



HEALTH SCIENCES

Evaluation of the anti-inflammatory potential of *Solidago microglossa* (Arnica-brasileira) *in vivo* and its effects on PPAR γ activity

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Abstract: *Solidago microglossa* is used as an anti-inflammatory agent in traditional Brazilian medicine, and this work evaluated the anti-inflammatory potential of the crude ethanolic extract of the flowers of *S. microglossa in vivo*, as assayed by paw edema models induced by carrageenan, prostaglandin E₂, bradykinin and compound 48/80. In the chemical profile, we identified compounds by electrospray ionization mass spectrometry and quantified them by HPLC-DAD. Additionally, this study analyzed the potential to activate the *in vitro* transcriptional activity of PPAR γ , which is a nuclear receptor linked to the anti-inflammatory response. It was possible to identify five compounds: quinic acid, quercetin, chlorogenic acid, hyperoside, and rutin. In the paw edema evaluation, it was possible to show the potential of reducing edema during the inflammatory process. The crude ethanolic extract of the flowers of *S. microglossa* activated PPAR γ compared to the full agonist rosiglitazone and in a dose-response manner. It is possible to conclude that the extract of the flowers of *S. microglossa* showed anti-inflammatory activity, and the phenolic compounds present in this species might be responsible for this activity.

Key Words: natural products, popular use, Asteraceae, inflammation, chemical profile.

INTRODUCTION

Solidago microglossa is a plant native to South America, and its botanical synonym is *Solidago chilensis*, which is used in traditional Brazilian medicine and appears in the list of Relação Nacional de Plantas Medicinais de Interesse ao Sistema Único de Saúde (Renuis). Infusions and macerates of the rhizomes, leaves or flowers of *S. microglossa* are used for the treatment of gastric disorders, intestinal ulcers, healing, rheumatism, and injuries as an

anti-inflammatory drug, popularly known as Brazilian Arnica (Goulart et al. 2007, Bieski et al. 2015). *Solidago* is the largest representative of the Asteraceae family, containing approximately 120 species distributed throughout the world. This genus is widely used in traditional medicine by many countries, particularly for the treatment of inflammatory and diuretic problems (Blumenthal et al. 1998). Phytochemical studies have identified the presence of monoterpenes, sesquiterpenes, sesquiterpene lactones,

triterpenes, steroids, saponins, flavonoids, and cinnamic acid derivatives (Souza et al. 2017).

In addition to being used in traditional medicine, especially in developing countries, natural products may be the basis for the study and development of new drugs. Between 1981 and 2014, 4% of newly released drugs were isolated from natural products, while 27% were synthetic compounds. Observing the source of other products, 59.1% were semisynthetic molecules, which are synthetic drugs with pharmacophoric groups based on the structures of natural products or structures that mimic natural products or botanical products (Newman & Cragg 2016). These data demonstrate the importance of natural product studies for the safety of communities that use these plants as well as for the development of new medicines.

Bearing this in mind, we evaluated the anti-inflammatory potential of the crude ethanolic extract of *S. microglossa* flowers *in vivo* using paw edema models induced by carrageenan, prostaglandin E2 and compound 48/80 and identified the compounds present by electrospray ionization mass spectrometry (ESI-MS/MS). To understand the molecular mechanism of the anti-inflammatory effects of the *S. microglossa* flower extract, we also investigated its effects on the transcriptional activity of PPAR γ , a nuclear receptor linked to the anti-inflammatory response.

MATERIALS AND METHODS

Plant material and preparation of the crude extract

S. microglossa (SM) was collected in the city of Nova Friburgo (22°19'54" S; 42°32'28" W) in January 2015. The specimen was identified and deposited in the herbarium of the

Instituto de Biologia da Universidade Estadual de Campinas, deposit number: UEC

177791. This study was registered in the Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado – SISGEN (no A3B47E6). The plant was stabilized and dried in a circulating air oven at a temperature of 40 °C, and the leaves were separated from the flowers. The flowers were ground and subjected to maceration in 99% ethanol with a powder/solvent ratio of 1:5 (mass/volume), and the solvent was changed every 24 h. The extract was dried in a rotary evaporator and stored in an amber bottle at a temperature between 2 and 8 °C.

Analysis of the chemical profile of the extract by ESI-MS/MS

Preliminary analysis of the extract of the plant species was performed by direct infusion in a TQD Acquity mass spectrometer (Micromass-Waters Manchester, England) using an ESI source in negative ionization mode. Samples (1 mg/ml) were dissolved in a solution containing 50/50 (v/v) chromatographic grade methanol (Merck, Darmstadt, Germany) and 0.5% ammonium hydroxide in water. The instrument parameters were as follows: source temperature of 100 °C, capillary voltage of 3.0 kV and a 30 V cone voltage, and a collision energy of 30 V. The constituents were identified by comparison of their ESI-MS/MS fragmentation spectra with commercial standards (quercetin (Sigma-Aldrich® $\geq 95\%$), quinic acid (Sigma-Aldrich®) and chlorogenic acid (Sigma-Aldrich® $\geq 95\%$)). All compounds were compared with data from the database www.massbank.eu.

HPLC-DAD quantification

Quantification of the main components of the extract were performed on an Agilent HPLC Infinity 1260 equipped with a quaternary gradient pump, automatic injector, temperature control column oven and diode array detector

(DAD). For HPLC analysis, an Inertsil® ODS-3 column, 15 cm in length with a 4.6 mm internal diameter and 5 µm particles was used. Analyses were performed at 30 °C, a flow rate of 0.8 mL/min, and an injection volume of 20 µL, and chromatograms were obtained at 326, 354, and 372 nm. The solvents used were 1% acetic acid in water (solvent A) and acetonitrile (solvent B) with the following gradient program: 0-5 min (20% B), 5-15 min (20-40% B), 15-25 min (40-80% B), 25-26 min (80-100% B), 26-30 min (100% B) 30-31 min (100-20% B) and 31-35 min (20% B). The extract was dissolved in methanol and water 80:20 v/v to obtain a 10 mg/mL dilution. This solution was cleaned with a C-18 solid phase extraction cartridge (SPE) previously activated with methanol and conditioned with methanol and water 80:20 (v/v). This sample was eluted and dissolved to obtain a 4 mg/mL dilution for quercetin quantification and a 0.4 mg/mL dilution for chlorogenic acid and rutin quantification. Samples were prepared in 6 replicates. Chlorogenic acid (≥ 98%, Sigma-Aldrich), quercetin (≥ 98%, Sigma-Aldrich) and rutin (≥ 98%, Sigma-Aldrich) standards were prepared at concentrations of 100 µg/mL in methanol. Then, chlorogenic acid and quercetin dilutions of 2, 4, 8, 12 and 16 µg/mL in methanol were obtained, while rutin dilutions in methanol were 5, 10, 15, 20 and 25 µg/mL. The patterns were analyzed in triplicate by HPLC, and from the peak area results, the respective analytical curves were obtained.

Animals

Adult female Swiss mice, 25-30 g, from the Núcleo de Animais de Laboratório of the Universidade Federal Fluminense (NAL-UFF) were used and kept in the Laboratório Multiusário de Pesquisa Biomédica de Nova Friburgo. The study was approved by the Committee of Ethics in Animal Experimentation of Universidade Federal

Fluminense (UFF) (no 925), according to the protocols described below.

Paw edema induced by carrageenan

Anti-inflammatory activity was determined using the carrageenan-induced hind paw edema method. Swiss mice were divided into four groups ($n = 7$ per group) and treated *per os* with vehicle (saline solution 0.9% + 1% Tween 80, negative control), indomethacin (10 mg/kg) or the extract of *S. microglossa* at doses of 125 and 250 mg/kg. After 30 min, 300 µg of carrageenan solution (a mixture of κ and λ carrageenans) (30 µL) was injected into the hind paw of each animal. Paw volumes were measured with digital caliper 0, 1, 2, 3 and 4 h after carrageenan administration. The results are expressed as the percent of paw thickness (Winter et al. 1962).

Paw edema induced by prostaglandin E₂, bradykinin and compound 48/80

Swiss mice were divided into four groups ($n = 8$ per group) and treated *per os* with vehicle (saline solution 0.9% + 1% Tween 80, negative control), reference drug or extract of *S. microglossa* at doses of 125 and 250 mg/kg. Thirty minutes later, edema was induced by intraplantar injection (right hind paw, 30 µL) of the inflammatory compounds, and the volume of the paws was measured before and 15, 30, 60 and 90 min after inflammation induction. Inflammatory inducers and reference drugs, when used, were as follows: PGE₂ (20 µg/paw, indomethacin 10 mg/kg), bradykinin (50 µg/paw, dexamethasone 1 mg/kg), compound 48/80 (10 µg/paw, positive control cyproheptadine, 4 mg/kg) (Vendramini-Costa et al. 2015). The paw thickness was measured with a digital caliper, and the results are expressed as the percent of paw thickness.

Transactivation and Reporter Gene Assay

Human cervical cancer HeLa cells (4×10^4 cells/well, in a 48-well plate), from the Cell Bank of Rio de Janeiro, Brazil, were cotransfected with expression vectors containing cDNA for the human PPAR γ receptor (pCMV-SPORT6-PPAR γ) and its responsive element fused to the luciferase reporter gene, which was kindly provided by Dr. Paul Webb from the Methodist Research Institute, TX, United States. Transfections were performed with Lipofectamine (Lipofectamine 2000 Transfection Reagent) according to the manufacturer's instructions. Cells were exposed for 24 h to vehicle (DMSO) or to the *S. microglossa* extract at concentrations ranging from 0.05 to 0.20 mg/ml. Luciferase activity was measured in a luminometer with a luciferase reporter assay kit (Promega) according to the manufacturer's instructions. The results were reported as luciferase activity induced by the extract of *S. microglossa* relative to the vehicle (DMSO). The experiment was performed in triplicate and repeated at least three times (Milton et al. 2017).

Statistical analyses

GraphPad Prism 5[®] software and R statistical program were used for the statistical analyses. The results are shown as the mean \pm SEM. In the experiments performed for the evaluation of paw edema induction, each group was analyzed by columns for each time point, and the analyses were performed by two-way ANOVA followed by Tukey's multiple comparisons of means 95% familywise confidence level. In the experiments performed for the evaluation of PPAR γ activation, the analyses were performed at a fixed time of treatment using one-way ANOVA followed by Tukey's *post hoc* test. Values of *p* less than 0.05 were considered significant.

RESULTS

Analysis of the chemical profile of the extract and fractions by ESI-MS/MS and HPLC-DAD

The extract of *S. microglossa* was analyzed by ESI-MS/MS in negative ion mode. Thus, by comparing its MS/MS fragments with data from the literature and a database, it was possible to identify five compounds: quinic acid, quercetin, chlorogenic acid, hyperoside, and rutin (Figures 1, 2 and 3). The spectra and fragmentation profiles of the identified molecules are described in Table I. In the HPLC-DAD analyses, it was possible to detect and quantify chlorogenic acid, quercetin, and rutin. The signals at 4.14 min, 7.50 min and 17.23 min showed appropriate resolution and were identified as chlorogenic acid, quercetin, and rutin, respectively (Figure 4). The results show that the methodology developed for HPLC-DAD quantification presented selectivity for the analyzed components.

The analytical curves (Figure 5) obtained from the results of 3 replicates for each point showed linearity, with determination coefficients (R^2) greater than 0.99 for the three substances. The relative standard deviations for each point on each curve were all below 2%. Area data (mAU) for curve preparation were obtained from chromatograms with detection at the most sensitive wavelengths for each substance, namely 326, 354 and 372 nm for chlorogenic acid, quercetin, and rutin, respectively. The results for the linearity analyses are summarized in Table II.

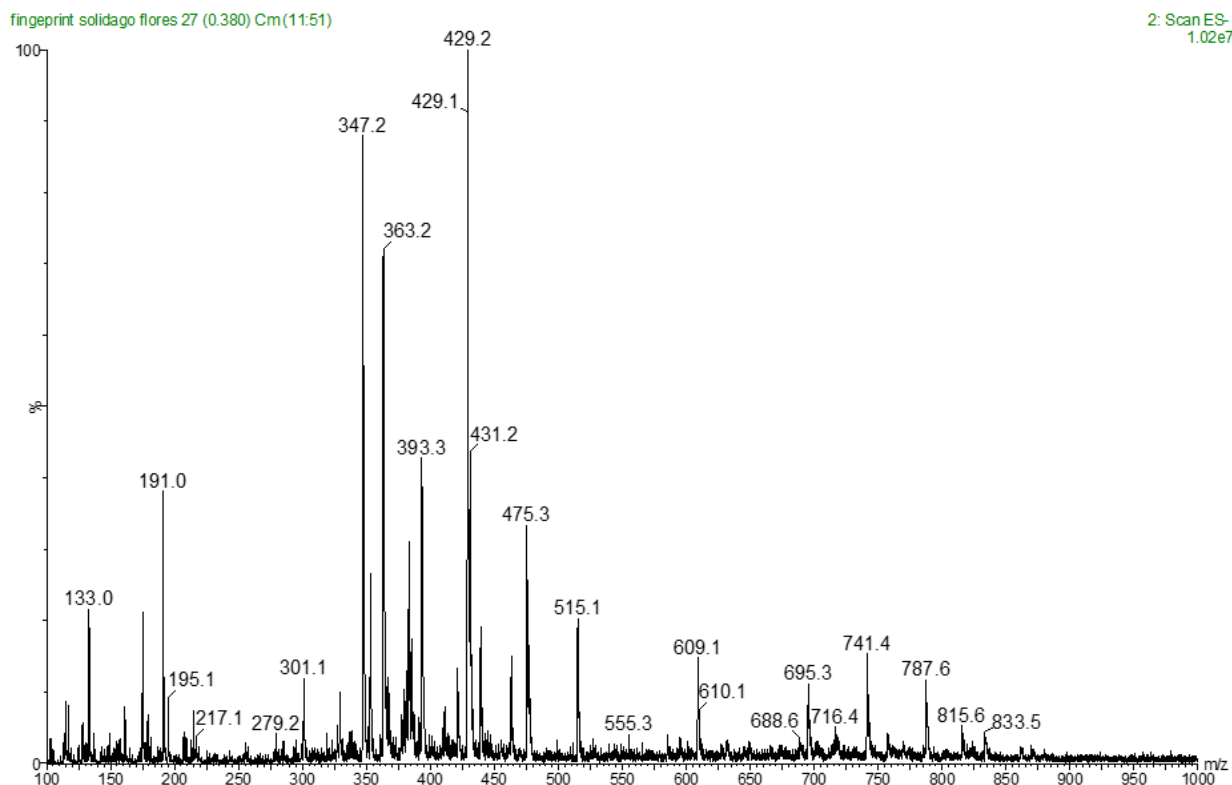
The quantification of the investigated components showed a relative standard deviation of 5.8% for chlorogenic acid, 5.7% for quercetin and 4.2% for rutin. These values can be considered suitable for the analysis of chemical markers in complex matrices. The concentration found for each substance was 7.3 mg chlorogenic acid per g extract, 3.4 mg quercetin per g stratum and 47.8 mg rutin per g extract (Table III).

Table I. Identified substances in the ethanolic extract of *S. microglossa* flowers by comparison of the ESI(-)-MS/MS spectrum with the database www.massbank.eu.

[M-H] ⁻ m/z precursor ion		m/z product ions
Substance		
Quinic acid	191	191 → 127, 93, 85
Quercetin	301	301 → 179, 151, 121, 107
Chlorogenic acid	353	353 → 191, 179, 135
Hyperoside	463	463 → 300
Rutin	609	609 → 300

Table II. Linearity data for chlorogenic acid, quercetin, and rutin.

Compound	Linear Regression	R ²	λ (nm)
Chlorogenic Acid	y = 18.80x + 97.64	0.998	326
Quercetin	y = 78.89x + 343.9	0.996	372
Rutin	y = 11.70x + 12.29	0.994	354

**Figure 1.** ESI(-)-MS spectrum of *S. microglossa* flowers.

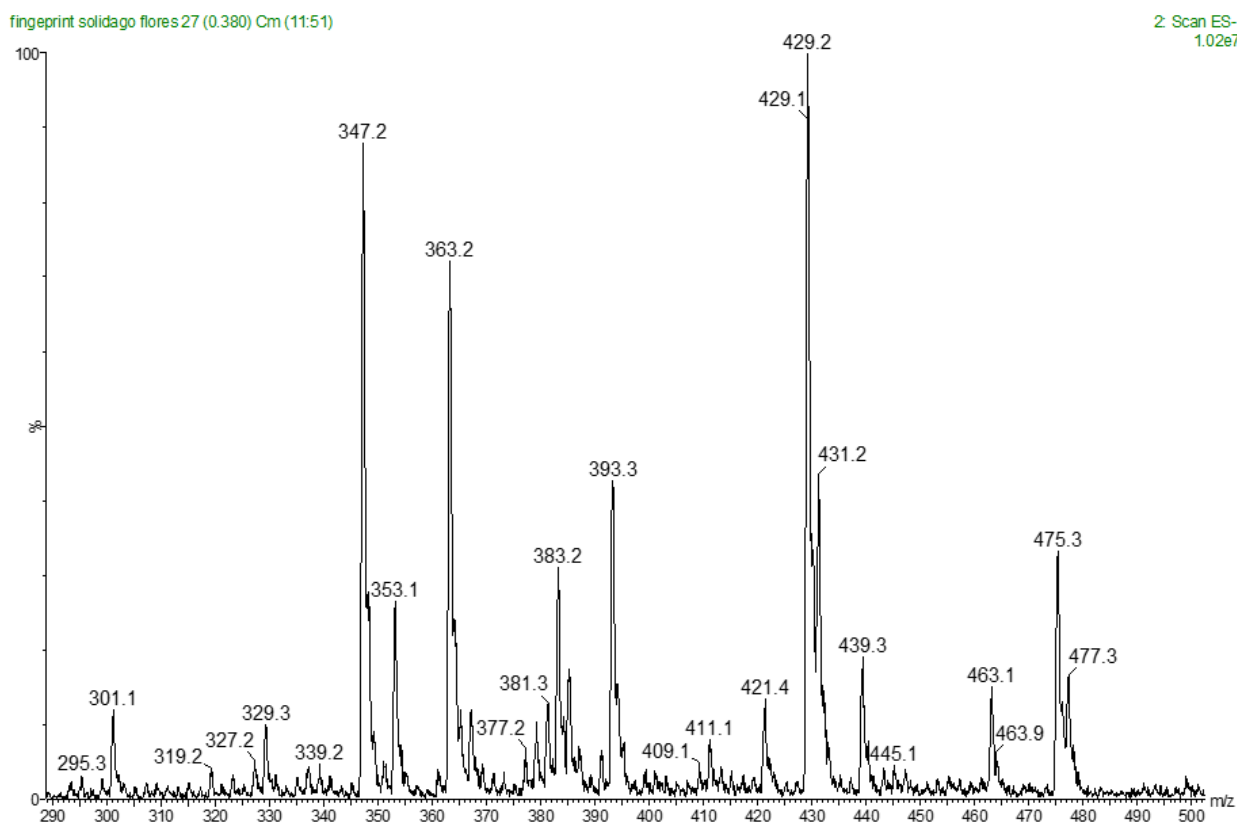


Figure 2. Enlargement of the ESI(-)-MS spectrum of *S. microglossa* flowers in the mass range of 300 to 500 *m/z*.

Table III. Data for the quantification of chlorogenic acid, quercetin and rutin in the *S. microglossa* extract: average area, relative standard deviation (DPR) and concentration of the substance in the extract.

Compound	Mean Area ¹ (mAU*s)	DPR (%)	Concentration in extract (mg/g)
Chlorogenic Acid	152.8	5.8	7.3
Quercetin	1425.3	5.7	3.4
Rutin	236.1	4.2	47.8

¹Mean, n=6.

Carrageenan-induced paw edema

For data analysis of the experiments with animals, the R statistical program was used. The results of the paw edema measurements were submitted to a two-way analysis of variance (ANOVA) (compound and time). Tukey's *post hoc* test was used to identify specific differences in the variables for which the *p* values found were higher than the established statistical significance criterion (*p* < 0.05).

According to Figure 6, the paw thickness percentiles increased with carrageenan treatment, ranging from 26.98% at the first hour to 40.86% at the fourth hour, while the negative control varied from 38.42% to 80.56% after 4 h of the experiment. The group treated with the reference drug ranged from 28.75 to 36.40% after 4 h of the experiment. Two-way ANOVA (compound and time) showed significant effects from time factors [*F* = 4,769; *p* < 0.01]

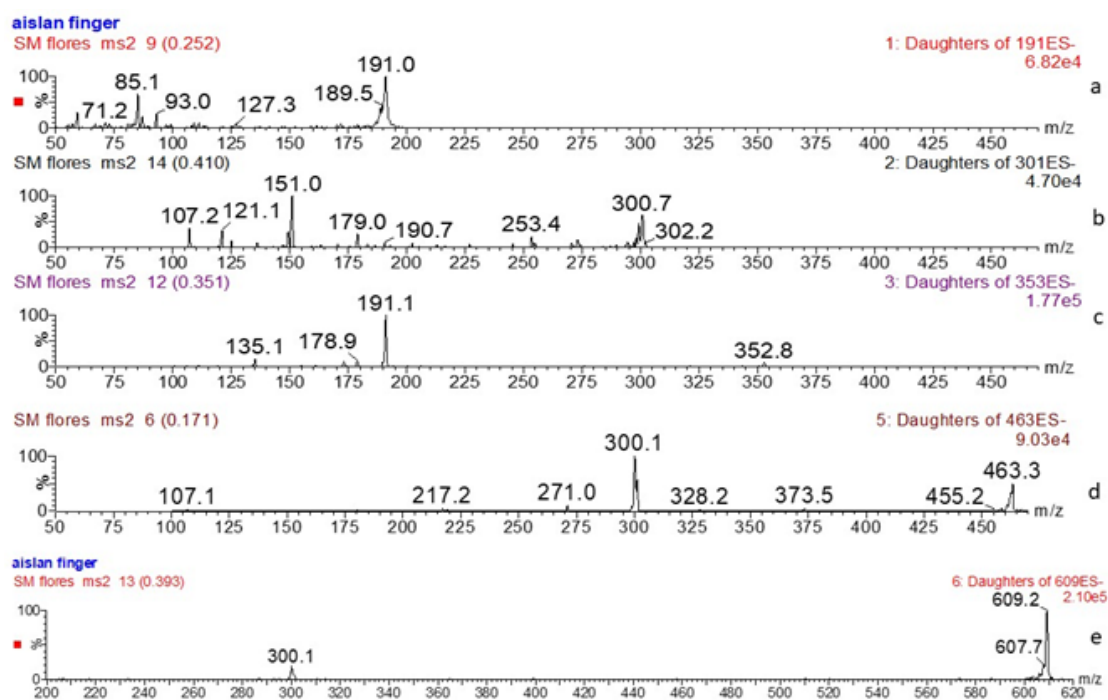


Figure 3. ESI(-)-MS/MS spectra of the compounds quinic acid (a), quercetin (b), chlorogenic acid (c), hyperoside (d) and rutin (e).

and compound factors [$F = 27,736$; $p < 0.001$], in addition to the interaction of compound and time factors [$F = 2,606$; $p < 0.01$] on the formation of carrageenan-induced paw edema.

Post hoc analysis showed that all compounds were more efficient in reducing paw edema relative to saline ($p < 0.001$). Indomethacin was more effective in reducing paw edema compared to *S. microglossa* 125 mg ($p < 0.01$). *S. microglossa* 250 mg was more effective than *S. microglossa* 125 mg ($p < 0.01$). There was no significant difference between *S. microglossa* 250 mg and indomethacin in reducing carrageenan-induced paw edema ($p > 0.1$).

Prostaglandin E₂-, bradykinin- and compound 48/80-induced paw inflammation

In the analysis of Prostaglandin E₂ (PGE₂)-induced paw inflammation, the increase in the thickness of the paw by the vehicle ranged from 53.84% to 7.14%, between 15 and 90 min.

Indomethacin-treated paws ranged between a 34.47% and 3.95% increase. Two-way ANOVA (compound and time) showed significant effects from time factors [$F = 131,164$; $p < 0.001$] and compound factors [$F = 24,391$; $p < 0.001$], in addition to the interaction of compound and time factors [$F = 3,602$; $p < 0.001$] on the formation of prostaglandin-induced paw edema. *Post hoc* analysis showed that only indomethacin was more efficient in reducing paw edema than saline ($p < 0.001$). There was no significant difference between animals treated with *S. microglossa* and saline in reducing prostaglandin-induced paw edema ($p > 0.1$) (Figure 7).

When the inflammatory process was induced by injecting bradykinin, only the group treated with dexamethasone presented a significant difference when compared with the vehicle (Figure 8).

In the assay where compound 48/80 was used, 4 mg/kg cyproheptadine was used as a

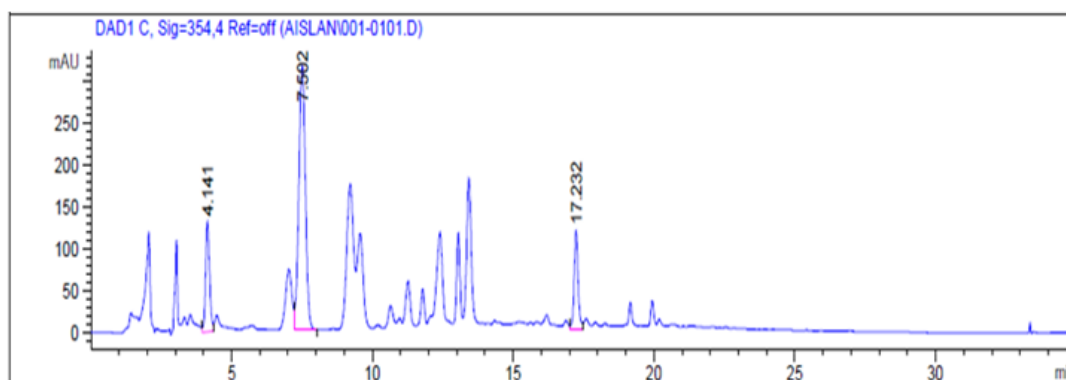


Figure 4. HPLC-DAD chromatogram of *S. microglossa* extract. Temperature 30 °C; flow rate 0.8 mL/min; 20 μ L injection volume; and detection at 354 nm. Gradient program of 1% acetic acid in water (solvent A) and acetonitrile (solvent B): 0-5 min (20% B), 5-15 min (20-40% B), 15-25 min (40-80% B), 25-26 min (80-100% B), 26-30 min (100% B) 30-31 min (100-20% B) and 31-35 min (20% B).

reference drug. Two-way ANOVA (compound and time) showed significant effects from time factors [$F = 51.12$; $p < 0.001$] and compound factors [$F = 75.39$; $p < 0.001$], in addition to the interaction of compound and time factors [$F = 35.71$; $p < 0.001$] on the formation of compound 48/80-induced paw edema. *Post hoc* analysis showed that all compounds were effective in reducing paw edema relative to saline ($p < 0.001$). *S. microglossa* at 250 mg/kg was more effective than *S. microglossa* at 125 mg ($p < 0.01$). Cyproheptadine was shown to have a more effective initial effect than *S. microglossa* 250 mg at time points 1 and 2 ($p < 0.001$), however *S. microglossa* 250 mg was shown to have a more efficient late effect than cyproheptadine at time point 4 ($p < 0.05$) (Figure 9).

Effects of *S. microglossa* on PPAR γ transcriptional activity

The extract of *S. microglossa* was able to increase the transcriptional activity of PPAR γ in a dose-dependent manner, indicating that it can have one or more compounds with agonist activity. The activation promoted by *S. microglossa* was 1.6 ± 0.25 -fold at 0.05 mg/ml, 2.9 ± 1.6 -fold at 0.075 mg/ml, 3.2 ± 1.2 -fold at 0.10 mg/ml, 4.3 ± 1.6 -fold at 0.15 mg/ml, and 4.5 ± 1.9 -fold at 0.2 mg/ml.

The EC₅₀ value was 0.075 mg/mL (Figure 10a). The highest activation promoted by *S. microglossa* was approximately 50% of that induced by rosiglitazone, a known agonist of PPAR γ , which increased the transcriptional activity 9.2 ± 1.4 -fold at 10^{-5} M (Figure 10b).

DISCUSSION

Natural products have been recognized as remedies since ancient times and have long been used as folk medicines for inflammatory conditions such as fevers, pain, migraines, and arthritis (Yuan et al. 2006). *S. microglossa* is present in the first edition of the Brazilian Pharmacopeia (Brazilian Pharmacopeia 1929, *apud* Liz et al. 2008) and is popularly used by the Brazilian population in substitution with *Arnica montana* as a diuretic, analgesic and anti-inflammatory agent to treat burns and rheumatic disease among other ailments (Mors et al. 2000, Lorenzi & Matos 2002).

The Ministério da Saúde Brasileiro initiated a Política Nacional de Práticas Integrativas e Complementares no SUS (MS 2018) to guarantee the safe access and rational use of medicinal and phytotherapeutic plants for the

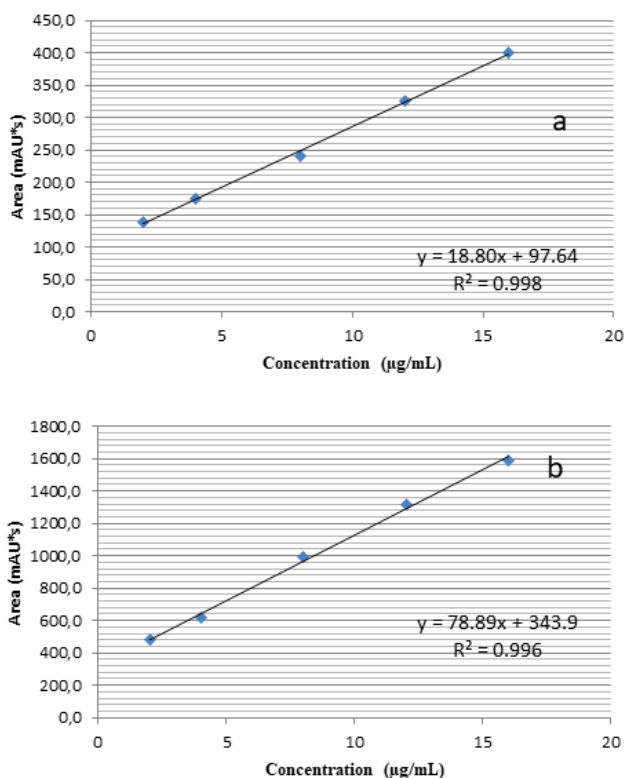
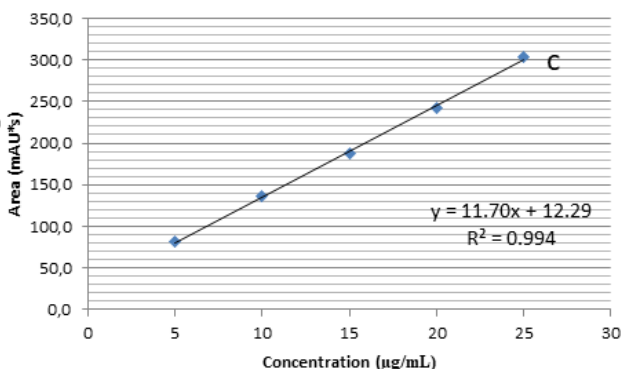


Figure 5. Analytical curves obtained for chlorogenic acid (a), quercetin (b) and rutin (c).



Brazilian population, as well as promoting the sustainable use of biodiversity and developing the productive chain and the national industry. This program encourages the study of several medicinal plants, including *S. microglossa*. Some studies have been conducted in recent years to confirm the popular use of Brazilian Arnica, but these works have focused on the leaves of the species. Our study was conducted with the flowers of *S. microglossa*, showing that the macerate containing the flowers can be effective in treating inflammation.

The analysis of the chemical profile with electrospray ionization of the samples in electrospray ionization mass spectrometry (ESI-MS/MS) is known as its *fingerprint* and has been used in the analysis of complex matrices, as well as in extracts of plant origin. By this method, it is possible to identify previously described compounds (Moller et al. 2007, Roesler et al. 2007). Flavonoids (quercetrin, quercetin, rutin)

and terpenes (pumiloxide, solidagenone, limonene, germacrene D, and γ -cadinene) have been described in this plant (Gastaldi et al. 2018). Some of these compounds have been found in this extract by ESI-MS/MS, corroborating data from the literature.

Baggio and colleagues conducted a study with *S. microglossa* grown in South Brazil and found the presence of the classes of saponins, flavonoids, tannins and alkaloids (Baggio et al. 2012). Another study on the leaves of *S. microglossa* from South Brazil demonstrated the capacity to inhibit reactive oxygen species and lipid peroxidation, and it was possible to identify the phenolic compounds rutin, quercetin, quercitrin and gallic acid by HPLC-DAD (Sabir et al. 2012). These compounds coincide with those described in the *S. microglossa* flowers collected from this study. However, studies on the toxicity and safety of these species are still scarce (Smolarek et al. 2009).

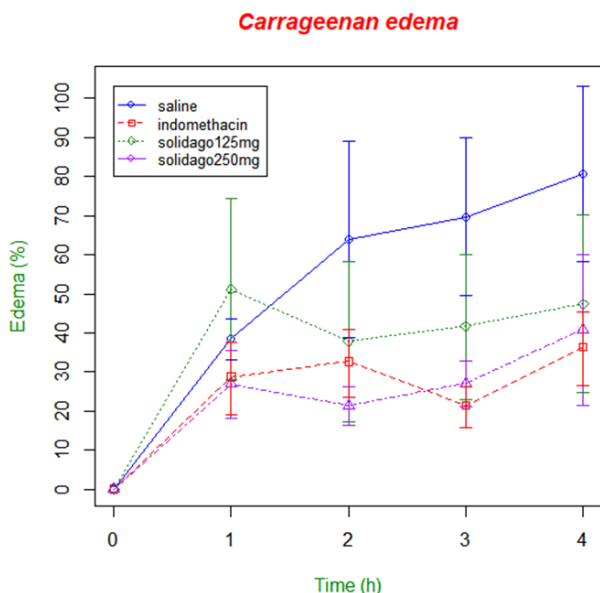


Figure 6. Variation in paw thickness percent 1 to 4 h after carrageenan administration. Animals were pretreated with vehicle (saline solution + 1% Tween 80, 10 ml/kg), indomethacin 10 mg/kg, *S. microglossa* extract 125 mg/kg, or *S. microglossa* extract 250 mg/kg 30 min before intraplantar injection of carrageenan (300 µg/paw). Edema was measured with a digital caliper at each time point marked. N=7 for all groups. Statistical analyses were performed between the treated groups and the group treated only with the vehicle at certain times (1 h, 2 h, 3 h, and 4 h). Analysis of variance (two-way ANOVA) followed by Tukey's multiple comparison of the means at the 95% familywise confidence level.

Some studies have shown the anti-inflammatory potential of the compounds that were identified in *S. microglossa*. Chlorogenic acid has anti-inflammatory effects in lipopolysaccharide-stimulated RAW 264.7 cells. This compound significantly inhibited NO production and the expression of COX-2 and iNOS without any cytotoxicity. Chlorogenic acid also attenuated proinflammatory cytokines (including IL-1 β and TNF- α) and other inflammation-related markers, such as IL-6, in a dose-dependent manner. Additionally, endotoxin-induced adhesion of macrophages and the expression level of ninjurin1 (Ninj1)

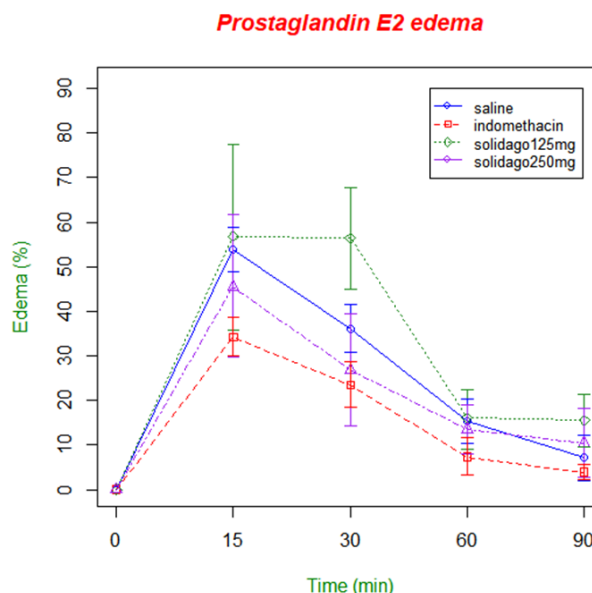


Figure 7. Variation in paw thickness percent 15 to 90 min after PGE2 administration. Animals were pretreated with vehicle (saline solution + 1% Tween 80, 10 ml/kg), indomethacin 10 mg/kg, *S. microglossa* extract 125 mg/kg or *S. microglossa* extract 250 mg/kg 30 min before intraplantar injection of PGE2 (20 µg/paw). Edema was measured with a digital caliper at each time point marked. N=8 for all groups. Statistical analyses were performed between the treated groups and the group treated only with the vehicle at certain times (15 min, 30 min, 60 min, and 90 min). Analysis of variance (two-way ANOVA) followed by Tukey's multiple comparison of the means at the 95% familywise confidence level.

decreased after chlorogenic acid treatment. Finally, chlorogenic acid inhibited the nuclear translocation of NF- κ B (Hwang et al. 2014).

Quercetin, one of the most studied flavonoids in recent years for different pathologies, is found in many fruits and vegetables. In a recent review, the action of quercetin on inflammation was described using *in vitro* cell line assays, which showed that quercetin inhibits lipopolysaccharide (LPS)-induced tumor necrosis factor α (TNF- α) production in macrophages and LPS-induced IL-8 production in lung A549 cells. In glial cells, it was shown that quercetin could inhibit the

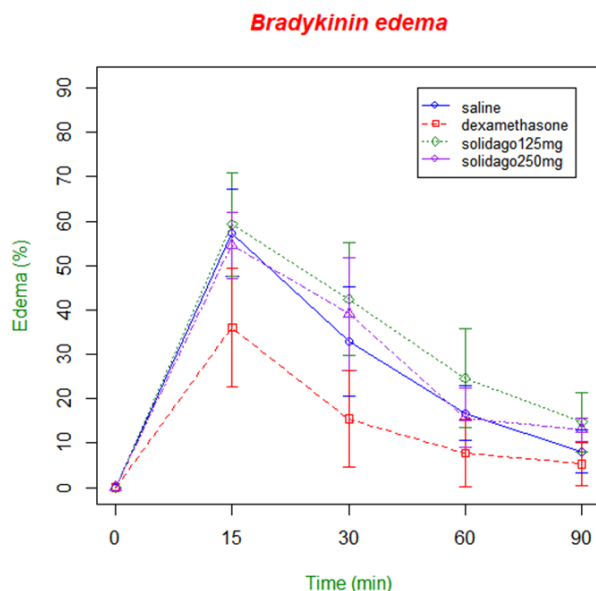


Figure 8. Variation in paw thickness percent 15 to 90 min after bradykinin administration. Animals were pretreated with vehicle (saline solution + 1% Tween 80, 10 ml/kg), dexamethasone 1 mg/kg, *S. microglossa* 125 mg/kg or *S. microglossa* 250 mg/kg 30 min before intraplantar injection of bradykinin (10 µg/paw). Edema was measured with a digital caliper at each time point marked. N=8 for all groups. Statistical analyses were performed between the treated groups and the group treated only with the vehicle at certain times (15 min, 30 min, 60 min, and 90 min). Analysis of variance (two-way ANOVA) followed by the Tukey's multiple comparison of the means at the 95% familywise confidence level.

LPS-induced mRNA level changes of TNF- α and interleukin (IL)-1 α . The effects of quercetin resulted in diminished apoptotic neuronal cell death induced by microglial activation. Quercetin inhibits the production of inflammation-producing enzymes (cyclooxygenase (COX) and lipoxygenase (LOX)) (Li et al. 2016).

Rutin and quercetin inhibited the acute and chronic phases of an experimental model of inflammation in rats (Guardia et al. 2001). Thus, evaluating the data described in the literature, we can recognize that phenolic compounds have been intensely studied for their pharmacological potential, so the compounds identified in the

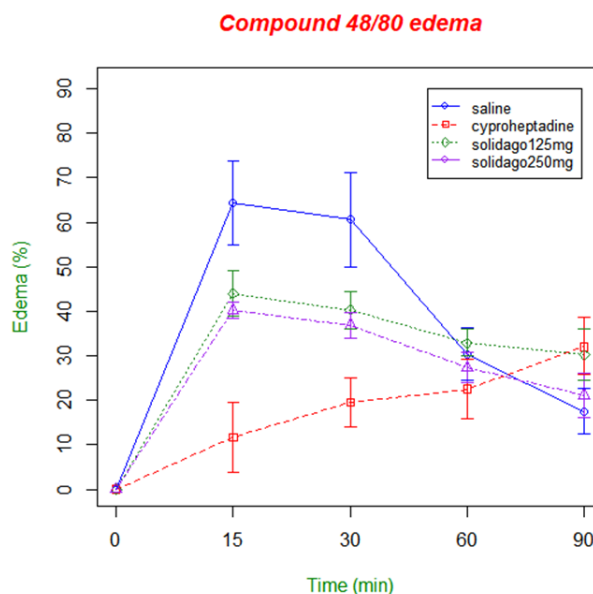


Figure 9. Variation in paw thickness percent 15 to 90 min after compound 48/80 administration. Animals were pretreated with vehicle (saline solution + 1% tween 80, 10 ml/kg), cyproheptadine 4 mg/kg, *S. microglossa* 125 mg/kg or *S. microglossa* 250 mg/kg 30 min before intraplantar injection of compound 48/80 (50 µg/paw). Edema was measured with a digital caliper at each time point marked. N=8 for all groups. Statistical analyses were performed between the treated groups and the group treated only with the vehicle at certain times (15 min, 30 min, 60 min, and 90 min). Analysis of variance (two-way ANOVA) followed by Tukey's multiple comparison of the means at the 95% familywise confidence level.

extract of the flowers of *S. microglossa* may be responsible for its anti-inflammatory activity (Shen et al. 2002, Kim et al. 2002, Morikawa et al. 2003).

In this work, the carrageenan-induced inflammation model was used for an initial screening in the evaluation of nonsteroidal anti-inflammatory drugs (NSAIDs), which is useful for evaluating the contribution of mediators involved in cellular changes, such as histamine, serotonin and kinins over the 4 h that it was evaluated (Winter et al. 1962, Kale et al. 2007). A bradykinin assay was performed to observe whether *S. microglossa* extract can reduce

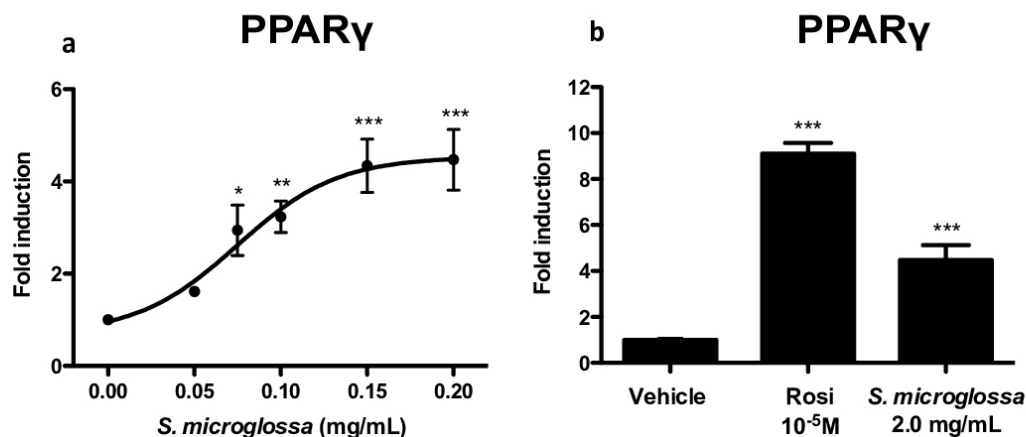


Figure 10. *S. microglossa* activates PPAR γ . HeLa cells were cotransfected with plasmids containing the expression vector pCMV-SPORT6-PPAR γ and the PPRE luciferase reporter and treated with vehicle (DMSO), rosiglitazone (Rosi) or increasing concentrations of *S. Microglossa*. *S. microglossa* activated PPAR γ in a dose-dependent manner with an EC₅₀ value of 0.75 mg/mL (a). The highest concentration of *S. Microglossa* (2.0 mg/mL) induced approximately 50% of the PPAR γ activation promoted by rosiglitazone at 10⁻⁵M (b). The luciferase activity was measured after 24 h. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$ (compared to vehicle). Data are presented as the mean (SD) of three independent experiments conducted in triplicate. The statistical significance of the difference between the groups was assessed by one-way ANOVA followed by Tukey's post hoc test. Values of p less than 0.05 were considered significant.

vasodilation and vascular permeability as well as release NO and regulate the production of prostaglandins and histamines. When the prostaglandin E2 assay was performed, the *S. microglossa* extract was able to reduce paw edema, which during the inflammatory process induces the vasodilation, chemotaxis and chemokinesis of leukocytes, the release of mediators of inflammation and the sensitization of nerve endings, causing hyperalgesia during the inflammatory response (Koeberle & Werz 2015, Robbins & Cotran 2005).

S. microglossa extract was not able to inhibit the inflammatory process induced by bradykinin within the evaluated doses. Bradykinin is a peptide with vasodilatory action formed by the activity of enzymes on kininogens. In the inflammatory process, bradykinin is formed due to the increase in vascular permeability, which causes the extravasation of plasma as well as in the release and modulation of the production of prostaglandins and histamines (Brown &

Robert 2001, Costa et al. 2006, Oliveira et al. 2010, Bender et al. 2017). Bradykinin can stimulate prostaglandins during an inflammatory process, whereas glucocorticoids inhibit prostaglandin production in endothelial cells by interacting with specific steroid receptors (Lewis et al. 1986). The *S. microglossa* extract was not able to act at this point of the inflammation cascade and showed that its anti-inflammatory activity is not the same as that of glucocorticoids.

In another experiment, compound 48/80 was used to identify the potential of the extract to inhibit the release of histamine and serotonin during the inflammatory process. According to previous work, when injected into the paw, serotonin at doses of 0.2-20 μ g produced dose-related edema (Cirino et al. 1989). Compound 48/80 is a polybasic mixture of low molecular weight polymers that induces an intense release of serotonin and histamine by tissue mast cells (Vendramini-Costa et al. 2015). *S. microglossa* extract, at the doses tested, was able to decrease

the edema caused by this agent only at 250 mg/kg for 30 min.

To elucidate the molecular mechanisms underlying the anti-inflammatory effects of the *S. microglossa* extract, we investigated its effects on PPAR γ transcriptional activity. A luciferase gene reporter assay showed that *S. microglossa* was able to activate PPAR γ in a dose-dependent manner. PPAR γ is a transcription factor that belongs to the superfamily of nuclear receptors and plays a central role in metabolic homeostasis of lipid and glucose metabolism and inflammation (Tyagi et al. 2011). It is a target of several compounds that exhibit anti-inflammatory effects, such as nonsteroidal anti-inflammatory drugs (Puhl et al. 2015) and natural products, such as *Amorpha fruticosa* L. (Weidner et al. 2012), *Cymbopogon citratus* (Katsukawa et al. 2010) and *Rosmarinus officinalis* (Rau et al. 2006).

The inhibitory effects of activated PPAR γ on prostaglandin E₂ and other proinflammatory molecules, such as thromboxane A₂ (TXA₂), inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX)-2, have been shown in both primary and secondary cell lines (Mendez & LaPointe 2003, Yamazaki et al. 2002). Other factors are likely to be involved in the anti-inflammatory effects of the *S. microglossa* extract observed in this study. However, the effects on PPAR γ certainly contributed to understanding its mechanisms of action.

Thus, the ethanolic extract of *S. microglossa* flowers has anti-inflammatory potential, and the phenolic compounds identified and quantified should be responsible for this activity since previous works have demonstrated this anti-inflammatory potential of this plant. The compounds present in the extract may interact with PPAR γ and possibly decrease the concentration of proinflammatory molecules, thereby decreasing the inflammatory process.

CONCLUSION

The extract of *S. microglossa* flowers showed anti-inflammatory activity. In a transactivation assay, the extract was able to increase PPAR γ transcriptional activity. These data demonstrate the pharmacological potential of this species, corroborating its popular use.

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