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Topical anti-inflammatory activity of *Solanum corymbiflorum* leaves

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ABSTRACT

Solanum corymbiflorum is popularly known as “baga-de-veado” and its leaves are applied on inflamed legs, scabies, tick bite, boils, mastitis, low back pain and otitis. The aim of this study was evaluate anti-inflammatory *in vivo* activity and relate this activity with antioxidant compounds present in the extract of *S. corymbiflorum* leaves. The extract from *S. corymbiflorum* leaves topically applied was able to reduce the croton oil-induced ear edema and myeloperoxidase (MPO) activity with maximum inhibition of 87 ± 3% and 45 ± 7%, rescpectively in the dose of 1 mg/ear. Similar results were found for positive control dexamethasone, which presented inhibitions of ear edema and MPO activity of 89 ± 3% and 50 ± 3%, respectively in a dose of 0.1 mg/ear. These findings are due, at least in part, the presence of polyphenols (195.28 mg GAE/g) and flavonoids, as chlorogenic acid (59.27 mg/g), rutin (12.72 mg/g), rosmarinic acid, caffeic acid and gallic acid found by high performance liquid chromatography (HPLC) analysis. This species showed potencial antioxidant by 1,1-diphenyl-2-picrylhydrazyl (DPPH), and carbonyl groups in proteins methods which may be related with the presence of this compounds. This species possess anti-inflammatory activity confirming their popular use for the local treatment of skin inflammatory disorders.

1. Introduction

Solanum corymbiflorum (Sendtn.) Bohs (syn. *Cyphomandra corymbiflora*), popularly known as “baga-de-veado”, is native in the southern states of Brazil and in Argentina, where is known as “ka’a Kururu” (Herb of frog) (Soares and Mentz, 2006). In the folk medicine its leaves can be applied on inflamed legs, scabies, tick bite, boils, mastitis, low back pain and otitis (Keller and Prance, 2012). However, to our knowledge, there are no studies related to popular use of *S. corymbiflorum* to treat skin inflammatory diseases. Taking into consideration the different uses of this species related to skin anti-inflammatory effects, there is a hypothesis that this activity can be confirmed using an inflammation model induced by topical application of croton oil (da Cunha et al., 2001).

The mechanism inflammatory is attributed, in part, to release of reactive species from activated cells, such as neutrophils and macrophage. Thus, free radicals are mediators that provoke or sustain inflammatory processes and consequently, their neutralization by radical scavengers can attenuate the inflammatory process (Conforti et al., 2008). In this sense, vegetal extracts with antioxidant effects have been established as a therapeutic approach for treating inflammation (Nijveldt et al., 2001). Moreover, phenolic compounds exhibit a wide range of biological effects including antibacterial, anti-inflammatory, antiallergic, hepatoprotective and anticarcinogenic actions. Many of these biological functions have been attributed to their free radical scavenging capacity (Krishnaiah et al., 2011).

The aim of this study was evaluate the anti-inflammatory *in vivo* activity of the extract of *S. corymbiflorum* leaves and relate their anti-inflammatory activity with antioxidant compounds present in this extract.

2. Material and methods

2.1. Chemicals

All chemicals were of analytical grade. Solvent for the extractions, folin–ciocalteau reagent, iron sulfate, hematoxylin–eosin and paraffin were purchased from Merck (Darmstadt, Germany). Croton oil, hexadecyltrimethylammonium bromide (HTAB), tetramethylbenzidine (TMB), dexamethasone, gallic, ascorbic, rosmarinic, chlorogenic and caffeic acids, quercetin, rutin, DPPH,

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Tris–HCl, thiobarbituric acid and DCFH-DA were acquired from Sigma Chemical Co. (St. Louis, MO, USA). Isoflurane (Baxter, São Paulo, Brazil), sodium acetate, acetone, absolute ethanol, acetic acid, formaldehyde (all from Vetec, Rio de Janeiro, Brazil) were used.

2.2. Plant collection and extractions

S. corymbiflorum leaves (300 g of an individual) were collected in Gaurama (Rio Grande do Sul state, Brazil) in October (2012). A dried voucher specimen is preserved in the herbarium of the Department of Biology at Federal University of Santa Maria by register number SMBD 13159. The leaves were dried at room temperature and powdered in a knife mill. The powder of leaves was macerated at room temperature with 70% ethanol for a week with daily shake-up. After filtration, the extract was evaporated under reduced pressure to remove the ethanol, the aqueous extract was dried in a stove (temperature above 40 °C) to produce the extract.

2.3. Anti-inflammatory activity

2.3.1. Animals

Male Swiss mice (25–30 g) were kept in a temperature-controlled room (22 ± 2 °C) under a 12 h light-dark cycle. Animals were acclimatized to the laboratory for at least 1 h before the experiments and were used only once. All of the experiments were carried out between 8:00 a.m. and 5:00 p.m. The data reported in this study were carried out in accordance with current ethical guidelines for the investigation of experimental pain in conscious animals (Zimmermann, 1983) and were approved by the Ethics Committee of the Federal University of Santa Maria (process number 5786050215/2015). The number of animals and the amount of irritant agent were the minimum necessary to demonstrate the consistent effects of the drug treatments.

2.3.2. Treatments

The extract of the *S. corymbiflorum* leaves, the irritant croton oil and dexamethasone were applied topically to the right ear of each mouse. Dexamethasone was used as a positive control.

2.3.3. Ear edema measurements

Skin dermatitis was induced by topical administration of croton oil and the inflammatory response was evaluated through of edema formation. The edema was quantified by the increase in ear thickness of mice upon inflammatory challenge. Ear thickness was measured before and after induction of the inflammatory response, using a digital micrometer (Digimess) in animals anesthetized with isoflurane (Silva et al., 2011). The micrometer was applied near the tip of the ear just distal to the cartilaginous ridges. The thickness was expressed in μm. To minimize variation, a single investigator performed the measurements throughout each experiment. The acetone (20 uL/ear) was used as vehicle group. The irritant agent (1 mg/ear), dexamethasone (0.1 mg/ear) and *S. corymbiflorum* extract (0.00001–1 mg/ear) were dissolved e applied topically in a constant volume of 20 μL of acetone to the right ear of each animal.

2.3.4. Croton oil-induced ear edema

Acute inflammation model was induced by a single topical application of croton oil at a concentration of 1 mg/ear in the right ear of the mice according to the method describe previously, with some modifications (da Cunha et al., 2001). The *S. corymbiflorum* extract (0.00001–1 mg/ear) or dexamethasone (0.1 mg/ear), used as a positive control, was applied topically immediately before of the croton oil treatment. Ear thickness was measured prior to and 6 h after the induction of inflammation. Six hours after the application of croton oil, the animals were sacrificed and ear samples (circles of tissue 6 mm in diameter) were collected for further analysis.

2.3.5. Myeloperoxidase activity (MPO) assay

MPO is an enzyme found in cells of myeloid origin and has been used as a biochemical marker of polymorphonuclear cells (mainly neutrophil) infiltration to the tissue. MPO activity was determined using an assay described previously (Oliveira et al., 2014), with some modifications. After 6 h of application croton oil, was assessed the MPO enzyme activity in the ear samples. Tissue samples were homogenized with a motor-driven homogenizer in 300 μl of acetate buffer (8 mM, pH 5.4) containing HTAB. The results were expressed as optical density (OD)/mL of the sample.

2.3.6. Histology

Separate groups of mice were used to verify the histological changes in mouse ear 6 h after croton oil administration or croton oil plus treatments. Mice were euthanized and the right ear was removed and fixed in an alfac solution (16:2:1 mixture of ethanol 80%, formaldehyde 40% and acetic acid). Each sample was embedded in paraffin wax, sectioned at 5 μm and stained with hematoxylin–eosin. A representative area was selected for qualitative light microscopic analysis of the inflammatory cellular response with a 20x and 40x objectives (Oliveira et al., 2014). To minimize a source of bias, the investigator did not know the group that they were analyzing.

2.4. Phytochemical analysis

2.4.1. Total polyphenols content

The amount of polyphenol was evaluated by method described by Chandra and Mejia (2004), using the Folin–Ciocalteau reagent. The extract samples were prepared at a concentration of 0.15 mg/mL. Absorbance was measured at 730 nm, in triplicate. Gallic acid was used in the calibration curve. The results were displayed in mg of gallic acid equivalents per g of extract (mg GAE/g).

2.4.2. Total flavonoids content

The flavonoids content was determined by the reaction with aluminum chloride using the method described by Woisky and Salatino (1998). Briefly, $AlCl_3$ solution was added to a aliquot of the sample and after 15 min the absorbance was verified at 420 nm. The data were calculated based on the calibration curve of rutin and expressed in mg of rutin equivalents per g of extract (mg RE/g).

2.4.3. Determination of total alkaloids

Total alkaloids were quantified by reaction of precipitation with Dragendorff's reagent by Sreevidya and Mehrotra (2003). The absorbance was measured at 435 nm and carried out in triplicate. For the results was used a calibration curve of bismuth nitrate which were expressed in mg of total alkaloids per g of extract (mg/g).

2.4.4. HPLC analysis polyphenols

HPLC analysis was performed on a Shimadzu HPLC system (Kyoto, Japan), Prominence Auto-Sampler (SIL-20A), equipped with Shimadzu LC-20 AT reciprocating pumps connected to a DGU 20A5 degasser, CBM 20A integrator, UV–VIS detector DAD SPD-M20A and LC Solution 1.22 SP1 software. Reversed phase chromatographic analyses were carried out under gradient conditions using a C-18 column (250 mm × 4.6 mm) packed with 5 μm diameter particles. The mobile phase comprising of solvent 1 (water containing 2% acetic acid) and Solvent 2 (methanol), according to method of Piana et al. (2013) with modifications.

The flow rate used was 0.6 mL/min and 40 μL of injection

volume. The identification of the phenolics was performed by comparing retention times and the Diode-Array-UV spectra with those of standards. Stock solutions (0.00625–0.250 mg/mL) of chlorogenic, caffeic, rosmarinic and gallic acids and rutin, as well as extract samples of *S. corymbiflorum* were dissolved in the mobile phase. Quantification was carried out by integration of the peaks using the external standard method. All chromatographic operations were performed in triplicate.

2.5. Antioxidant activity

2.5.1. DPPH test

The radical scavenging capacity of the *S. corymbiflorum* extract was evaluated in the presence of 1,1-diphenyl-2-picrylhydrazyl (DPPH) stable radical, according to a method described by Choi et al. (2002). Briefly, the extract samples were tested at 7.81, 15.62, 31.25, 62.50, 125 and 250 μg/mL. Each sample was mixed with 1.0 mL of DPPH 0.3 mM in ethanol solution, after 30 min the absorption was measured at 518 nm. Ascorbic acid and quercetin (positive controls) also was assessed in the same concentrations. The test was performed in triplicate and the calculation of the radical scavenging capacity (%) followed the equation:

Where: Abs_{sample} is absorbance of each fracti

on; Abs_{blank} is absorbance of the samples without adding the DPPH; $Abs_{control}$ is absorbance the solution of ethanol in DPPH.

$$\%\ \text{inhibition} = 100 - \frac{(ABS_{sample} - ABS_{blank}) \times 100}{Abs_{control}} \quad (1)$$

2.5.2. Protein carbonyl content

Human blood samples were employed to obtain the serum used in the protein carbonyls evaluation assays. The experiments were carried out according to the research ethics committee of the Federal University of Santa Maria (Rio Grande do Sul, Brazil), and approved under number 23081.012/2006-94.

The dosages of protein carbonyl were performed, according to Morabito et al. (2004). The extract samples were diluted in PBS buffer in the different concentrations (25, 50, 100 and 200 μg/mL). Human serum and extract diluted in PBS buffer were incubated during at 37 °C in assay tube. After 30 min was added H_2O_2 and incubated for 60 min at same temperature. Then, human serum in the absence or presence of the samples in different dilutions was incubated with 20 mM 2,4-dinitrophenylhydrazine (DNPH) solution for 60 min. The proteins were precipitated from the solution with the use of 20% trichloroacetate and resuspended in 6 M guanidine at 37 °C for 15 min. The content was determined in the absorbance at 366 nm. Ascorbic acid and quercetin were used as positive controls. The assays were assessed in triplicate and results expressed as nmol/g protein.

2.6. Statistical analysis

For phytochemical composition was used a calibration curve, the experimental values were expressed as mean ± S.D. For antioxidant activity values of SC_{50} (scavenging concentration required for to inhibit the DPPH radical or decrease carbonyl protein levels in 50%) were obtained by linear regression and expressed as mean ± SE. In the anti-inflammatory activity the results are presented as mean ± SEM with exception of the ID_{50} values (dose required to reduce the responses of the treated groups by 50% relative to the control group), which are reported as geometric means plus their respective 95% confidence limits. The maximum effect (E_{max}) was calculated based on the response of the control groups. The statistical significance between the groups was assessed by one-way ANOVA followed by a post hoc Newman–Keuls test. The accepted level of significance for the test was $P < 0.05$.

3. Results

3.1. Anti-inflammatory activity

3.1.1. Extract of S. corymbiflorum leaves on croton oil- induced cutaneous inflammation

The anti-inflammatory activity of the extract of *S. corymbiflorum*in in an acute contact dermatitis model induced by croton oil was assessed. A single topical application of croton oil on ear induced an increase of the ear thickness with an E_{max} of 130 ± 9 μm 6 h after the induction, while topical application of the vehicle (acetone) alone did not change ear thickness (Fig. 1 A).

The extract from *S. corymbiflorum* leaves (0.00001 – 1 mg/ear) topically applied resulted in a dose-dependent inhibition of croton oil-induced ear edema, with an ID_{50} value of 0.39 (0.12 – 1.16) μg/ear and a maximum inhibition of 87 ± 3% (at 1 mg/ear). Similar result was found for dexamethasone, used as positive control, which inhibited croton oil-induced ear edema in 89 ± 3% (Fig. 1A).

3.1.2. MPO enzyme activity

MPO is a marker of polymorphonuclear leukocytes and its activity is directly related to the amount of neutrophil infiltration, which is indicative of an inflammatory reaction (Suzuki et al., 1983). In order to verify the effects of the extract on croton oil-induced neutrophil infiltration, MPO activity was assessed. Croton oil caused an increase in MPO activity when compared with the vehicle (acetone), and the application of the extract from *S. corymbiflorum* (0.00001, 0.01 and 1 mg/ear in acetone) promoted a dose-dependent inhibition of MPO activity with a maximum

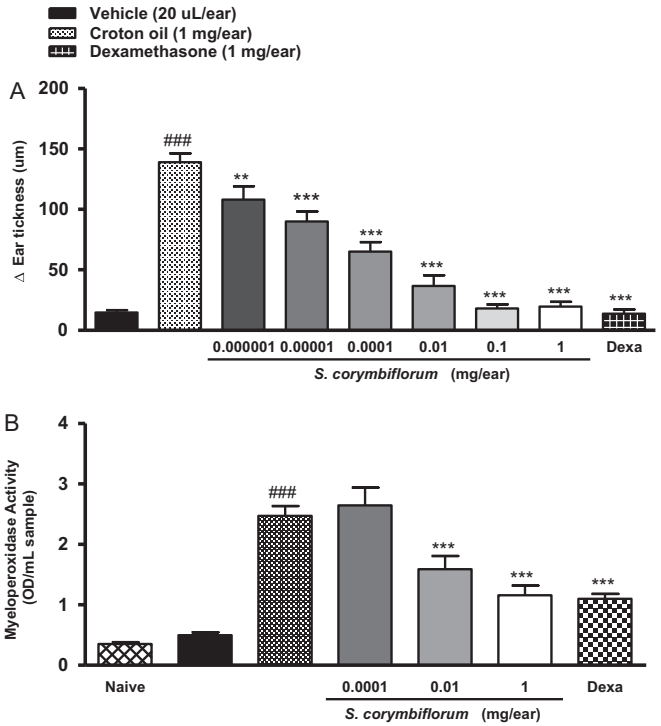

Fig. 1. Effect of the extract of *S. corymbiflorum* leaves and dexamethasone (Dexa) administered topically on inflammatory parameters croton oil-induced. Ear edema (A) and MPO activity (OD/mL sample) (B) of the ear tissue of mice 6 h after croton oil or croton oil plus treatments. Each bar represent the mean + S.E.M ($n = 5–7$); ### $P < 0.05$ when compared with the vehicle (A) and naïve (B) group. ** $P < 0.05$ and *** $P < 0.01$ when compared with the croton oil group (one-way ANOVA followed by post hoc Newman–Keuls test).

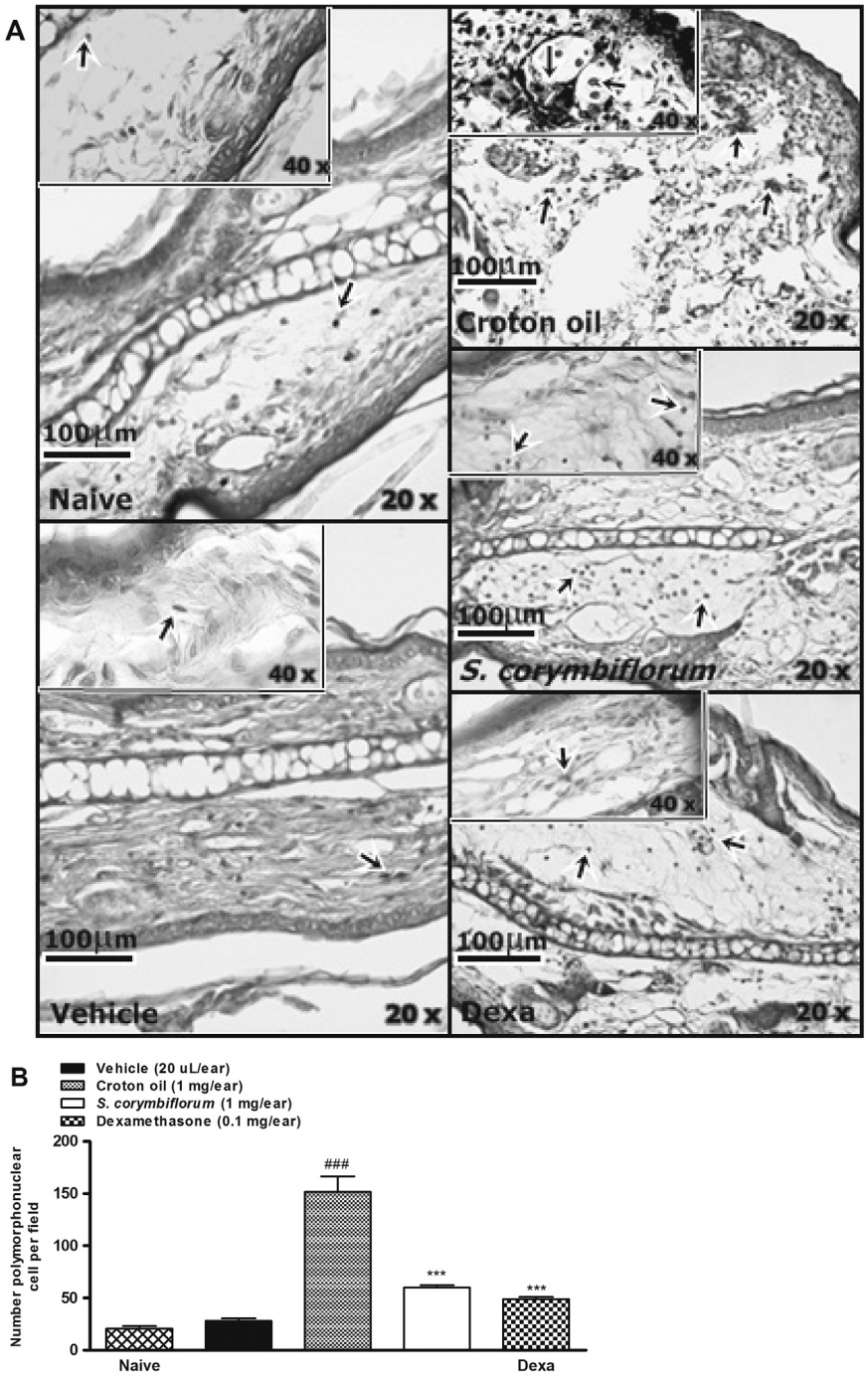

Fig. 2. Effect of the extract of *S. corymbiflorum* leaves and dexamethasone (Dexa) administered topically on cell infiltration croton oil-induced. Ear representative light microphotograph (A arrows indicate polymorphonuclear cells) and quantification of polymorphonuclear cells per field (B) of the ear tissue of mice 6 h after croton oil or croton oil plus treatments. Each bar represent the mean + S.E.M ($n = 5–7$); $^{###}P < 0.05$ when compared with the vehicle group. $^{***}P < 0.01$ when compared with the croton oil group (one-way ANOVA followed by post hoc Newman–Keuls test).

inhibition of 45 ± 7% (1000 μg/ear). Moreover, the positive control dexamethasone also reduced the MPO activity with a maximum inhibition of 50 ± 3% (Fig. 1B).

3.1.3. Histological assessment of ear tissue

Once we observed an increase in the leukocyte infiltration by the measurement of the MPO (marker for neutrophil) enzyme activity, we carried out histological analysis to confirm the cell infiltration. We observed that the single topical application of croton oil (151.5 ± 14.9 polymorphonuclear cells per field) promoted intense leukocyte infiltration when compared with the naïve (20.5 ± 2.5 polymorphonuclear cells per field) or vehicle (28 ± 2.5 polymorphonuclear cells per field) groups. The topical application of *S. corymbiflorum* (1 mg/ear; 60 ± 2.4 polymorphonuclear cells per field) and the positive control, dexamethasone (0.1 mg/ear; 49 ± 2.1 polymorphonuclear cells per field) decreased the cell infiltration when compared to croton oil group (151.5 ± 14.9 polymorphonuclear cells per field) (Fig. 2A and B).

3.2. Phytochemical analysis

The yield of the extract was 7.82% (w/w). The results of the

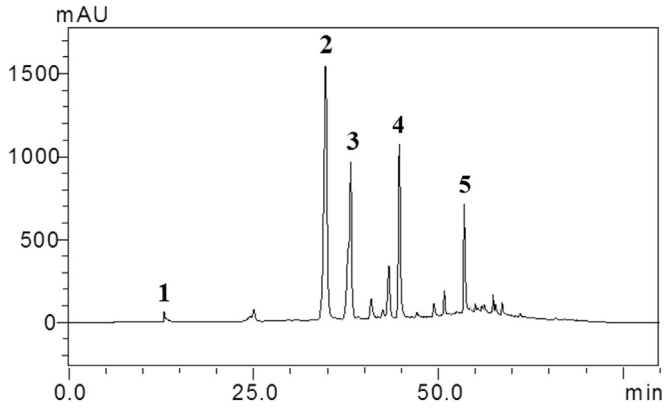

Fig. 3. Chromatogram of the extract of *S. Corymbiflorum* leaves. 1 correspond to gallic acid peak, 2 chlorogenic acid, 3 caffeic acid, 4 rosmarinic acid and 5 rutin.

phytochemical analysis revealed the presence of polyphenols (195.28 ± 0.57 mg GAE/g), flavonoids (69.94 ± 0.36 mg RE/g) and alkaloids (14.44 ± 0.79 mg/g) as active constituents of the extract. HPLC analysis showed high amount of phenolic compounds (Fig. 3) such as chlorogenic acid (59.27 ± 0.83 mg/g), caffeic acid (38.85 ± 0.82 mg/g), rosmarinic acid (40.52 ± 0.67 mg/g), gallic acid (0.77 ± 0.05 mg/g) and rutin (12.72 ± 0.08 mg/g).

3.3. Antioxidant activity

The extract of *S. corymbiflorum* leaves showed good results in the DPPH test which is reported method of antioxidant capacity screening. The SC_{50} value to extract was 23.94 ± 0.33 μg/mL, similar to ascorbic acid and quercetin standards, which present SC_{50} value of 16.57 ± 0.78 μg/mL and 14.79 ± 0.81 μg/mL, respectively.

Another important question evaluated is action of reactive species on proteins, which has been demonstrated by increase of the formation of carbonyl groups (Zadra et al., 2012). Here, we demonstrated that the extract decrease the protein carbonyl levels when incubated with human blood serum (SC_{50}=54.79 ± 0.67 μg/mL). Similarly, quercetin (SC_{50}=84.86 ± 0.76 μg/mL) and ascorbic acid (SC_{50}=38.20 ± 0.94 μg/mL) standards also decrease the protein carbonyl levels. The extract was more potent in reducing the protein carbonyl levels than quercetin but presented a lower potency than ascorbic acid.

4. Discussion

Traditionally, *S. corymbiflorum* leaves are empirically used in folk medicine to treat skin inflammatory disorders. Here, we demonstrated, by first time, the anti-inflammatory activity of the extract of *S. corymbiflorum* leaves, confirming its traditional use in inflammatory process of skin. This anti-inflammatory effect may be related to its antioxidant activity.

The extract of *S. corymbiflorum* leaves was able to reduce the ear edema and the inflammatory cell infiltration in a contact dermatitis model induced by croton oil. These results indicate that the anti-edematogenic activity presented by extract of *S. corymbiflorum* is related to reduction of inflammatory cells infiltration. These results are in according to other studies carried out with other Solanum species which presented anti-inflammatory activity, among them are *Solanum nigrum* (Elango et al., 2012), *Solanum lycopersicum* (Li et al., 2014) and *Solanum trilobatum* (Emmanuel et al., 2006). HPLC analysis demonstrated the presence of four phenolic acids (chlorogenic acid which is present in high amount-59.27 mg/g, rosmarinic acid, caffeic acid and gallic acid all presents in small quantities) and of the flavonoid rutin which is found in considerable amount (12.72 mg/g). Additionally, previous studies showed that chlorogenic acid found in the eggplant of *Solanum aethiopicum* and *Solanum macrocarpon* are correlated with its anti-inflammatory activity (Plazas et al., 2014). Moreover, the chlorogenic acid content was also associated with the inhibition of nitric oxide synthesis, which is a free radical involved in many physiological processes in the human body including inflammatory diseases. Dos Santos et al. (2006) demonstrated that chlorogenic acid presents anti-edematogenic activity probably due the inhibitory action on release of inflammatory mediators such as tumor necrosis factor α and nitric oxide. Furthermore, *S. corymbiflorum* presents high amounts of phenolic compounds and considerable amount of flavonoids, which exhibit a wide range of biological effects, among them the anti-inflammatory activity (García-Lafuente et al., 2014). Addition of phenolic compounds present anti-inflammatory activity, Piana et al. (2013) showed that the flavonoid rutin, also found in the extract of *S. corymbiflorum* leaves, contributed largely in the anti-inflammatory activity of *Viola tricolor* flower.

Several studies have reported that besides polyphenols and flavonoids, the alkaloids are also related to anti-inflammatory activity of species of the same genus as the study of Aboyade et al. (2010) with *Solanum aculeastrum*. So, it is possible that the alkaloids also contribute to anti-inflammatory activity of *S. corymbiflorum* leaves.

The extract inhibited the DPPH radical *in vitro* and decreased the protein carbonyl levels demonstrating the antioxidant potential of this species. However, the result was not the same for the oxidation of DCFH-DA and TBARS assays. It is probable that these different activities were due the distinct mechanisms involved in the steps of the oxidation process (Pacheco et al., 2014).

5. Conclusion

In this study was verified that the extract of *S. corymbiflorum* leaves showed anti-edematogenic and anti-inflammatory activity in a contact dermatitis model croton oil-induced, confirming their popular use for the local treatment of skin inflammatory diseases. These results may be associated to high amount of phenolic compounds as chlorogenic acid and rutin quantified by HPLC.

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References

Aboyade, O.M., et al., 2010. Comparative studies on the anti-inflammatory and analgesic activity of the aqueous extracts from fresh, dried and boiled berries of *Solanum aculeastrum* Dunal. Afr. J. Biotechnol. 9, 3011–3015.

Chandra, S., Mejia, E.G., 2004. Polyphenolic compounds, antioxidant capacity, and quinine reductase activity of an aqueous extract of *Ardisia compressa* in comparison to mate (*Ilex paraguariensis*) and green (*Camellia sinensis*) teas. J. Agric. Food Chem. 52, 3583–3589.

Choi, C.W., et al., 2002. Antioxidant activity and free radical scavenging capacity between Korean medicinal plants and flavonoids by assay-guided comparison. Plant Sci. 163, 1161–1168.

Conforti, F., et al., 2008. In vivo anti-inflammatory and in vitro antioxidant activities of Mediterranean dietary plants. J. Ethnopharmacol. 116, 144–151.

da Cunha, F.M., et al., 2001. Additional evidence for the anti-inflammatory and anti-allergic properties of the sesquiterpene polygodial. Life Sci. 70, 159–169.
Dos Santos, M.D., et al., 2006. Evaluation of the anti-inflammatory, analgesic and antipyretic activities of the natural polyphenol chlorogenic acid. Biol. Pharm. Bull. 29, 2236–2240.
Elango, V., et al., 2012. Anti-inflammatory activity of the flower extracts of *Solanum nigrum* in Rats. Hygeia J. Drugs Med. 4, 59–62.
Emmanuel, S., et al., 2006. Antiinflammatory activity of *Solanum trilobatum*. Fitoterapia 77, 611–612.
García-Lafuente, A., et al., 2014. In vitro anti-inflammatory activity of phenolic rich extracts from white and red common beans. Food Chem. 161, 216–223.
Keller, H.A., Prance, G.T., 2012. Etnobotánica de las especies de *solanum*, subgénero *bassovia*, sección *pachyphylla*1 (solanaceae) de Misiones, Argentina. Bonplandia 21, 45–54.
Krishnaiah, D., et al., 2011. A review of the antioxidant potential of medicinal plant species. Food Bioprod. Process. 89, 217–233.
Li, H., et al., 2014. Bioaccessibility, in vitro antioxidant activities and in vivo anti-inflammatory activities of a purple tomato (*Solanum lycopersicum* L.). Food Chem. 159, 353–360.
Morabito, F., et al., 2004. Lipid peroxidation and protein oxidation in patients affected by Hodgkin's lymphoma. Mediat. Inflamm. 13, 381–383.
Nijveldt, R.J., et al., 2001. Flavonoids: a review of probable mechanisms of action and potential applications. Am. J. Clin. Nutr. 74, 418–425.
Oliveira, S.M., et al., 2014. Antinociceptive effect of 3-(4-fluorophenyl)-5-trifluoromethyl-1H-1-tosylpyrazole. A *Celecoxib* structural analog in models of pathological pain. Pharmacol. Biochem. Behav. 124, 396–404.
Pacheco, N.R., et al., 2014. *Cecropia pachystachya*: A species with expressive in vivo topical anti-Inflammatory and in vitro antioxidant effects. Biomed. Res. Int. http://dx.doi.org/10.1155/2014/301294
Piana, M., et al., 2013. Antiinflammatory effects of *Viola tricolor* gel in a model of sunburn in rats and the gel stability study. J. Ethnopharmacol. 150, 458–465.
Plazas, M., et al., 2014. Reducing Capacity, chlorogenic acid content and biological activity in a collection of scarlet (*Solanum aethiopicum*) and gboma (*S. macrocarpon*) eggplants. Int. J. Mol. Sci. 15, 17221–17241.
Silva, C.R., et al., 2011. The involvement of TRPA1 channel activation in the inflammatory response evoked by topical application of cinnamaldehyde to mice. Life Sci. 88, 1077–1087.
Soares, E.L.C., Mentz, L.A., 2006. As espécies de *solanum* subgênero *bassovia* seção *pachyphylla* (= *cyphomandra* mart. ex sendtn.-solanaceae) no Rio Grande do Sul, Brasil. Pesqui. Botânica 57, 231–254.
Sreevidya, N., Mehrotra, S., 2003. Spectrophotometric method for estimation of alkaloids precipitable with Dragendorff's reagent in plant materials. J. AOAC Int. 86, 1124–1127.
Suzuki, K., et al., 1983. Assay method for myeloperoxidase in human polymorphonuclear leuko- cytes. Anal Biochem 132, 345–352.
Woisky, R.G., Salatino, A., 1998. Analysis of propolis: Some parameters and procedures for chemical quality control. J. Apic. Res. 37, 99–105.
Zadra, M., et al., 2012. Antioxidant activity and phytochemical composition of the leaves of *Solanum guaraniticum* A. St.-Hil. Molecules 17, 12560–12574.
Zimmermann, M., 1983. Ethical guide lines for investigations of experimental pain in conscious animals. Pain 16, 109–110.