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Original Article

Determination of phenolic profile by HPLC-ESI-MS/MS and anti-inflammatory activity of crude hydroalcoholic extract and ethyl acetate fraction from leaves of *Eugenia brasiliensis*

Diogo A. Siebert[a], Juliana Bastos[b], Daniel A. Spudeit[b], Gustavo A. Micke[b], Michele D. Alberton[a,*]

[a] Laboratório de Pesquisa em Produtos Naturais, Departamento de Ciências Farmacêuticas, Universidade Regional de Blumenau, Blumenau, SC, Brazil
[b] Laboratório de Eletroforese Capilar, Departamento de Química, Universidade Federal de Santa Catarina, Florianópolis, SC, Brazil

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ABSTRACT

Eugenia brasiliensis Lam., Myrtaceae, is used in folk medicine for anti-inflammatory diseases such as arthritis and rheumatism. This study investigated the anti-inflammatory activity and phenolic profile of the crude hydroalcoholic extract and ethyl acetate fraction from *E. brasiliensis* leaves. Crude hydroalcoholic extract and the ethyl acetate fraction were analyzed by HPLC-ESI-MS/MS in comparison to standard phenolic compounds. The anti-inflammatory activity of the crude hydroalcoholic extract (1, 10 and 25 mg kg^{-1}) and the ethyl acetate fraction (10, 25 and 50 mg kg^{-1}) was evaluated in a swiss mouse model of acute pleurisy induced by carrageenan, being the total cell count, exudation and analysis of nitrite/nitrate the inflammation parameters. HPLC-ESI-MS/MS analysis revealed apigenin, catechin, galangin, isoquercetin, myricetin, quercetin and rutin. Crude hydroalcoholic extract and ethyl acetate fraction were effective in inhibiting cell migration in all tested doses. Crude hydroalcoholic extract was effective in inhibiting exudation only at the 10 mg kg^{-1} dose; ethyl acetate fraction was effective in all tested doses. Results for nitrite/nitrate levels reveals that only the ethyl acetate fraction was effective at the tested doses. This is the first report of the presence of isoquercetin, galangin and apigenin in this species. Results from the phytochemical analysis enhance the chemical knowledge of this species. In the future, together with more studies, validation of its popular use in inflammatory diseases is possible.

Introduction

Eugenia brasiliensis Lam., Myrtaceae, popularly known in Brazil as "grumixama", "grumixameira" and "brazilian-cherry", is endemic to the Atlantic Rainforest in Brazil (Legrand and Klein, 1969). This plant is used in folk medicine as diuretic, for the treatment of diarrhea, arthritis and rheumatism (Revilla, 2002). Some articles in the literature have reported biological activities from extracts, essential oil and isolated compounds from this species. The essential oil have shown promising antibacterial activity against *Staphylococcus aureus* (Magina et al., 2009a). The crude extract from the leaves have demonstrated an interesting antioxidant activity, which is probably related to the phenolic and flavonoid content (Magina et al., 2010), and also exerted an antidepressant-like effect in the tail suspension test in mice (Colla et al., 2012). Regarding previous anti-inflammatory studies, this plant demonstrated effect in an croton oil-induced and arachidonic acid-induced ear edema in mice model of inflammation (Pietrovski et al., 2008). Finally, recent work with ethanolic extracts of leaves, pulp, and seeds of *E. brasiliensis*, evaluated that oral administration of these extracts reduced the *in vivo* carrageenan-induced neutrophil migration by 47, 41, and 50%, respectively (Infante et al., 2016).

Monoterpenes, such as α-pinene and β-pinene, oxygenated monoterpenes as α-terpineol, sesquiterpenes as caryophyllene and oxygenated sesquiterpenes as spathulenol, α-cadinol and τ-cadinol have been identified in the essential oil (Fischer et al., 2005; Ramos et al., 2006; Moreno et al., 2007; Lima et al., 2008; Magina et al., 2009a; Siebert et al., 2015). Some flavonoids such as myricetin, quercetin and rutin, as well as other phenolic compounds such as catechin, gallocatechin and gallic acid (Pietrovski et al., 2008), also triterpenes such as α-amyrin, β-amyrin, betulin, 29-hydroxy-oleanolic acid, ursolic acid, betulinic acid and oleanolic acid have been identified from their leaves (Frighetto et al., 2005; Magina et al., 2012; Lima et al., 2014). Moreover, in studies regarding the specie's fruits, some phenolic compounds such as anthocyanins,

* Corresponding author.
E-mail: michele@furb.br (M.D. Alberton).

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flavanols, flavonols, ellagitannins and carotenoids were identified, using techniques that include HPLC-ESI-MS/MS (Reynertson et al., 2008; Flores et al., 2012; Silva et al., 2014; Teixeira et al., 2015). Most of the substances above mentioned have proven anti-inflammatory and other biological activities (Di Carlo et al., 1999; Huguet et al., 2000; Magina et al., 2009b).

Considering the popular use of this *Eugenia* species and previous report regarding the anti-inflammatory activity, this study aims to investigate the effect of *E. brasiliensis* leaves in a mouse model of pleurisy induced by carrageenan. Therefore, we evaluated the effect of the crude hydroalcoholic extract (CHE) and ethyl acetate fraction (EAF) on leukocyte migration, exudate concentrations and nitrate/nitrite (NO_x) levels. Moreover, analysis and identification of phenolic compounds by high-performance liquid chromatography tandem mass spectrometry using the electrospray ionization (HPLC-ESI-MS/MS) were performed.

Materials and methods

Plant material

Leaves from *Eugenia brasiliensis* Lam., Myrtaceae, were collected in Florianópolis, Santa Catarina state (27°36′13.65″ S, 48°31′14.75″ W), in March 2012. Plant material was identified by Dr. Daniel de Barcellos Falkenberg from the Botany Department of Universidade Federal de Santa Catarina (UFSC), and a voucher specimen was deposited in the herbarium FLOR of the same institution under registry number 34675.

Preparation of the CHE

The plant material was dried and milled, totaling 1813 g of material. This material was macerated in hydroalcoholic solution (92.8%, w w^{-1}) for seven days. The extract was filtered and solvent evaporated in rotatory evaporator (below 60 °C) coupled with a vacuum condenser, and concentrated to a reduced volume. After total evaporation of the solvent, 192.5 g of crude extract was obtained, which represent a yield of 10.62% of plant material.

Preparation of EAF fraction

An aliquot of 117 g from the CHE was resuspended in water and the mixture was stored under refrigeration (2–8 °C) over night and filtered. The aqueous solution was defatted by washing with dichloromethane. Thus, ethyl acetate fraction was prepared by liquid-liquid partition, yielding 23.87 g.

Determination of phenolic compounds in the CHE extract and EAF fraction of Eugenia brasiliensis *by HPLC-ESI-MS/MS*

The CHE and the EAF were analyzed by HPLC-ESI-MS/MS in LabEC/INCT-Catalise (UFSC). Analysis were conducted in a Agilent® 1200 (Agilent Technologies, Germany) liquid chromatograph system, with a Phenomenex Synergi® 4μ Polar-RP 80A column (150 mm × 2 mm, particle size of 4 μm) at the temperature of 30 °C. The eluents were formed by mixing solvents A (H_2O + 0.1% formic acid) and B (acetonitrile/H_2O, 95:5) as follows: 1st stage – 80% solvent A (isocratic mode) for 10 min; 2nd stage – linear gradient of solvents A and B (from 80 to 5% of A) for 15 min; 3rd stage – 5% solvent A and 95% B (isocratic mode) for 5 min with a flow rate of 200 μl min^{-1} of mobile phase. In all analyses, the injected volume was 5 μl.

The liquid chromatograph was coupled to a mass spectrometry system consisting of a hybrid triple quadrupole/linear ion trap mass spectrometer Qtrap® 3200 (Applied Biosystems/MDS SCIEX, USA) with TurboIonSpray® as ionization source, in positive ionization mode, with the following source parameters: ion spray interface at 400 °C; ion spray voltage of 4500 V; curtain gas, 10 psi; nebulizer gas, 45 psi; auxiliary gas, 45 psi; collision gas, medium. The Analyst® (version 1.5.1) software was used for recording and processing the data. Pairs of ions were monitored in MRM (Multiple Reaction Monitoring) mode.

For the identification of the compounds, 29 standard phenolic compounds (4-methylumbelliferone, apigenin, apigenin 7-glucoside, aromadendrine, caffeic acid, catechin, chlorogenic acid, cinnamic acid, coniferaldehyde, coumarin, epicatechin, ferulic acid, galangin, hispidulin, isoquercetin, kaempferol, luteolin, myricetin, naringin, *p*-coumaric acid, quercetin, resveratrol, rutin, scopoletin, sinapaldehyde, sinapic acid, syringaldehyde, syringic acid, vanillic acid) dissolved in methanol (1 mg l^{-1}) were analyzed in same conditions as described above (Bertin et al., 2014).

Evaluation of anti-inflammatory activity of Eugenia brasiliensis

Animals

Experiments were carried out using 30-day-old Swiss mice of both sexes (18–25 g). Animals were kept at controlled room temperature (22 ± 3 °C) under a 12 h light-dark cycle, with access to water and food *ad libitum*. All procedures used in the study were approved by the Ethics Committee on Animal Use (protocol number 008/12) from Universidade Regional de Blumenau (FURB). The number of animals (n = 5–6) was the minimum necessary to demonstrate the consistent effects of treatment.

Carrageenan-induced pleurisy

The pleurisy procedure was performed according to the methodology described by Saleh et al. (1999, 1996). According to the experimental protocol, different groups of animals were pretreated (30 min) with different concentrations of the CHE (1, 10 and 25 mg kg^{-1}) and the EAF (10, 25 and 50 mg kg^{-1}), administered intraperitoneally (*i.p.*).

Then, the pleurisy was induced by administration of 0.1 ml of grade IV λ carrageenan (Cg) (1%) in the right pleural cavity (*i.pl.*). After 4 h, the animals were sacrificed with an overdose of pentobarbital and the pleural cavity exposed and washed with 1 ml of a solution of heparin (20 IU ml^{-1}) diluted with phosphate buffered saline (PBS, pH 7.6). For the indirect quantification of exudation, 10 min after the administration of Cg, the animals received 0.2 ml of Evans blue dye solution at 25 mg kg^{-1} intraorbitally (*i.o.*). To verify the levels of nitrite/nitrate (NO_x), groups of animals were carried out separately, with the best dose of each test sample without the administration of Evans blue dye in order to avoid interference with the results. The experiments were always accompanied by positive control groups (animals that received only Cg 1%, 0.1 ml, *i.pl.*) and negative control groups (animals that received only 0.1 ml saline *i.pl.*). In addition, experiments were performed using dexamethasone (4 mg kg^{-1}, *i.p.*) as anti-inflammatory reference drug.

Total and differential leukocyte count

Total cell counts were performed in Neubauer chambers by means of an optical microscope (magnification 400×) after diluting a sample of the collected fluid from the pleural space with Türk solution (1:200). Cellular smears were prepared with an aliquot of pleural lavage to determine the differential leukocyte count. The slides were stained with May–Grünwald–Giemsa dye, and the analysis was carried out under oil immersion objective (Saleh et al., 1996, 1999).

Indirect quantification of exudation

Samples obtained from the pleural cavity were separated into aliquots of 500 μl and the levels of Evans blue dye estimated colorimetrically at 620 nm by interpolation from a constructed standard

Table 1
Phenolic compounds identified in *Eugenia brasiliensis* by HPLC-ESI-MS/MS.

	Compounds	*Rt* (min)	Calculated mass (M)	Experimental mass $[M+H]^+$	MS/MS (*m/z*)
1	Catechin	10.94	290.26	291.1	139.0
2	Myricetin	11.85	318.24	319.1	153.0
3	Isoquercetin	12.34	464.38	465.1	303.1
4	Rutin	12.46	610.52	611.1	303.1
5	Quercetin	12.47	302.23	303.0	153.1
6	Galangin	17.35	270.24	271.1	77.1
7	Apigenin	17.79	270.24	271.0	153.1

curve of the dye in the range of 0.05–50 $\mu g\,ml^{-1}$, according to the methodology described by Saleh et al. (1996, 1999). Results were expressed as $\mu g\,ml^{-1}$.

Quantitative analysis of nitrite/nitrate (NO_x)

To verify the levels of nitrite/nitrate (NO_x), groups of animals with the best dose of each test sample without the administration of Evans blue dye were performed to avoid interference with the results. Based on the results of cell migration and exudation, were tested the CHE at the dose of $10\,mg\,kg^{-1}$, and the EAF at the dose of $10\,mg\,kg^{-1}$.

Nitric oxide (NO) was measured indirectly by its breakdown products nitrite (NO_2^-) and nitrate (NO_3^-), using the Griess reaction (Green et al., 1982), and the pre-processing proposed by (Miranda et al., 2001). Nitrite concentrations were estimated by interpolation from a standard curve of sodium nitrite (0–150 mM) by colorimetric measurements at 540 nm in an ELISA plate reader. Results were expressed as mM.

Statistical analysis

Statistical analysis of the results of anti-inflammatory activity, were conducted by the ANOVA parametric test supplemented when necessary by Dunnett's test, or *t* test (nonparametric test) by means of the GraphPad PRISM® (version 3.0) statistical software. *p* values < 0.05 were considered significant.

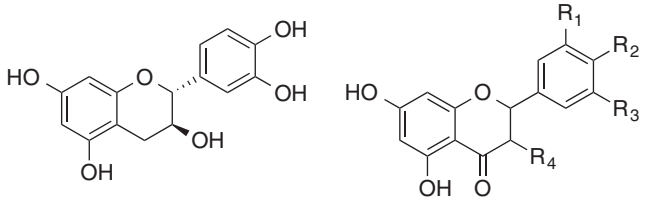
Results and discussion

Determination of phenolic compounds in the CHE and EAF fraction of Eugenia brasiliensis

In order to record a profile of phenolic compounds which are present in the CHE and EAF, these samples were analyzed by the hyphenate technique HPLC-ESI-MS/MS, that combines the versatility of the liquid chromatography and the sensibility and specificity of a tandem mass spectrometer, therefore being extremely reliable (Wu et al., 2013).

The fingerprint of the extracts and fractions of *E. brasiliensis* led to the identification of phenolic compounds based on their molecular formula, fragmentation pattern and comparison with the retention times of standards commercially available. In this method, the samples were compared qualitatively with standards of 29 phenolic compounds. Table 1 summarizes the seven phenolic compounds identified in CHE or EAF fraction, their retention time, protonation $[M+H]^+$ and molecular weight.

The results revealed the presence of the flavonoids catechin (**1**), myricetin (**2**), isoquercetin (**3**), rutin (**4**), quercetin (**5**) and galangin (**6**) in the CHE. In the EAF, the compounds catechin (**1**), isoquercetin (**3**), galangin (**6**) and apigenin (**7**) have been identified.



1

2 R_1=R_2=R_3=R_4=OH
3 R_1=R_2=OH; R_3=H; R_4=*O*-glu
4 R_1=R_2=OH; R_3=H; R_4=*O*-rut
5 R_1=R_2=OH; R_3=H; R_4=OH
6 R_1=R_2=R_3=H; R_4=OH
7 R_1=R_3=R_4=H; R_2=OH

Although some of the substances identified have already been described in the leaves of this species, like catechin, myricetin, rutin and quercetin, this is the first report of the presence of isoquercetin, galangin and apigenin in *E. brasiliensis*.

Anti-inflammatory activity of CHE and EAF fraction from Eugenia brasiliensis

The results for cell migration and exudation (Figs. 1 and 2) showed that the CHE was effective in inhibiting cell migration, which increases with higher doses, along with the EAF, however with the same intensity (no significant difference) at all tested doses. CHE inhibited 43 ± 12% ($10\,mg\,kg^{-1}$ dose) and 58 ± 11% ($25\,mg\,kg^{-1}$ dose), and the EAF inhibited 43 ± 5% ($10\,mg\,kg^{-1}$ dose), 43 ± 12% ($25\,mg\,kg^{-1}$ dose) and 60 ± 11% ($50\,mg\,kg^{-1}$) the total cell count, compared with the non-treated group ($p < 0.001$).

On exudation levels, CHE was effective in inhibiting them only at the $10\,mg\,kg^{-1}$ dose (55 ± 10%), and the EAF inhibited exudation significantly in all tested doses (38 ± 9%, $10\,mg\,kg^{-1}$; 46 ± 7%, $25\,mg\,kg^{-1}$; 42 ± 9%, $50\,mg\,kg^{-1}$ dose) compared with the non-treated group ($p < 0.001$).

The results for NO_x levels (Fig. 3) reveals that EAF was effective in inhibiting the production of this inflammatory mediator (76 ± 19%) even more than the standard drug, dexamethasone (41 ± 25%). However, the CHE was not able to inhibit significantly the production of NO in the pleural cavity, at the tested dose.

Although pleurisy induced by carrageenan is not an asthma model, the administration of carrageenan into the pleural cavity provides an inflammatory environment similar to that observed in patients with neutrophilic asthma phenotype. The samples were evaluated on the early phase of inflammation (4 h after administration of carrageenan 1%), which is characterized by an enhancement in the total number of cells (due primarily to neutrophil influx, rather than mononuclear) exudation and increase of NO (Saleh et al., 1996).

The obtained results suggest an anti-inflammatory activity of *E. brasiliensis* in the pleurisy model, evidenced by the inhibition of the leukocyte migration, diminished the exudation, and reduced the

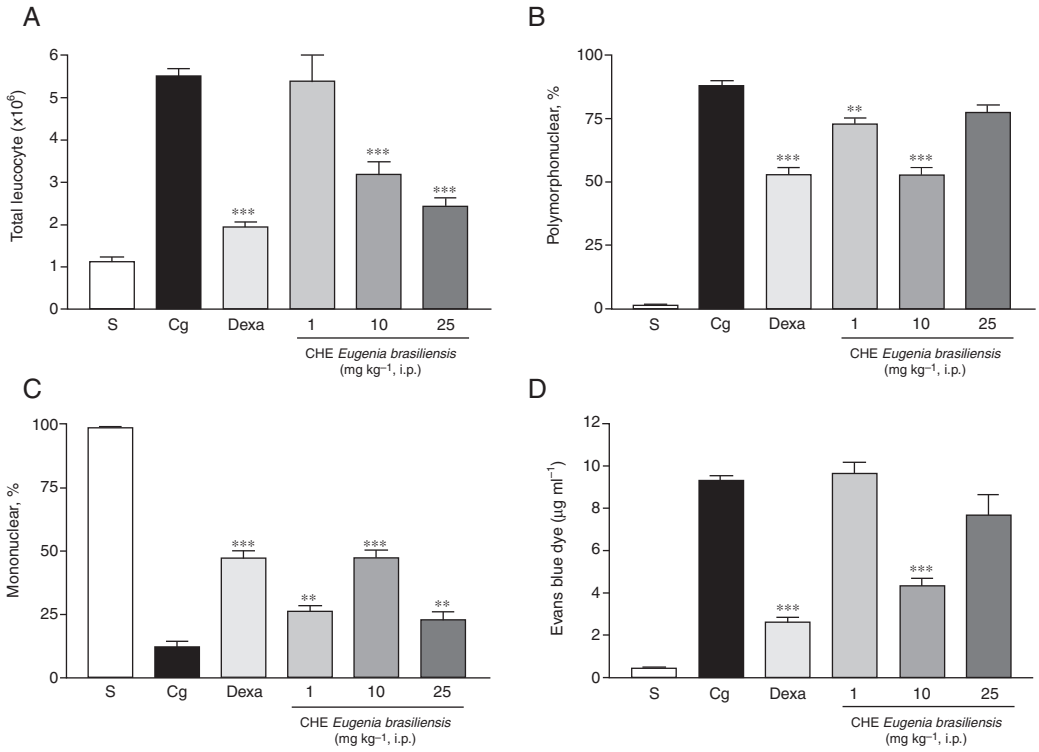


Fig. 1. Effect of crude hydroalcoholic extract of *Eugenia brasiliensis* (1, 10, 25 mg kg^{-1}, *i.p.*) on the number of total leukocytes (A), percentage of polymorphonuclear cells (B), percentage of mononuclear cells (C) and exudation (D) on the pleural cavity in the mouse model of pleurisy (4 h) induced by carrageenan. Control animals were treated only with saline (S), carrageenan (0.1 ml of 2%, *i.pl.*) (Cg) and dexamethasone (4 mg kg^{-1}, *i.p.*) (Dexa). Each group represents the mean of 4–6 animals. $^{***}p < 0.001$; $^{**}p < 0.01$ and $^{*}p < 0.05$.

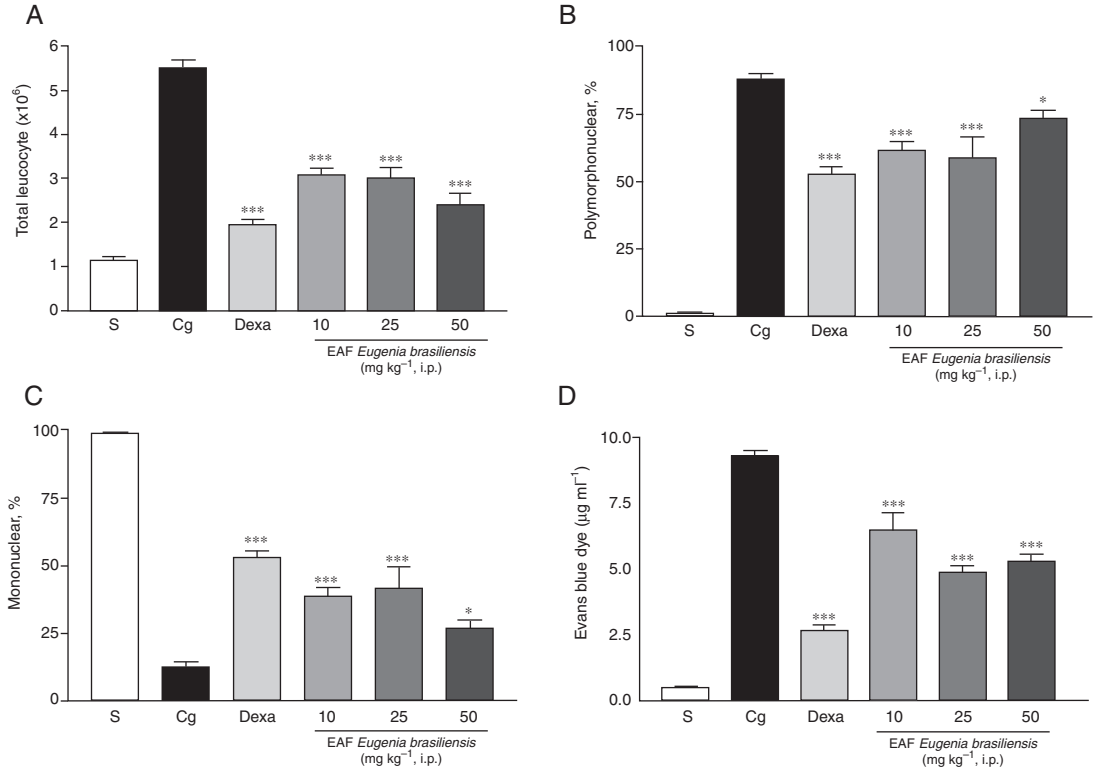


Fig. 2. Effect of ethyl acetate fraction of *Eugenia brasiliensis* (10, 25 and 50 mg kg^{-1}, *i.p.*) on the number of total leukocytes (A), percentage of polymorphonuclear cells (B), percentage of mononuclear cells (C) and exudation (D) on the pleural cavity in the mouse model of pleurisy (4 h) induced by carrageenan. Control animals were treated only with saline (S), carrageenan (0.1 ml at 2%, *i.pl.*) (Cg) and dexamethasone (4 mg kg^{-1}, *i.p.*) (Dexa). Each group represents the mean of 4–6 animals. $^{***}p < 0.001$; $^{**}p < 0.01$ and $^{*}p < 0.05$.

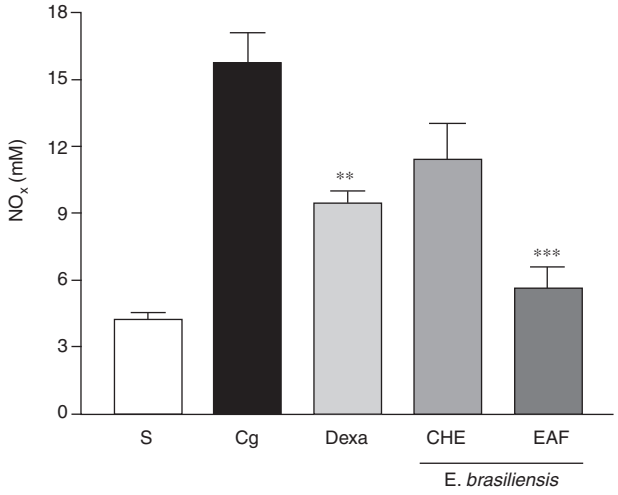


Fig. 3. Effect of crude hydroalcoholic extract (10 mg kg^{-1}, *i.p.*) and ethyl acetate fraction of *Eugenia brasiliensis* (10 mg kg^{-1}, *i.p.*) on NO_x levels in the pleural cavity in the mouse model of pleurisy (4 h) induced by carrageenan. Control animals were treated only with saline (S), carrageenan (0.1 ml at 2%, *i.pl.*) (Cg) and dexamethasone (4 mg kg^{-1}, *i.p.*) (Dexa). Each group represents the mean of 4–6 animals. $^{***}p < 0.001$; $^{**}p < 0.01$ and $^{*}p < 0.05$.

NO production. These results corroborate with study from the same species using the croton oil-induced and arachidonic acid-induced ear edema in mice (Pietrovski et al., 2008), that also observed a promising topical anti-inflammatory activity from the hidroalcoholic extract, hexane, dichloromethane and ethyl acetate fractions, as well as the isolated compounds quercetin, catechin and gallocatechin. Also, stay in agreement with study by Infante et al. (2016), which showed that ethanolic extracts of leaves, pulp, and seeds of *E. brasiliensis*, reduced the *in vivo* carrageenan-induced neutrophil migration. Extracts from other species of the *Eugenia* genus have already demonstrated anti-inflammatory effect, therefore agreeing with the present study (Slowing et al., 1994; Ramirez et al., 2012; Basting et al., 2014; Soares et al., 2014).

Nitric oxide is a soluble gas produced from enzymes called nitric oxide synthases (NOS). Three NOS isoforms are described (Nathan and Xie, 1994), of which two are constitutive (cNOS) and expressed under physiological conditions, and another is induced (iNOS). A number of agents, such as inflammatory cytokines and oxidants, induce their expression in different cell types such as macrophages (Morris and Billiar, 1994), neutrophils (Eiserich et al., 1998), mononuclear cells (Salvemini et al., 1989), eosinophils and vascular smooth muscle cells (Guo et al., 1995).

Increase of NO in the carrageenan-induced pleurisy model in mice was previously demonstrated and suggests the induction of cNOS and iNOS enzymes in the pleural cavity cells of inflamed animals. The fact that NO concentrations are not related either to neutrophils or mononuclear cells is indicative that this compound comes from different sources besides leukocytes (Barnes et al., 1998). The exudation levels are directly linked to the increase in the amount of NO produced, and the released NO plays an important role as vasodilator and contributes to exudation (Luz et al., 2016).

Many phenolic compounds are present in the extract and fraction of the plant, as evidenced by HPLC-ESI-MS/MS analysis. The anti-inflammatory effect of phenolic compounds, such as apigenin, quercetin and myricetin as well as their capability to inhibit NO production from macrophages, is known for some time (Formica and Regelson, 1995; Kim et al., 1999). However, the possibility that other classes of compounds are contributing to the observed result, cannot be excluded.

Similar results with anti-inflammatory activity of phenolic compounds were observed by Zhu et al. (2009), which showed a significant inhibition of leukocyte infiltration into the inflammatory site in the air pouch model with *N*-formylmethionyl-leucyl-phenylalanine bacterial in mice when they were treated with catechins extracted from green tea (*Camellia sinensis*). Furthermore, Wang et al. (2011) found that the extract enriched with green tea catechins was effective in reducing paw edema induced by carrageenan in rats, and the ear edema induced by xylene in mice. Furthermore, quercetin, another identified compound from *E. brasiliensis*, has previously demonstrated anti-inflammatory effect in work conducted by Morikawa et al. (2003), when a significantly decrease in the total number of leukocytes and exudation, in the air pouch model induced by carrageenan in rats, was showed.

Another study evaluated the anti-inflammatory effect of quercetin using other models of inflammation related to atherosclerosis, suggesting a promising anti-inflammatory activity, therefore agreeing with this study (Kleemann et al., 2011). In a recent review article (Leiherer et al., 2013), the authors listed a number of activities, among them anti-inflammatory, for various phenolic compounds, including catechins and quercetin (Suzuki et al., 2007; Qureshi et al., 2011a; Yoon et al., 2011; Abd El-Aziz et al., 2012). Moreover, rutin demonstrated anti-inflammatory activity in a model of intestinal oxidative damage induced by methotrexate in rats and was also able to inhibit 75.6, 81.0 and 80.4% of cyclooxygenase-1 and 2, and 15-lipoxygenase respectively *in vitro* (Gautam et al., 2016).

Previous work demonstrated a significant reduction in NO levels in embryonic myogenic culture from cells of the rat heart, when they were treated with the flavonoid quercetin before the inflammatory stimuli inducted with bacterial lipopolyssacaride (LPS) (Angeloni and Hrelia, 2012). Moreover, the previous treatment with quercetin diminished the NO production in LPS-stimulated macrophages (Qureshi et al., 2011b). The flavanol catechin was also efficient to reduce NO plasmatic levels in tumor angiogenesis induced mice, and diminish the NO production in LPS-stimulated macrophages (Guruvayoorappan and Kuttan, 2008). More recently, researchers demonstrated that leaf extracts from Korean *Chrysanthemum* species, that have in their composition many phenolic compounds such as apigenin, dose-dependently suppressed NO production stimulated by LPS, inhibited production of LPS-induced PGE_2 and reduced the LPS-induced expressions of inducible NO synthase and cyclooxygenase-2 (Kim et al., 2015).

In another study, galangin was able decrease NO production, reduce messenger RNA levels of cytokines, including IL-1β and IL-6, and proinflammatory genes, such as inducible nitric oxide synthase (iNOS), in a dose-dependent manner, decreased the protein expression levels of iNOS and inhibit IL-1β production in LPS-stimulated RAW 264.7 murine macrophages. Galangin was found to elicit anti-inflammatory effects by inhibiting extracellular signal-regulated kinases and NF-κB-p65 phosphorylation (Jung et al., 2014).

Myricetin showed a significant inhibition on ear edema and hind paw edema caused by xylene and carrageenan, respectively. Furthermore, it also inhibited the increase in capillary permeability induced by the production of acetic acid in the human body. Myricetin significantly decreased the serum levels of malondialdehyde and, in turn, increased the serum levels of superoxide dismutase in the carrageenan-induced paw edema model, and significantly decreased leukocyte count. During chronic inflammation, myricetin inhibited the formation of granuloma tissue. These results, collectively, demonstrate that myricetin possesses a potent anti-inflammatory function on acute and chronic inflammation and the anti-inflammatory mechanisms are probably associated with the inhibition of antioxidant activity (Wang et al., 2010).

Conclusions

Results from the phytochemical study stay in agreement with the literature to the *Eugenia* genus, being the first ever report of the presence of isoquercetin, galangin and apigenin in this species, enhancing the chemical knowledge of this species.

Our results shown that CHE and EAF from *E. brasiliensis* were effective in inhibiting leucocytes migration to the inflammatory site, and this effect was due to ability of this plant to inhibit the neutrophil influx. The other signal of inflammation evaluated, the exudate, was also significantly reduced by treatment with *E. brasiliensis*. These results, at least for the EAF fraction, are directly related with the reduction of NO levels in the pleural samples from treated animals which suggest an anti-inflammatory activity in the model tested. This effect may be, at least in part, due to the presence of the phenolic compounds identified by HPLC-ESI-MS/MS. Furthermore, this fact aids the validation of the plant use in folk medicine were it is already being used in the treatment of inflammatory diseases.

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 no patient data appear in this article.

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

Authors' contributions

DAS (MSc student) collected plant sample, performed the experiments, interpreted the results and drafted the manuscript. JB and DAS conducted the HPLC-ESI-MS/MS analysis. GAM supervised the HPLC-ESI-MS/MS analysis and laboratory work. MDA designed the study, supervised the laboratory work, interpreted the results and contributed to critical reading of the manuscript. All the authors have read the final manuscript and approved the submission.

Conflicts of interest

The authors declare no conflicts of interest.

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