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Topical anti-inflammatory activity of *Eugenia brasiliensis* Lam. (Myrtaceae) leaves

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Abstract

Eugenia brasiliensis Lam., a plant from the south of Brazil, is used in the popular medicine for rheumatism treatment. This study reports that topical application of hydroalcoholic extract, fractions and isolated compounds from *E. brasiliensis* caused an inhibition of ear oedema in response to topical application of croton oil on the mouse ear. For oedema inhibition, the estimated ID50 values (dose reducing the inflammatory response by 50% relative to the control value) for hydroalcoholic extract and fractions (hexane, ethyl acetate and dichloromethane) were 0.17, 0.29, 0.13 and 0.14 mg/ear, respectively, with inhibition of 79 ± 7%, 87 ± 6%, 88 ± 5% and 96 ± 2%, respectively. Isolated phenolic compounds (quercetin, catechin and gallocatechin) were also effective in inhibiting the oedema (inhibition of 61 ± 5%, 66 ± 2% and 37 ± 9%, respectively). Moreover, both extract and isolated compounds caused inhibition of polymorphonuclear cells influx (inhibition of 85 ± 6%, 81 ± 5%, 73 ± 6% and 76 ± 6%, respectively). The histological analysis of the ear tissue clearly confirmed that the extract and compounds of *E. brasiliensis* inhibited the influx of polymorphonuclear cells to mouse ear skin after application of croton oil. Furthermore, hydroalcoholic extract was also effective in inhibiting the arachidonic acid-mediated mouse ear oedema (ID50 value was 1.94 mg/ear and inhibition of 60 ± 7%). Therefore, these results consistently support the notion that *E. brasiliensis* possesses topical anti-inflammatory activity.

Introduction

The skin represents the largest organ in the body, providing the principal physical barrier to the environment. Therefore, the skin is the main target tissue for exogenous noxes, protecting us from harmful environmental hazards, UV-irradiation and endogenous water loss (Welss et al 2004).

Skin injuries are responsible for initiation of the inflammatory response, which is mediated by an early infiltration of neutrophils and subsequent recruitment of macrophages to the wound site. These cells release pro-inflammatory cytokines into the wound surroundings, acting as a start for downstream repair processes (Grose & Werner 2002). These signals cause inflammatory skin diseases such as atopic dermatitis, contact dermatitis and psoriasis. Inflammatory skin illness has a large incidence in the population. Some of these pathologies such as psoriasis affect ~1–3% of the world-wide population (Gottlieb 2005).

Medicines currently available to the practising doctor, such as non-steroidal anti-inflammatory drugs, antihistamines, glucocorticoids and immunosuppressors, are far from optimal to effectively deal with chronic inflammatory processes of the skin, like psoriasis and atopic dermatitis (Disepio et al 1999; Cauwenbergh 2002; Mendonça & Burden 2003). Therefore, natural products sources, including composites derived from plants, have been used as a strategy to discover new biologically active composites. The Myrtaceae family, which possesses about 3000 species and 80 genuses, stands out as one of the most important families due its wide distribution. In this family is included the *Eugenia* genus, one of the largest genuses with more than 500 species, of which about 400 species are located in Brazil. These species have their special relevance by virtue of utilization as medicinal plants

(Oliveira et al 2005). *Eugenia brasiliensis* Lam. is a plant from the south of Brazil, known by the popular names of grumixama, grumixaba, grumichameira and ibaporoiti. In popular medicine, *E. brasiliensis* leaves, fruits and bark are used in preparations for the treatment of rheumatism, an infusion of its leaves is applied as a diuretic, and their mature fruits are used as nutriment (Revilla 2002).

The biological activity of *E. brasiliensis*, in popular use for inflammatory processes like arthritis, has been poorly studied. *E. jambolona*, another species of the genus, has been described as anti-inflammatory (De Lima et al 1998; Ravi et al 2005). In this study, therefore, the potential anti-inflammatory activity of *E. brasiliensis* leaves was evaluated. For that, topical anti-inflammatory properties of the hydroalcoholic extract from the *E. brasiliensis* leaves were examined by classic models of skin inflammation–arachidonic acid- and croton oil-induced mouse ear oedema.

Materials and Methods

Drugs

The following substances were used: croton oil, arachidonic acid(AA), indometacin, dexamethasone, hexadecyltrimethylammonium bromide, tetramethylbenzidine hydrogen peroxide, formaldehyde, phosphate-buffered saline (PBS), eosin, haematoxylin (all from Sigma, St Louis, MO), sodium acetate, dimethylformamide, acetone, formaldehyde, absolute ethanol, sodium tetraborate and methanol (all from Merck, Darmstadt, Germany).

Plant material

Leaves of *Eugenia brasiliensis* Lam. (Myrtaceae) were collected in Santo Amaro da Imperatriz, Santa Catarina, and identified by Dr Daniel de Barcellos Falkenberg, Department of Botany, Universidade Federal de Santa Catarina. A voucher specimen (FLOR 34,675) was deposited in the Herbarium of the Department of Botany, Universidade Federal de Santa Catarina, Santa Catarina, Brazil.

Preparation of extracts

Botanical material was dried under air circulation and minced. The air-dried leaves of *E. brasiliensis* (1.570 g) were extracted with ethanol 70% at room temperature for 15 days. The solvent was removed by rotary evaporation (< 55°C) under reduced pressure, to yield the crude hydroalcoholic extract.

Preparation of fractions

The hydroalcoholic crude extract (170 g) was suspended in ethanol–H_2O (20:80 v/v) and successively partitioned with hexane, dichloromethane and ethyl acetate (EtOAc). The solvents were evaporated to dryness by rotary evaporation (< 55°C) under reduced pressure, to yield hexane (30.4 g), dichloromethane (15.5 g) and EtOAc (33.4 g) fractions.

Isolation of compounds

EtOAc fraction (17.0 g) was chromatographed on a silica gel column eluting with hexane–EtOAc–ethanol mixtures with increasing gradient of polarity, to afford seven fractions (A–G). Fraction C was re-chromatographed on a silica gel column using a stepwise gradient solvent system of hexane–EtOAc (from 8:2 to EtOAc 100%) to afford quercetin (24 mg), catechin (150 mg) and gallocatechin (142 mg). The isolated compounds were identified by spectroscopic analysis (IR, ^{1}H NMR, ^{13}C NMR and DEPT), in comparison with those reported in the literature (Agrawal 1989; Foo et al 2000). The isolated compounds were co-eluted by TLC in comparison with standard compounds, using as eluent $CHCl_3$–methanol–acetic acid (8:2:0.5).

Analysis of EtOAc fraction by capillary eletrophoresis (CE)

Instrumentation

The CE experiments were carried out on an Agilent capillary electrophoresis system (Agilent CE^{3D}), equipped with on-column diode-array detection (DAD) and a temperature control device set at 25°C. Samples were introduced onto the capillary via hydrodynamic injection by applying 50 mbar/4 s.

Fused-silica capillaries (Polymicro Technologies, Phoenix, AZ) with dimensions 45.0 cm total length, 36.5 cm effective length, 50 μm i.d. and 375 μm o.d. were used. The electrophoresis system was operated under normal polarity and constant voltage conditions of 30 kV. At the beginning of the day, the capillary was conditioned by flushes of 1 M NaOH solution (5 min), de-ionized water (5 min) and electrolyte solution (10 min). In between runs, the capillary was reconditioned by a pressure flush with the electrolyte (2 min). Electrolytes, composed of 40 mM STB, 10% (v/v) methanol (pH 9.3), were prepared daily by direct weighing of reagents.

All reagents for analysis of EtOAc fraction by CE were of analytical grade and water was purified by de-ionization (Milli-Q system; Millipore, Bedford, MA). The EtOAc fraction was prepared in methanol (2.0 g L^{-1}), and the standards of phenolic compounds (rutin, galangin, myricetin, quercetin and gallic acid) were prepared in methanol (20 mg L^{-1}) and injected into the CE equipment.

Hydrolysis conditions

The hydrolysis procedure of the sample was carried out by adding 300 μL of extract 2.0 g L^{-1} (in methanol) in a 2-mL tube, then 60 μL of HCl 6 M were transferred to the tube. Thereafter the tube was closed and heated at 95°C for 45 min. The mixture was dried with a flow of nitrogen gas and dissolved in methanol to the final volume of 300 μL.

Animals

Male Swiss mice, 25–35 g, housed at 22 ± 2°C under a 12-h light–dark cycle and with free access to food and water, were used in these experiments that were performed during the light phase of the cycle. The mice were allowed to adapt to the laboratory for at least 1 h before testing and were used only once. Experiments reported in this study were performed

after approval of the protocol by the institutional ethics committee of our University and were carried out in accordance with current guidelines for the care of laboratory animals and ethical guidelines for the investigation of experimental pain in conscious animals (Zimmermann 1983).

Ear oedema measurement

Oedema was expressed as the increase in ear thickness due to the inflammatory challenge. Ear thickness was measured before and after induction of the inflammatory response by using a micrometer (Great, MT-045B). The micrometer was applied near the tip of the ear just distal to the cartilaginous ridges and the thickness was recorded in μm. To minimize variation due to technique, a single investigator performed the measurements throughout any one experiment. Extracts were applied topically in 20 μL acetone (Otuki et al 2005).

Croton oil-induced dermatitis

Oedema was induced on the right ear by topical application of 0.1 mg/ear of croton oil dissolved in 20 μL of acetone. Hydroalcoholic extract (0.03–1.0 mg/ear), hexane, dichloromethane and ethyl acetate fractions (0.003–0.6 mg/ear), the compounds quercetin, catechin and gallocatechin (0.6 mg/ear) from *E. brasiliensis*, and dexamethasone (0.05 mg/ear, used as a positive control) were applied topically simultaneously with croton oil. The thickness of the ear was measured before and 6 h after induction of inflammation (Swingle et al 1981; Tragni et al 1985).

Arachidonic acid-induced dermatitis

Hydroalcoholic extract of *E. brasiliensis* (0.03–3.0 mg/ear) or indometacin (1 mg/ear, used as a positive control) were applied topically simultaneously with AA (2 mg/ear) to the right ear. The thickness of the ear was measured before and 1 h after induction of inflammation (Young et al 1984; Crummey et al 1987).

Tissue myeloperoxidase activity assay

The activity of tissue myeloperoxidase was assessed 24 h after croton oil application to the mouse ear according to the technique reported by Suzuki et al (1983) and modified by De Young et al (1989). A biopsy (6 mm ear tissue punch) was placed in 0.75 mL of 80 mM PBS pH 5.4, containing 0.5% hexadecyltrimethylammonium bromide, then homogenized (45 s at 0°C) in a motor-driven homogenizer. The homogenate was decanted into a microfuge tube, and the vessel was washed with a second 0.75-mL volume of hexadecyltrimethylammonium bromide in buffer. The wash was added to the tube and the 1.5-mL sample was centrifuged at 12000 *g* at 4°C for 15 min. Triplicate 30-μL samples of the resulting supernatant were added to 96-well microtitre plates. For assay, 200 μL of a mixture containing 100 μL of 80 mM PBS pH 5.4, 85 μL of 0.22 M PBS pH 5.4, and 15 μL of 0.017% hydrogen peroxide were added to the wells. The reaction was started by the addition of 20 μL of 18.4 mM tetramethylbenzidine HCl in dimethylformamide. Plates were incubated at 37°C for 3 min and then placed on ice. The reaction was stopped by the addition of 30 μL of 1.46 M sodium acetate, pH 3.0. Enzyme activity was determined colorimetrically using a plate reader (EL808; BioTech Instruments, INC) set to measure absorbance at 630 nm and expressed as mOD mg/tissue.

Histology

Ear samples were bouin-fixed. Each sample was cut longitudinally into equal halves, one of which was embedded in paraffin wax, sectioned at 5 μm and stained with haematoxylin–eosin. A representative area was selected for qualitative light microscopic analysis of the inflammatory cellular response with 10× and 20× objectives (Recio et al 2000). To minimize a source of bias, the investigator did not know the group that he was analysing.

Statistical analysis

The results are presented as means ± s.e.m., except the ID50 values (i.e. the dose of extract or fractions reducing the inflammatory response by 50% relative to the control value), which are reported as geometric means accompanied by their respective 95% confidence limits. Data were subjected to analysis of variance or *t*-test and complemented by Newman Keul's post-hoc test. $P<0.05$ was considered as indicative of significance. The ID50 values were determined by linear regression from individual experiments using GraphPad Software (California, USA).

Results

Electrophoretic determination of phenolic profile on EtOAc fraction

Figure 1 shows the results of the analysis of the EtOAc fraction by capillary electrophoresis (CE). The electropherograms show the phenolic profile of the EtOAc fraction before (Figure 1A) and after (Figure 1B) acid hydrolysis with HCl, obtained from 2 to 15 min. Analysis of the fraction before hydrolysis shows several peaks between 6 and 8 min, which disappear after hydrolysis. Figure 1C shows the eletropherogram of standard solution, comprised of flavonoids rutin, galangin, myricetin, quercetin and gallic acid, a simple phenolic compound. Figure 2 shows the uv spectrum of the glycosylated flavonoid rutin, with the presense of two maximum wavelengths, in the region of 260 and 380 nm, in comparison with the spectrum of compound *a.

Topical anti-inflammatory activity

The hydroalcoholic extract and its fractions and compounds caused a significant and dose-dependent inhibition of the croton oil-induced skin inflammation (i.e. both oedema and cell migration). The hydroalcoholic extract presented an estimated mean ID50 value of 0.17 (0.12–0.24) for oedema with 79 ± 7% (0.6 mg/ear) and 85 ± 6% (1.0 mg/ear) inhibition values for oedema and myeloperoxidase activity, respectively. In the same way, the used reference drug dexamethasone caused inhibition of oedema and myeloperoxidase activity with

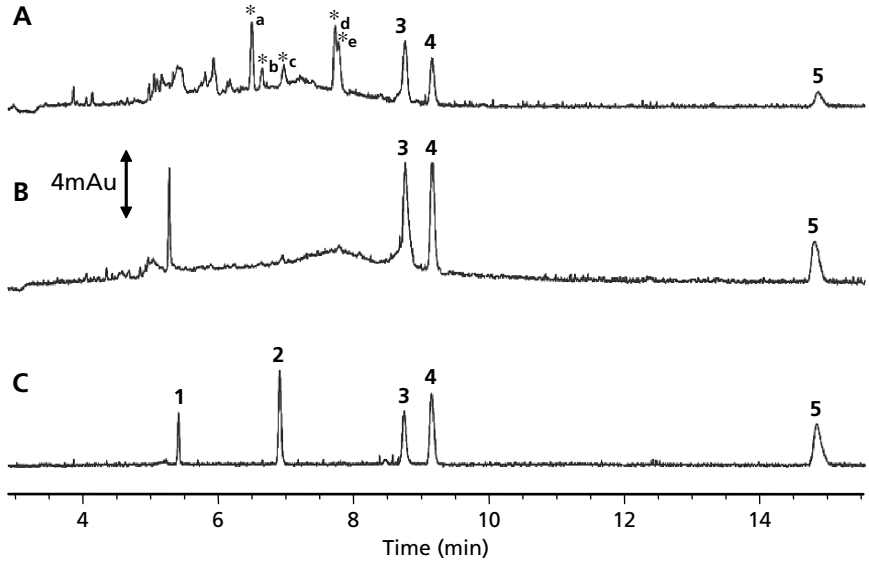

Figure 1 Electropherograms of EtOAc fraction 2000 mg L^{-1} in methanol before (A) and after (B) hydrolysis (*a–e, flavonoid glycosides). C. Standard solution: 1, rutin; 2, galangin; 3, myricetin; 4, quercetin; 5, gallic acid. Electrophoretic conditions: fused-silica capillary, 50 μm i.d., 45 cm total length (36.5 cm to detector); electrolyte composition, sodium tetraborate 40 mM, pH 9.3, 10% methanol (v/v); separation voltage, 30 kV; hydrodynamic injection, 4 s at 50 mbar; temperature, 25°C, detection at 280 nm.

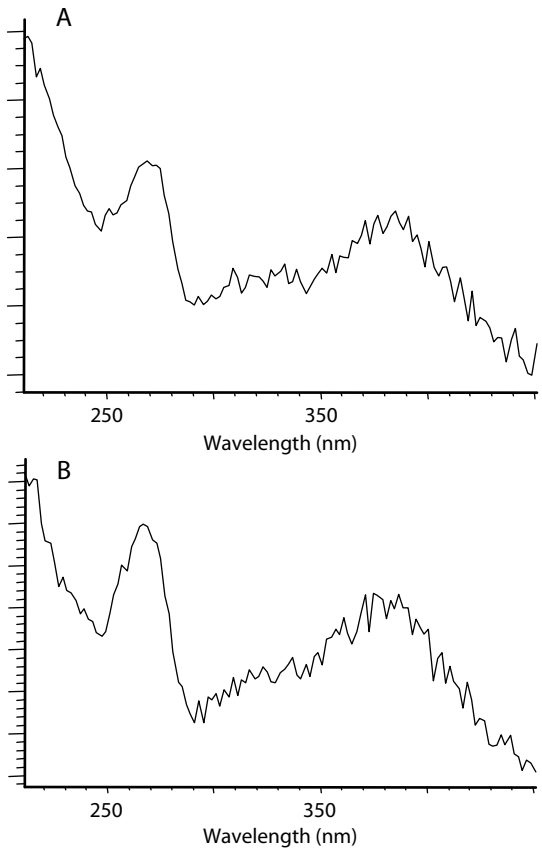

Figure 2 UV spectra of *a (flavonoid glycoside, EtOAc fraction) (A) and rutin (B).

values of 92 ± 4% and 85 ± 1% (0.05 mg/ear), respectively. The hexane, ethyl acetate and dichloromethane, of *Eugenia* caused an inhibition of the croton oil-induced ear oedema with ID50 values of 0.29 (0.20–0.43), 0.13 (0.06–0.26) and 0.14 (0.03–0.63) mg/ear, respectively, and inhibition of 87 ± 6%, 88 ± 5% and 96 ± 2% (0.6 mg/ear), respectively. In this experiment, dexamethasone inhibition value was 96 ± 3% (0.05 mg/ear). The inhibition values for the oedema and myeloperoxidase activity of compounds isolated from *Eugeria,* quercetin, catechin and gallocatechin, were 61 ± 5%, 66 ± 2%, 37 ± 9% and 81 ± 5%, 73 ± 6% and 76 ± 6%, respectively (0.6 mg/ear) (Figures 3, 4 and 5).

Optical microscopic analysis of the mouse ears, 24 h after application of croton oil, revealed epidermal hyperplasia and marked infiltration of inflammatory cells associated with dilated blood vessels. These events were greatly reduced after topical application of hydroalcoholic extract (0.6 mg/ear) as well as by the positive control dexamethasone (0.05 mg/ear; Figure 6).

Thus, we next investigated whether topical application of the hydroalcoholic extract would inhibit AA-induced ear oedema in mice. Topical application of AA provokes a rapid and intense inflammatory response in the ear that was blocked by lipoxygenase inhibitors (Young et al 1984; De Young et al 1989). Similarly to indometacin (1.0 mg/ear; cyclooxygenase/lipoxygenase inhibitor), the topical application of hydroalcoholic extract caused a significant and dose-dependent inhibition of the AA-induced mouse ear oedema. The estimated mean ID50 value from the oedema inhibition was 1.94 (1.24–3.03) mg/ear and inhibition of 60 ± 7% (3.0 mg/ear). The calculated indometacin inhibition was 84 ± 5% (1.0 mg/ear) (Figure 7).

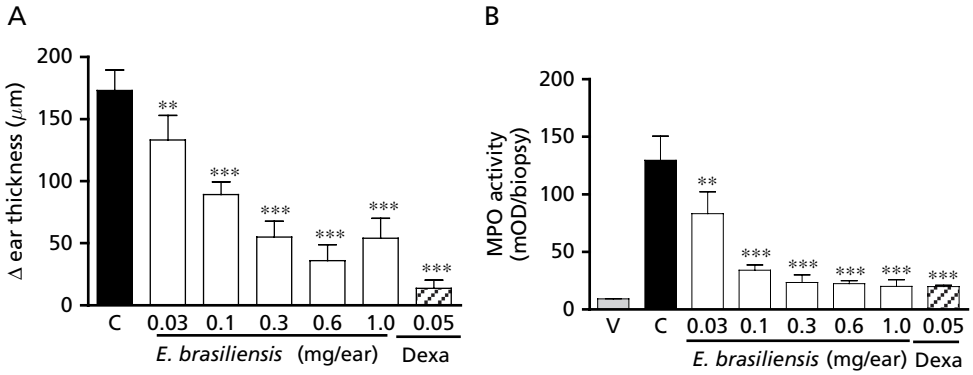

Figure 3 Effects of *E. brasiliensis* and dexamethasone (Dexa) administered topically on croton oil-induced ear oedema (A) and myeloperoxidase (MPO) activity (B) in supernatants of homogenates from croton oil-treated ears. Ear oedema and myeloperoxidase activity were measured at 6 and 24 h after croton oil treatment, respectively. In the vehicle mouse (V) only acetone was applied. Each point represents the mean ± s.e.m. of 6–10 mice. $^{**}P < 0.01$ and $^{***}P < 0.001$ compared with control groups.

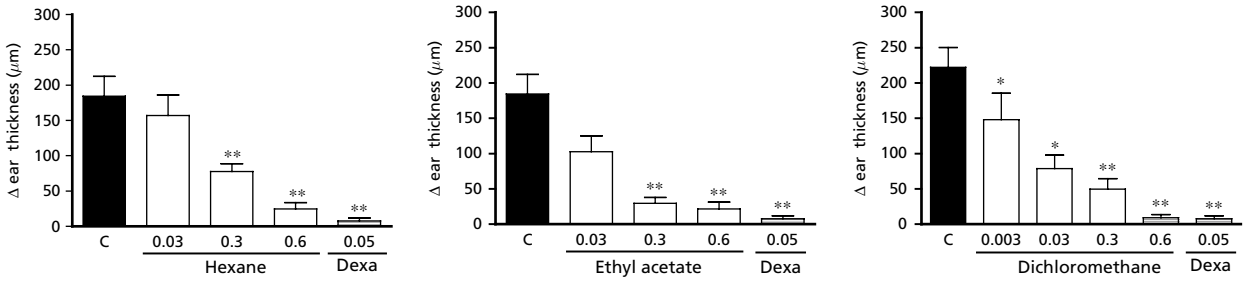

Figure 4 Effect of *E. brasiliensis* hexane fraction, ethyl acetate fraction and dichloromethane fraction, and dexamethasone (Dexa) administered topically on croton oil-induced ear oedema in mice. Ear oedema was measured at 6 h after croton oil treatment. Each point represents the mean ± s.e.m. of 6–10 mice. $^{*}P < 0.05$ and $^{**}P < 0.01$ compared with control groups.

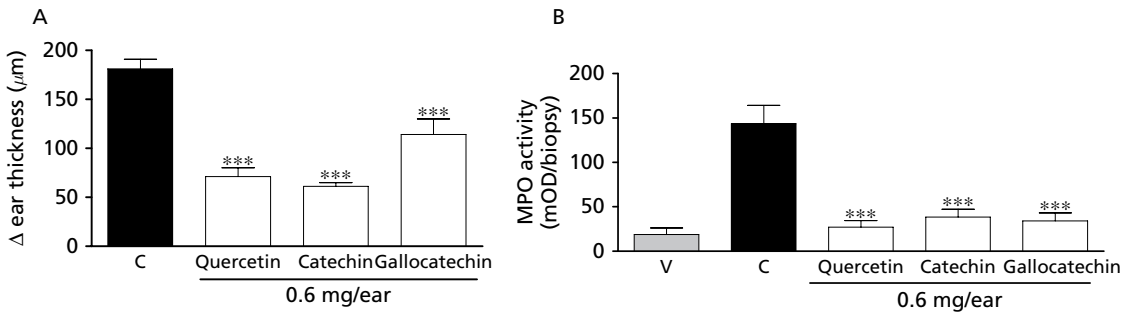

Figure 5 Effect of *E. brasiliensis* compounds quercetin, catechin and gallocatechin administered topically on croton oil-induced mouse ear oedema (A) and myeloperoxidase (MPO) activity (B) in supernatants of homogenates from croton oil-treated ears. Ear oedema and myeloperoxidase activity were measured at 6 and 24 h after croton oil treatment, respectively. In the vehicle mouse (V) only acetone was applied. Each point represents the mean ± s.e.m. of 6–10 mice. $^{***}P < 0.001$ compared with controls.

Discussion

Capillary electrophoresis (CE) has been used as an alternative method for analysis of phenolic compounds, like flavonoids, because of its powerful resolving ability and it is a simpler, more efficient and less costly procedure in comparison with other methods, like HPLC. The combination of speed, resolution, simplicity and low operating costs make the technique an attractive option for the development of improved methods

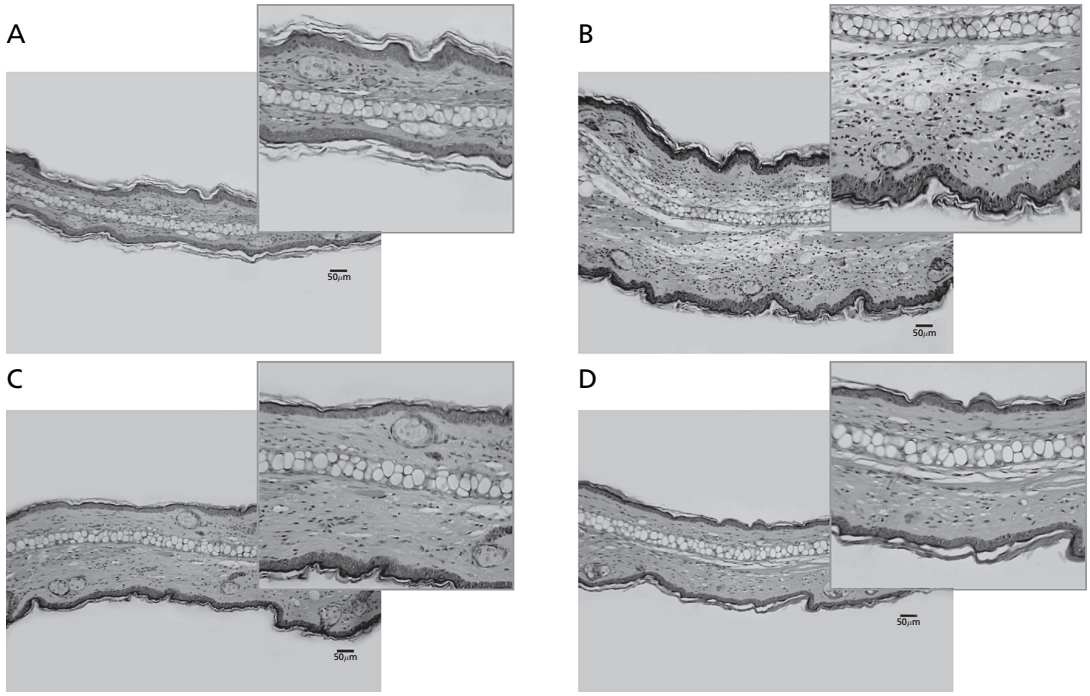

Figure 6 Histology of vertical sections of mouse ears (10× and 20×, Haematoxylin & eosin-stained) were evaluated 24 h after croton oil application, showing ears without treatment (A) and treated with croton oil (B) and croton oil plus *E. brasiliensis* (0.6 mg/ear) (C), or with dexamethasone (0.05 mg/ear) (D).

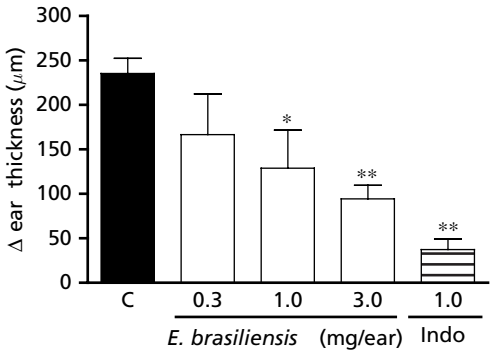

Figure 7 Effect of *E. brasiliensis* administered topically on AA-induced mouse ear oedema. Ear oedema was measured at 1 h after AA treatment. Each point represents the mean ± s.e.m. for 6–10 mice. $*P<0.05$ and $**P<0.01$ compared with control groups. Indo, indometacin.

for determining phenolic compounds (Gómez-Caravaca et al 2006). Some researchers reported the successful use of CE for separation and determination of flavonoid compounds extracted from herbal plants and wine (Demmianova et al 2003; Nong et al 2005). As flavonoids are weakly acidic their separation requires a buffer of pH close to 10 to be successful.

The analysis of the EtOAc fraction was performed by capillary electrophoresis (CE) and compared with standards of phenolic compounds. The electropherogram of the EtOAc fraction presented several peaks. The analysis of the UV spectra of some peaks in the region of 6–8 min showed a great similarity to the spectra presented by flavonoids linked to sugars (peaks *a–e in Figure 1A). This fact was confirmed when the UV spectra of the glycosylated flavonoid rutin was observed, where there is the presence of two maximum wavelengths, in the region of 260 and 380 nm, in comparison with the spectrum of compound *a (Figure 2). In examining the electropherogram before acid hydrolysis, the presence of free flavonoids was also observed, as aglycones myricetin and quercetin, corresponding to peaks 3 and 4 (Figure 1A, C). Besides flavonoids, there was also the presence of simple phenolic compounds, such as gallic acid, corresponding to peak 5.

After the acid hydrolysis (Figure 1B), the signals in the region of 6–8 min decreased considerably, and the peaks corresponding to the aglycones myricetin and quercetin showed an increase. This reinforces the fact that these signals can deal with flavonoids linked to sugars, whose aglycones would probably be these flavonoids. An increased peak corresponding to gallic acid suggests that these derivatives can be linked to units of this phenolic compound.

These profiles are interesting because the *Eugenia* genus is rich in phenolic compounds, such as catechins and flavonoids. These compounds have been isolated from many *Eugenia* species, such as *E. jambolana* (Timbola et al 2002), *E. jambos* (Slowing et al 1994; Srivastava et al 1995), *E. edulis* (Hussein et al 2003) and others. Due to the high phenolic content, this species presents several biologic actions related to these compounds, including antioxidant (Einbond et al 2004), anti-inflammatory and xanthine oxidase inhibiting activity,

all related to the pathology of arthritis (Schmeda-Hirschmann et al 1987; Theoduloz et al 1988).

This study provides evidence that *E. brasiliensis* fractions and compounds present a significant topical anti-inflammatory effect in different models of cutaneous inflammation in mice. Moreover, the plant reduced important stages of the inflammatory process, such as the oedema and the cellular migration of polymorphonuclear leucocytes as shown by the measure of myeloperoxidase enzymatic activity.

The hydroalcoholic extract caused a significant and dose-dependent inhibition of the croton oil-induced skin inflammation. This phlogistic agent is an extract of *Croton tiglium* L. (Euphorbiacea) seeds and has an irritant and vesiculant effect on the skin. Croton oil contains phorbol esters, of which the main one is 12-*O*-tetradecanoylphorbol-13-acetate (TPA). Epicutaneous application of TPA or croton oil results in vascular leakage, leucocyte infiltration and epidermal hyperplasia. These effects are due to direct activation of protein kinase C, which triggers an increased release of AA and its metabolites, especially leukotriene B4, which is a potent chemotactic agent. Moreover, it also promotes enzyme induction and increased protein, RNA and DNA synthesis (Gábor 2000; Garrido et al 2006). As mentioned previously, this whole inflammatory cascade is activated in skin diseases, so compounds that reduce this process, such as the ones in *E. brasiliensis*, can open a new therapeutic perspective.

Topical application of the hydroalcoholic extract of *E. brasiliensis* reduced not only the oedema, but also the cellular migration, demonstrated by the determination of myeloperoxidase activity. This enzyme is a major neutrophil protein and it is also present in monocytes. In neutrophils, it is stored in azurophilic granules and released during phagocytosis. Thus, myeloperoxidase is an indirect marker of neutrophil presence, resulting from its infiltration in inflamed tissue (Winterbourn et al 2000; Lau & Baldus 2006). The decrease in myeloperoxidase levels, as demonstrated in the treatment with hydroalcoholic extract of *E. brasiliensis*, confirms the topical anti-inflammatory effect and suggests an extract effect regarding inflammatory cell migration neutrophils. The reported action is interesting because the accumulation of neutrophils plays a critical role in cutaneous inflammatory disease such as dermatitis (Sanchez & Moreno 1999; Schaerli et al 2004; Otuki et al 2006). Confirming these results, histological analysis demonstrated that the plant extract reduced the epidermal hyperplasia and dilated blood vessels associated with marked infiltration of inflammatory cells.

Following this, we analysed the activity of the fractions from *Eugenia*: hexane, ethyl acetate and dichloromethane. All tested fractions presented a significant effect in reducing oedema, especially the ethyl acetate and dichloromethane fractions, which presented similar ID50 values. Furthermore, we isolated three phenolic compounds from the ethyl acetate fraction with anti-oedematogenic activity–quercetin, catechin and gallocatechin.

The presented results suggest that these three compounds are involved in plant anti-inflammatory action. The compounds possibly can act at different points in the inflammatory cascade to produce the final therapeutic effect. The three compounds studied belong to the flavonoid family and present antioxidant effects. Quercetin has been suggested to modulate inflammatory responses in various models and exerts multiple pharmacological effects: prevention of tumour development and carcinogen activation; arrest of the cell cycle and induction of apoptosis; inhibition of heat shock protein; inhibition of the enzymatic activity of alkaline phosphatase, phospholipase A_2 and protein kinases; and other effects (Mamani-Matsuda et al 2006; Pawlikowska-Pawlega et al 2007). Other phenolic compounds are catechins, which include catechin and gallocatechin compounds. Catechins have attracted much attention in relation to their physiological role as anti-mutagenic and anti-tumorigenic agents (Medina et al 2007). Catechins also present an anti-inflammatory effect induced by suppression of several inflammatory factors, including NF-κB, adhesion molecules, monocyte chemoattractant protein (MCP)-1 and matrix metalloproteinases (MMPs) (Suzuki et al 2007). Several other tests are necessary for better understanding of the mechanism of action for the plant anti-inflammatory effect, as well a more detailed analysis of chemical compounds present to determine which compounds possess biological activity.

The extract was also effective in inhibiting, significantly and in a dose-dependent manner, the oedema induced by the prostanoid precursor, and results in the fast production (concentration peak in 15 min) of PGE_2 and LTC_4/D_4. These mediators are able to promote vasodilatation and increase of vascular permeability and synergically can participate in the oedema production (Carlson et al 1985; Gábor 2000). Therefore, differences exist in the inflammatory response mechanism induced by TPA. In contrast, phospholipase A_2 and cyclooxygenase inhibitors have little or no effect on AA-induced ear oedema, but are highly effectiveness against the inflammation caused by croton oil (Sanchez & Moreno 1999; Otuki et al 2005). Therefore, it is possible that *E. brasiliensis* extract possesses an inhibitory effect on phospholipase A_2 and cyclooxygenase due to its effectiveness in the croton oil model. However, at this stage in our work, it is difficult to determine the precise mechanism through which the extract promotes its effect. Moreover, AA ear inflammation has been regarded for many years as a suitable screen for detecting lipoxygenase inhibitors in-vivo. On the other hand, several studies have shown that AA-induced inflammation can be inhibited by mechanisms other than cyclooxygenase/lipoxygenase enzyme inhibition, such as modulation of histamine and serotonin response (Chang et al 1985; Pignat et al 1986; Sanchez & Moreno 1999; Gabor 2000). Consequently, one or more targets could be involved in this anti-inflammatory response. It is important to emphasize that the plant presents activity in both models, TPA and AA, which represent different mechanisms, emphasizing the relevance of *E. brasiliensis* in the treatment of cutaneous inflammatory diseases.

Conclusions

This work confirmed the anti-inflammatory effect of the *E. brasiliensis* plant, as suggested by its popular use. Initially fractionation was carried out to separate the compounds involved in this effect. As a result, the three obtained fractions presented anti-oedematogenic activity. From the ethyl

acetate fraction three compounds were isolated and all were effective. Thus, this result demonstrates that there is a group of plant substances providing the anti-inflammatory effect. Experiments are being carried out in our laboratory to evaluate the efficacy and safety of chronic treatment with *E. brasiliensis* its in topical formulations in other animal models of skin inflammation, as well as to investigate the cellular and molecular mechanisms by which the plant exerts its topical anti-inflammatory action. Thus, this study demonstrates that *E. brasiliensis*, its fractions and compounds, possess a topical anti-inflammatory activity in mice, and this could be one more promising candidate in the development of a new medicine for the treatment of skin diseases, as well as a source of biologically active compounds.

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