Oral MEST treatment (100 and 300 mg/kg) did not alter the increase in MPO activity induced by carrageenan. The positive control (dexamethasone) induced inhibitory activity in the MPO analysis when compared with the control group (Fig. 1C). Scientific works with *S. terebinthifolius* showed that extracts from leaves exhibited topical (doses of 0.1, 0.3 and 1 mg/ear) (Fedel-Miyasato et al., 2014a) and systemic anti-inflammatory properties in another inflammatory model including croton oil-induced ear oedema, arthritis and air pouch models in mice (Fedel-Miyasato et al., 2014a; Rosas et al., 2015). Previous studies using wound-healing models showed that extracts from the leaves of *S. terebinthifolius* were effective for wound healing (Nunes et al., 2006; Coutinho et al., 2006; Martorelli et al., 2011). In rats, Fedel-Miyasato et al. (2014a) showed that MEST (80 mg/ml) topical application significantly decreased the diameter of the wound. In the present work, the extract was tested systemically (oral route) in a different model of inflammation and compound **3** was assayed *via* both an oral route and local injection (intraplantar injection).

Compound **2** also exhibited anti-edematogenic activity, inhibiting approximately 43 ± 9% (100 mg/kg) and 58 ± 5% (300 mg/kg) (Fig. 2A). Dexamethasone inhibited oedema formation (51 ± 4%) 2 h after inflammatory stimulus (Fig. 2A). Animals treated with intraplantar compound **2** injections displayed 34 ± 2% (10 µg/paw) and 39 ± 8% (100 µg/paw) inhibition (Fig. 2C). Oral (100 and 300 mg/kg) or intraplantar (10 and 100 µg/paw) administration of compound **2** did not alter MPO activity compared to the control group (Fig. 2B and D). The anti-inflammatory effects of MEST were associated with increased levels of methyl gallate (**2**), which is also found in other Aceraceae family plants including *Acer rubrum* L, *A. saccharinum*

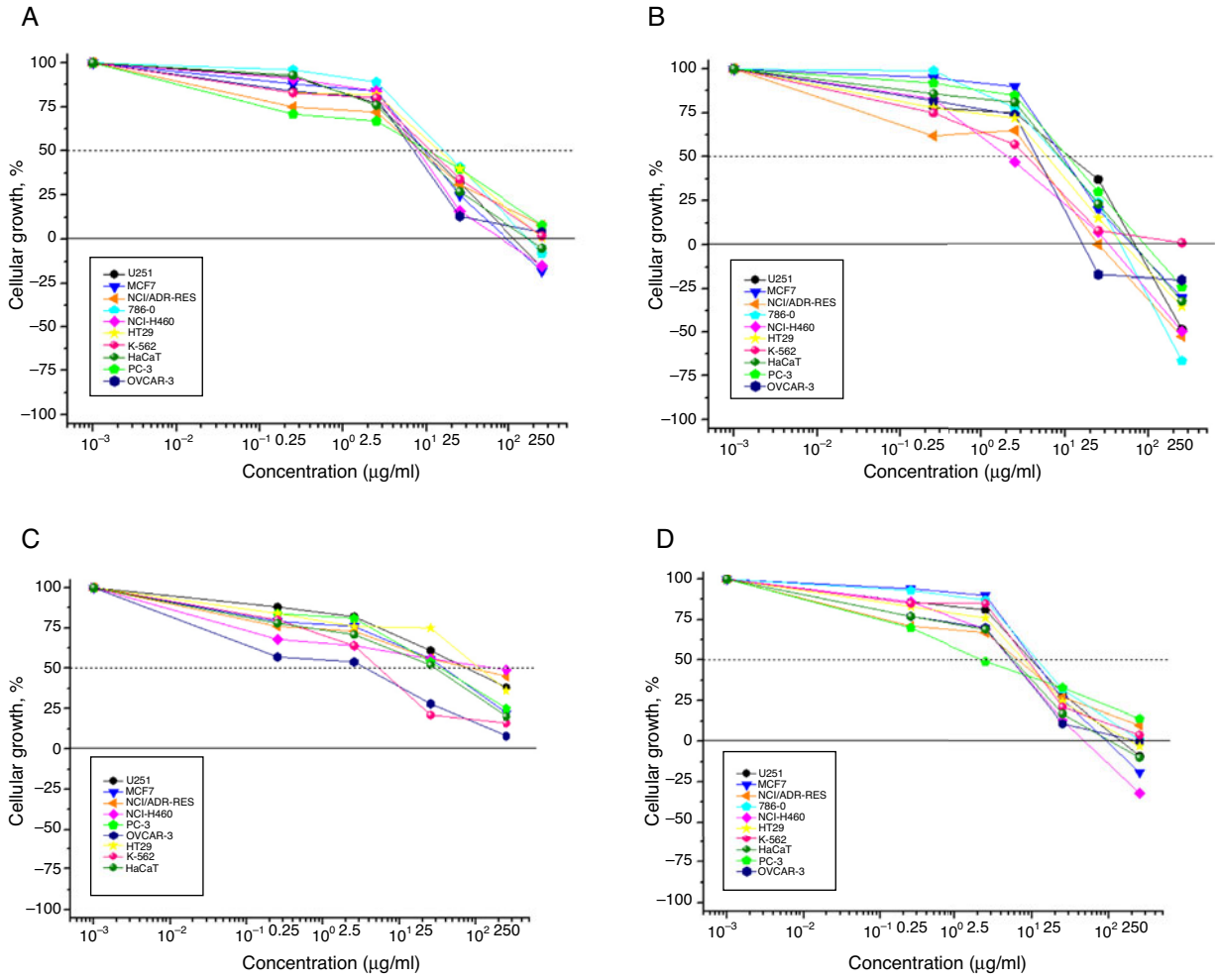


Fig. 3. Antiproliferative activity. In *A*: MEST; *B*: compound **1**; *C*: compound **5**; *D*: compound **6**. U251 (glioma, CNS), MCF-7 (breast), NCI-ADR/RES (ovarian expressing the multiple drug resistance phenotype), 786-0 (renal), NCI-H460 (lung, non-small cells), PC-3 (prostate), OVCAR-3 (ovarian), HT-29 (colon), K-562 (leukaemia) and HaCaT (human keratinocytes, immortalized non-tumoural cells). GI_{50}: concentrations that elicit 50% inhibition of cell growth (in μg/ml).

L, and *A. saccharum* Marsh (Whang et al., 2005; Kim et al., 2006; Abou-Zaid et al., 2009).

The results demonstrated that sample MEST possesses *in vitro* anticancer activity, with GI_{50} values ranging from 6.3 to 9.4 μg/ml with selectivity for prostate (PC-3) (GI_{50} = 6.3 μg/ml), ovarian (OVCAR-3) and resistant ovarian- (NCI-ADR/RES) (GI_{50} = 6.5 μg/ml), breast (NCI/H460) (GI_{50} = 7.6 μg/ml), glioma (U251) (GI_{50} = 9.1 μg/ml) and breast (MCF-7) (GI_{50} = 9.4 μg/ml) cancer and for HaCat (GI_{50} = 8.1 μg/ml) non-tumoural cells (Fig. 3A). Furthermore, 1,2,3,4,6-penta-*O*-galloyl-O-β-glucopyranoside (**1**) demonstrated high activity with GI_{50} < 5.00 μg/ml against resistant ovarian (NCI-ADR/RES), breast (NCI/H460) (GI_{50} = 1.9 μg/ml), leukaemia (K-562) (GI_{50} = 2.2 μg/ml), ovarian (OVCAR-3) (GI_{50} = 2.5 μg/ml) and colon (HT-29) (GI_{50} = 4.9 μg/ml) cancer cells (Fig. 3B). Quercetrin (**5**) showed inhibitory activity against prostate (PC-3) (GI50 = 2.5 μg/ml), ovarian (OVCAR-3) (GI50 = 4.1 μg/ml), (HaCaT) (GI50 = 4.3 μg/ml), ovarian (NCI-ADR/RES) and breast (NCI/H460) (GI50 = 4.4 μg/ml) cancer cell lines (Fig. 3CD). Luteolin (**6**) showed activity against OVCAR-3 (GI_{50} = 1.3 μg/ml), K562 (GI_{50} = 4.5 μg/ml) and HaCaT (GI_{50} = 17.4 μg/ml) cell lines (Fig. 3D). Compound **3**, robustaflavone, did not show inhibitory activity against any cell lines (GI_{50} > 100 μg/ml) (data not shown). In general, *S. terebinthifolius* and its compounds showed the highest growth inhibitory activities towards ovarian cancer cells (NCI-ADR/RES; OVCAR-3) which showed GI_{50} values in the 1.3–6.5 μg/ml range. The Anacardiaceae plant species has already been recorded in the literature, with varied growth inhibition potential for different cancer cell lines (Kim et al., 2013). Studies report that α-pinene isolated from *S. terebinthifolius* leaves induces apoptosis and confers antimetastatic protection in a melanoma model (Matsuo et al., 2011).

In another study by our group, the *in vitro* antiproliferative activity against ten human cancer cell lines of a series of galloyl derivatives bearing substituted-1,3,4-oxadiazole and carbohydrazide moieties was evaluated. The results demonstrated that methyl gallate and intermediary galloyl hydrazide showed great antiproliferative activity, with GI_{50} values < 5.54 μM, against all human tumour cell lines tested (Da Silva et al., 2015). Therefore, since **2** exhibited several activities, this antioxidant agent is a good natural product lead for structural modification studies yielding new agents (Asnaashari et al., 2014). This result is in accordance with Lipinski's Rule of Five, TPSA and % ABS, which are important for further development of drugs based upon these moieties.

Conclusions

This study demonstrated that MEST, obtained from leaves collected in Dourados-MS, has potential anti-inflammatory activity, which supports previous claims regarding the traditional use of *S. terebinthifolius*. Six phenolic constituents were isolated. To the best of our knowledge, this study is the first report of luteolin from

S. terebinthifolius leaves. The methanolic extract and compounds showed the highest growth inhibitory activity, with particular effectiveness against ovarian cancer (NCI-ADR/RES; OVCAR-3) cell lines.

Ethical disclosures

Protection of human and animal subjects. The authors declare that the procedures followed were in accordance with the regulations of the relevant clinical research ethics committee and with those of the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Confidentiality of data. The authors declare that they have followed the protocols of their work center on the publication of patient data.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Authors' contributions

MCV identified and collected plant material. ASNF, MMS and KPS prepared the methanolic extract and phytochemistry study, assessed antioxidant activity and helped to write and edit the manuscript. CALK and EKKI designed the anti-inflammatory assays. MAF, JEC and ALTGR contributed to the antiproliferative assay. All authors have read and approved the final manuscript for submission.

Conflicts of interest

The authors declare no conflicts of interest.

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