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Topical anti-inflammatory activity of yacon leaf extracts

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Abstract: *Smallanthus sonchifolius* (Poepp.) H. Rob., Asteraceae, known as yacon, is an herb that is traditionally used for the treatment of diabetes in folk medicine. However, recent studies have demonstrated that this plant has other interesting properties such as anti-microbial and anti-inflammatory actions. Thus, the purpose of this study was to evaluate the topical anti-inflammatory property of different extracts prepared from yacon leaves and analyze the role of different chemical classes in this activity. Three yacon leaf extracts were obtained: aqueous extract, where chlorogenic acid derivatives and sesquiterpene lactones were detected; leaf rinse extract, rich in sesquiterpene lactones; and polar extract, rich in chlorogenic acid derivatives. All the extracts exhibited anti-edematogenic activity *in vivo* (aqueous extract: 25.9% edema inhibition at 0.50 mg/ear; polar extract: 42.7% inhibition at 0.25 mg/ear; and leaf rinse extract: 44.1% inhibition at 0.25 mg/ear). The leaf rinse extract furnished the best results regarding neutrophil migration inhibition, and NO, TNF-α and PGE_2 inhibition. These data indicate that both sesquiterpene lactones and chlorogenic acid derivatives contribute to the anti-inflammatory action, although sesquiterpene lactones seem to have more pronounced effects. In conclusion, yacon leaf extracts, particularly the sesquiterpene lactone-rich extract, has potential use as topical anti-inflammatory agent.

Article

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Introduction

Smallanthus sonchifolius (Poepp.) H. Rob., popularly known as yacon, is a medicinal herb belonging to the Asteraceae family. It is native to the Andean region, where its tuberous roots, rich in inulin, are consumed as food (Goto et al., 1995; Pedreschi et al., 2003; Genta et al., 2005). Yacon cultivation has been expanded to several countries such as Japan, New Zealand, Czech Republic, and Brazil (Vilhena et al., 2000; Genta et al., 2010), and its leaves are used in folk medicine for the treatment of hyperglycemia as well as kidney and skin disorders (Goto et al., 1995; Pedreschi et al., 2003; Genta et al., 2005).

The hypoglycemic activity of different yacon leaf extracts has been investigated in normal and diabetic animals (Aybar et al., 2001; Miura et al., 2004; Miura, 2007; Baroni et al., 2008; Genta et al., 2010). In these studies, administration of polar extracts resulted in significant hypoglycemic effect. Further investigations showed that organic fractions of the foliar extracts have in vitro anti-oxidant activity and diminished glucose production in rat hepatocytes (Valentová et al., 2005). This anti-oxidant action has been attributed to the presence of higher concentrations of phenolic compounds such as chlorogenic acid and other caffeic acid derivatives in yacon leaves (Yan et al., 1999; Simonowska, et al., 2003; Takenaka et al., 2003; Valentová & Ulrichová, 2003; Valentová et al., 2004; 2005). The existence of several chlorogenic acid derivatives (CGA) such as 3,4-dicaffeoylquinic, 3,5-dicaffeoylquinic, 4,5-dicaffeoylquinic, 2,3,5-tricaffeoylaltraric, and 2,4,5-tricaffeoylaltraric acids has been described in yacon leaves and roots (Takenaka et al., 2003; Terada et al., 2009). The two altraric acids isolated from yacon leaves elicit potent inhibition of the enzyme α-glucosidase, and a patent for these compounds as oral hypoglycemic agents has been filed (Takenaka et al., 2003).

Additional studies have demonstrated that yacon display other interesting properties such as anti-fungal, antibacterial, and anti-inflammatory actions (Inoue et al., 1995; Pinto et al., 2001; Gonçalez et al., 2003; Lin et al., 2003; Pak et al., 2006; Schorr et al., 2007). These activities have been associated with the presence of sesquiterpene

lactones (STL) (Lin et al., 2003; Pak et al., 2006), which are present in higher concentrations in the leaves (Inoue et al., 1995; Schorr & Da Costa, 2003). Enhydrin, uvedalin, sonchifolin, and polimatin B are the main STL identified in yacon leaves (Schorr et al., 2007). Some of these STL, such as enhydrin and uvedalin, have been shown to exhibit anti-inflammatory activity through inhibition of the NF-κB transcription factor (Schorr et al., 2007; Siedle et al., 2004).

Although several biological activities have been reported for yacon leaf extracts, it has been recently demonstrated that the chronic oral consumption of aqueous yacon leaf extract is toxic to rats, culminating in kidney damage (Oliveira et al., 2011). The phytochemical analysis of this aqueous extract revealed that it contains both STL and CGA. In this same study, treatment with an STL-rich extract was compared with treatment using a CGA-rich extract. It was found that toxicity is due to the presence of STL, while the extract rich in CGA is safer.

Both STL and CGA have been described as anti-inflammatory agents in the literature (Shin et al., 2004; Siedle et al., 2004; Santos et al., 2006; Schorr et al., 2007), and yacon leaf extracts may be a promising therapeutic agent in topical applications. Thus, the aim of this work was to compare the effect of three different yacon leaf extracts using a topical model of anti-inflammatory activity as well as in vitro assays, in order to gain insight into the classes of compounds present in these extracts and the mode of action involved in their activities.

Materials and Methods

Plant material

Smallanthus sonchifolius (Poepp.) H. Rob., Asteraceae, leaves were collected in a cultivated area of the university *campus* of Ribeirão Preto, University of Sao Paulo, SP, Brazil. Intact leaves were air-dried at 40 °C, and a voucher specimen was deposited at the SPFR herbarium of the same university under the code R.B. Oliveira 495. The identification was confirmed by R.B. Oliveira.

Chemicals and drugs

Analytical grade MeOH and acetone (Dinamica) were employed for preparation of the extracts. Glacial acetic acid, acetonitrile (Merck), and ultra-pure water (Millipore) were HPLC grade. Chlorogenic acid (95% of purity) and quercetin (95% of purity) (Sigma-Aldrich), as well as enhydrin (96% of purity by HPLC analyses) available in our laboratory (Schorr & Da Costa, 2003; Schorr et al., 2007) were utilized as HPLC standards. The following drugs were used for the anti-inflammatory tests: acetone (Dinamica), croton oil (Sigma), glycerol (Synth), indomethacin (99% of purity) (Sigma), dexamethasone (0.001 g in 1.0 g of vehicle, Medley), Cremophor® EL (Basf), hexadecyltrimethyl ammonium bromide (HTAB) (Sigma), TMB substrate reagent (BD-OptEIATM), DEMEM (Gibco), gentamicin (Gibco), bovine serum (Gibco), sulfanilamide (Sigma), *N*-(1-naphthyl) ethylenediamine (Sigma), and PGE2 (Invitrogen) and TNF-α (Invitrogen) ELISA Kits.

Extract preparation

Three extracts from air-dried yacon leaves were prepared as described previously (Oliveira et al., 2011). Briefly, the aqueous extract (AE) was obtained from 500 g of dried intact leaves. These leaves were divided in 24 portions of 20 g. In each portion of 20 g was added 1,000 mL of boiling water. The resulting extracts were cooled at room temperature (*ca*. 26 °C), filtered, lyophilized, and frozen at -20 °C until the experiment was conducted (total yield 45 g residue). The leaf-rinse extract (LRE) was produced by rinsing 1 kg of dried yacon leaves with acetone for 10 s, which furnished an extract rich in STL (STL are present in glandular trichomes on the leaf surface). This extract was then filtered, and the solvent was evaporated under vacuum. The solid material was re-suspended with MeOH-H_2O (7:3, v/v) and submitted to liquid-liquid partition with n-hexane. The hydroalcoholic fraction was evaporated under vacuum, lyophilized, and frozen at -20 °C (total yield 26.9 g) until the assay was performed. The polar extract (PE) was prepared with powdered air-dried yacon leaves previously rinsed in acetone (LRE preparation). Extraction was carried out by three 24-h maceration procedures with 70% MeOH. The extract was filtered, the solvent was evaporated under vacuum, and the sample was submitted to liquid-liquid partition with *n*-hexane. The hydroalcoholic fraction was dried under vacuum, lyophilized, and frozen at -20 °C (total yield 95 g) until the experiment was accomplished.

Quantification of major compounds

Phytochemical analysis of the three extracts was carried out by reversed-phase HPLC-UV-DAD profiling as described previously (Oliveira et al., 2011). For the quantification procedure, the HPLC system consisted of a Shimadzu SCL 10Avp liquid chromatograph equipped with a Shimadzu SPD-M10Avp photodiode array detector-DAD and a C-18 column (Shimadzu, ODS Shim-pack 5 μm, 4.6 x 250 mm). Elution was conducted in the following way: initial gradient elution with a binary mobile phase consisting of H_2O (0.5% AcOH) and MeCN (0.5% AcOH) in a linear gradient of 0 to 5% MeCN for 30 min, followed by isocratic elution with 45% MeCN from 30 to 50 min and final linear gradient of 45 to 100% MeCN from 50 to 80 min. The flow rate was 1.3 mL/min, and the injection volume was 20 μL. The UV data were acquired between

190 and 600 nm, and chromatograms were simultaneously recorded at 254 and 325 nm. The chromatographic data were processed using Class VP software (version 5.02; Shimadzu).

Quantifications were performed by using standard curves of enhydrin to STL, chlorogenic acid to CGA, and quercetin to flavonoids. The standard solutions were injected by means of an automatic injector, in triplicate. The concentrations were obtained by serial dilutions of 35.25-0.24 µg/mL for chlorogenic acid and quercetin, and 2,000-15.63 µg/mL for enhydrin, in a total of eight points for each curve. The peak areas were used for calculation of the concentration of the compounds in each extract using the obtained equations and by comparison of their UV spectra at 254 nm.

Animals

Adult male Balb/c mice (20-25 g) were provided by the animal housing facility of Faculdade de Ciências Farmacêuticas de Ribeirão Preto, USP, and were maintained under standard laboratory conditions (25±2 °C at 40-60% relative humidity and 12-h light-dark cycle). The animals were allowed free access to food and water. All the animals were euthanized in a CO_2 chamber. The study was approved on August 1, 2007 by the Institutional Ethical Animal Committee of the University of Sao Paulo (protocol number: 07.1.636.53.5), which followed the rules of the Brazilian Committee on Animal Care (COBEA).

Croton oil-induced mouse ear edema

Topical anti-inflammatory activity was evaluated as the inhibition of the croton oil-induced ear edema in mice, using a methodology modified from Tubaro et al. (1985). Briefly, cutaneous inflammation was induced by application of 20 µL acetone solution containing 5% irritant croton oil on the inner surface of the left ear of the mouse (n = 6 per group). The right ears remained untreated. The target leaf extracts (0.125, 0.25, and 0.5 mg/ear) dissolved in glycerol were topically applied in the inner surface of the left ears 30 min after croton oil administration. Indomethacin (0.5 mg/ear) was used as reference compound, and control animals received irritant and vehicle only. The vehicle was acetone-glycerol (1:8, v/v) for LRE, indomethacin, and control; and water-glycerol (1:8, v/v) for AE, PE, and control. The mice were euthanized six h later, and a six-mm diameter plug was removed from both the treated and untreated ears with the aid of a dermatologic punch. The edematous response was measured as the weight difference between the two plugs. The anti-inflammatory activity was expressed as the percentage of edema reduction in treated mice as compared to control mice.

Myeloperoxidase assay

The myeloperoxidase (MPO) kinetic-colorimetric assay was used for evaluation of leukocyte migration to the subcutaneous tissue of mouse ears. Left ear plugs obtained from the croton oil ear edema assay (see above) were kept in 200 µL NaEDTA/NaCl buffer (pH 4.7) at -20 ºC until the experiment was initiated. The plugs were homogenized with Polytron (PT03100) and centrifuged at 956 x *g* for 15 min, at 4 ºC. The pellet was re-suspended in 200 µL hexadecyltrimethyl ammonium bromide (HTAB) 0.5% buffer (pH 5.4) and homogenized again. The samples were re-centrifuged, and 20 µL supernatant was employed for MPO quantification. MPO was quantified by using 20 µL supernatant mixed with 30 µL $NaPO_4$ 0.08 M. The enzymatic reaction was assessed with 50 µL TMB substrate reagent. After 10 min, the reactions were stopped with H_2SO_4 (2.5 M). The absorbance was measured at 450 nm (Bio-Rad model 680 - Microplate Reader, Brazil), and the MPO activity in the samples was compared to a standard curve of neutrophils. The results are presented as the MPO activity (O.D./mg of tissue).

In vitro *anti-inflammatory activity*

Murine macrophages of the RAW 264.7 cell lineage were cultivated at 37 °C and 5% CO_2 in DEMEM (Dulbecco's Modified Eagle Medium) supplemented with 10% bovine serum and gentamicin. The cells were gently detached from the bottle with cell scrapes, transferred to 50-mL tubes, and centrifuged for 10 min at 264 x *g* and 18 °C. The supernatant was discarded, and the cells pellet was re-suspended in 10 mL DEMEM. The cell concentration was adjusted to 5 x 10^5 cells/mL, and the cells were placed into 96-well plates at 37 °C for 24 h, to allow for cell adhesion. The medium was then substituted with 100 µL medium containing the yacon leaf extracts at different concentrations (0.25, 0.5, and 1.0 µg/mL), as previously determined by means of the MTT viability cell assay, or the positive controls indomethacin or dexamethasone (1.0 µg/mL). Three independent experiments were conducted. Each independent experiment was performed in triplicate. After two hours, the cells were stimulated with LPS (lipopolysaccharide from E. coli 0111.B4 cellular wall) at 2 µg/mL. The cells were incubated for 24 h at 37 °C and 5% CO_2. The supernatants were employed for NO (nitric oxide), TNF-α, and PGE2 quantification.

NO, TNF-α, and PGE_2

NO was quantified by using supernatants from the RAW 264.7 cell culture. To this end, an aliquot of 100 µL supernatant was incubated with an equal volume of Griess reagent (1:1 v/v 5% H_3PO_4 containing 1%

sulfanilamide and *N*-(l-naphthyl)ethylenediamine 1%) for 5 min. Absorbance was measured at 550 nm, and the total nitrite concentration was determined by comparison with a standard $NaNO_2$ curve. The remaining supernatants from the RAW 264.7 culture were used for TNF-α and PGE_2 quantification by means of ELISA commercial kits, using the manufacturer's instructions. The results were compared with standard curves and are expressed in pg/mL.

Statistical analysis

Data are represented as mean±SEM. Results were analyzed by one-way ANOVA followed by Tukey's multiple comparison test.

Results

The HPLC-UV-DAD phytochemical profiles of the extracts AE, PE, and LRE have been published previously (Oliveira et al., 2011). Polar compounds related to chlorogenic acid were identified as the major components in AE and PE, whereas several STL and flavonoids were detected in LRE (Table 1). However, STL were also present in AE, albeit in smaller amounts. Herein, we have constructed analytical curves for enhydrin (r^2 = 0.992), chlorogenic acid (r^2 = 0.999), and quercetin (r^2 = 0.996) during the quantification experiments. Table 1 shows that LRE contains high STL concentrations and low concentrations of flavonoids [3-*O*-methylquercetin (**5**) and 3,4'-di-*O*-methylquercetin (**6**)], while no CGA (**1**–**4**) is

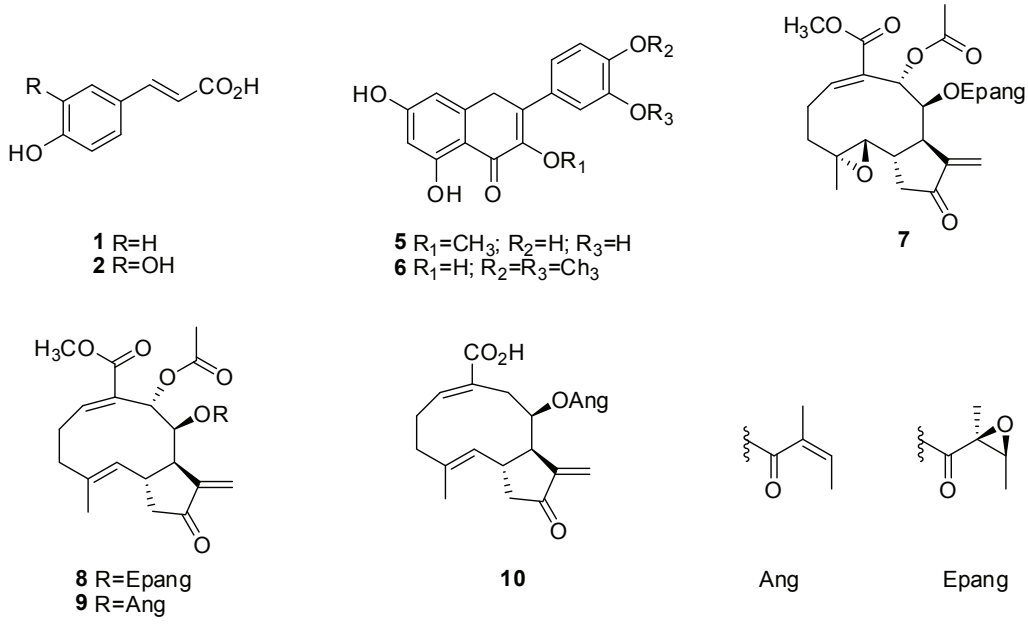

Table 1. Concentrations (µg/mL) of the compounds present in the three yacon leaf extracts investigated in this work as obtained by HPLC-UV-DAD analytical curve.

Compound #	Class	Identified as	AE	PE	LRE
(**1**)	CGA	coumaric acid	0.3	3.5	0.0
(**2**)	CGA	caffeic acid	0.3	1.3	0.0
(**3**)	CGA	*CGA_1	2.0	9.9	0.0
(**4**)	CGA	*CGA_2	1.3	9.6	0.0
(**5**)	Flavonoid	3-*O*-methylquercetin	0.0	0.0	20.4
(**6**)	Flavonoid	3,4'-di-*O*-methylquercetin	0.0	0.0	7.2
(**7**)	STL	enhydrin	99.7	0.0	1,997.7
(**8**)	STL	uvedalin	319.0	0.0	1,257.4
(**9**)	STL	polymatin B	129.3	0.0	616.2
(**10**)	STL	sonchifolin	102.7	0.0	519.1

The compounds were identified by comparison with authentic standards available in our laboratory. *Chemical structures unidentified. The UV profile suggests that these compounds correspond to CGA (UV max: 298 and 325 nm). CGA_1 and CGA_2 correspond to the most intense peaks in AE and PE at 325 nm. AE: aqueous extract, PE: polar extract, LRE: leaf-rinsed extract.

detected in this extract. The STL enhydrin (**7**), uvedalin (**8**), polymatin B (**9**) and sonchifolin (**10**) appear at lower concentrations in AE as compared to LRE, but their concentrations are still higher as compared to those of CGA in AE. PE, in turn, contains larger CGA concentrations as compared to AE, but this extract lacks STL.

In the croton oil-induced ear edema assay, the topical treatment of the animals with AE led to reduction of the ear edema, but a statically significant response (25.9% edema inhibition) was only verified at the highest tested dose (0.5 mg/ear) (Figure 1A). This effect was statically similar to that elicited by indomethacin (28% edema inhibition). The topical effect of the extract PE was statically significant for all the assayed doses (25.2, 42.7, and 30.1 % ear edema inhibition for doses of 0.125, 0.25, and 0.5 mg/ear, respectively). Topical treatment with LRE showed statically significant outcome at all tested doses (0.125 mg/ear, 44.1% ear edema inhibition; 0.25 mg/ear, 38.5% ear edema inhibition; and 0.5 mg/ear, 18.9% ear edema inhibition) (Figure 1A).

MPO activity was statically decreased as compared to the control group at all the investigated extract doses as well as in the presence of indomethacin (Figure 1B). All the extracts had a more marked influence on MPO than indomethacin (0.5 mg/ear, 2.34 O.D./mg of tissue). Meanwhile, PE (0.5 mg/ear, 0.35 O.D./mg of tissue) and LRE (0.5 mg/ear, 0.30 O.D./mg of tissue) had a more pronounced effect on MPO than AE (0.5 mg/ear, 0.83 O.D./mg of tissue).

Treatment of RAW 264.7 cells with AE and PE exercised no effect on NO production (Figure 2A). However, treatment of the cells with LRE resulted in a statistically dose-dependent inhibition of NO production (0.25 µg/mL: 23.2% inhibition; 0.5 µg/mL: 38.5% inhibition; 1.0 µg/mL: 72.96 % inhibition). The LRE activity at doses of 0.25 and 0.5 µg/mL was similar to the activity of the positive control dexamethasone (1.0 µg/mL: 33.6% inhibition), whilst LRE activity at a dose of 1.0 µg/mL was statistically higher than that of dexamethasone.

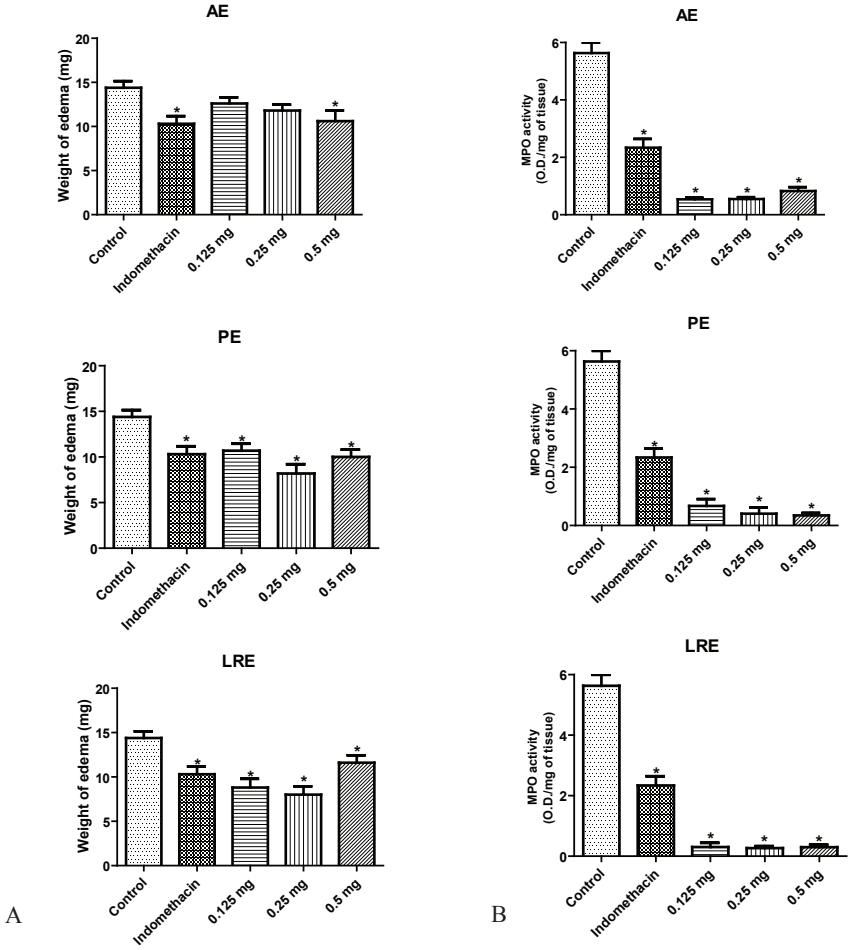

Figure 1. Effect of the three yacon leaf extracts on the topical anti-inflammatory activity induced by croton oil. A. Weight of edema measured as the weight difference between the two plugs of left ear (with edema induction) and right ear (without edema induction). B. MPO activity in the ears with edema induction. Data are expressed as means±SEM. *p<0.05 in relation to the control (one-way ANOVA following Tukey's multiple comparison test. AE: aqueous extract, PE: polar extract, LRE: leaf-rinsed extract.

All the extracts were able to moderately inhibit TNF-α production (Figure 2B), and treatment with LRE evidenced a dose-dependent trend (0.25 µg/mL: 33.25% inhibition; 0.5 µg/mL: 42.13% inhibition; 1.0 µg/mL: 48.32% inhibition). LRE activity at a dose of 0.25 µg/mL was statistically similar to that of indomethacin (1.0 µg/mL: 27.41% inhibition), while LRE at doses of 0.5 and 1.0 µg/mL was more active than indomethacin (Figure 2B). AE (1.0 µg/mL: 38.42% inhibition) and PE (1.0 µg/mL: 36.37% inhibition) led to statistically similar effects at all the tested doses (Figure 2B).

In the same way, all the extracts were able to inhibit *in vitro* PGE_2 production, being the actions of AE (1.0 µg/mL: 73.45% inhibition) and LRE (1.0 µg/mL: 77.01% inhibition) more pronounced than that of PE (1.0 µg/mL: 41.57% inhibition). Treatment with LRE was the only one that presented a dose-dependent effect on PGE2 production (Figure 2 C). AE and LRE displayed activity that was statistically similar to that of indomethacin (1.0 µg/mL: 79.11% inhibition).

Discussion

As previously reported (Oliveira et al., 2011), HPLC analysis identified PE as a polar extract rich in CGA and lacking STL, LRE as an extract rich in STL and flavonoids, and AE as an extract containing both CGA and STL. Here, we have quantified the different classes of the main compounds in these three extracts. We verified that AE displays lower STL concentrations as compared to LRE. However, STL concentrations in AE are larger if compared to CGA concentrations in this same extract. This suggests that STL play an important role in the biological activities observed for the aqueous extracts that are used orally by the population (Oliveira et al., 2011). PE contains high CGA concentrations, while STL are the major compounds in LRE. Thus, we have achieved our goal, which was to produce an extract rich in CGA and another one rich in STL, in order to gain insight into the role of these two classes of compounds in the anti-inflammatory activity of yacon leaves.

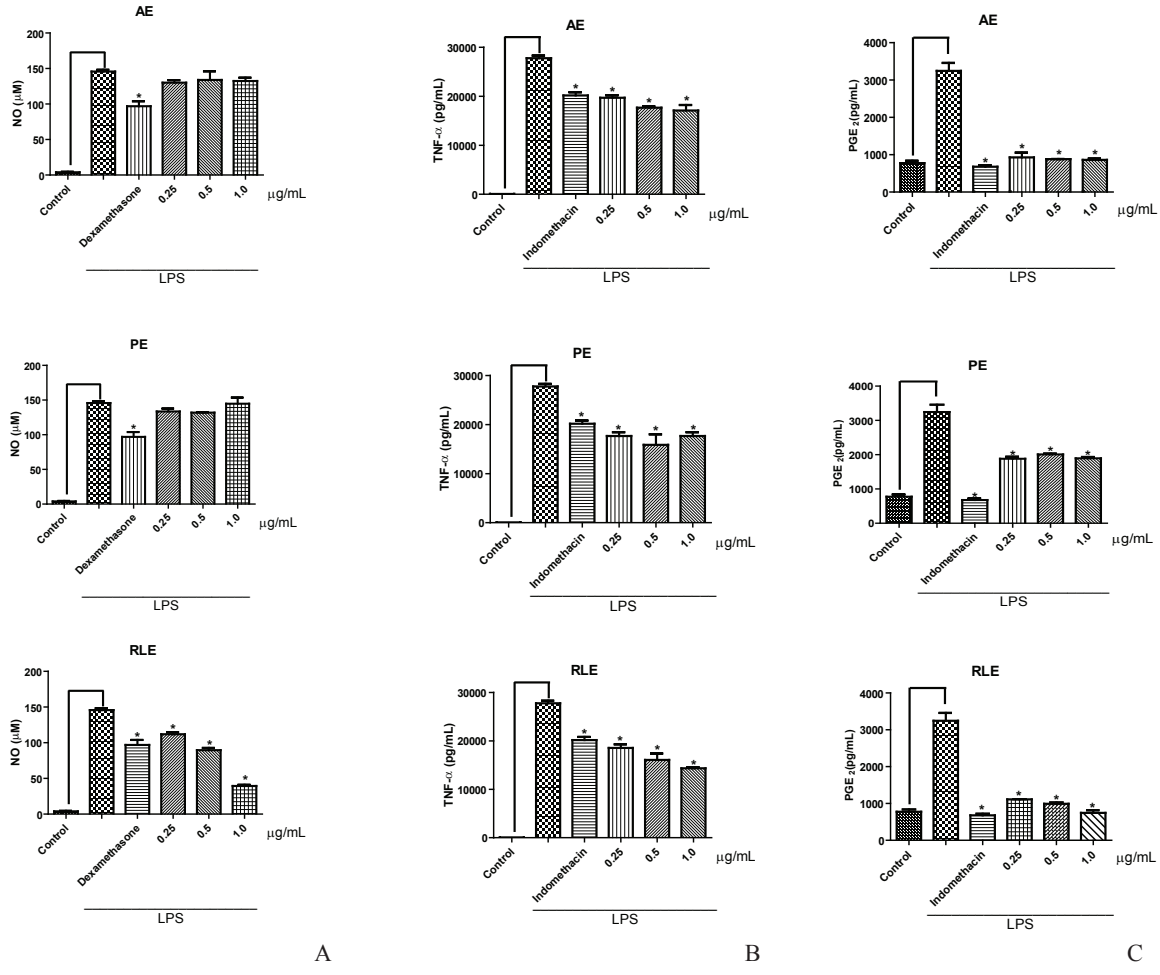

Figure 2. *In vitro* anti-inflammatory assay in RAW 264.7 cells culture induced with LPS after treatment with the three different yacon leaf extracts. A. Nitric oxide determination. B. TNF-α determination. C. PGE_2 determination. Data are expressed as mean±SEM. *p<0.05 in relation to the control without LPS. AE: aqueous extract, PE: polar extract, LRE: leaf-rinsed extract.

We have found that all the prepared extracts have topical anti-edematous and anti-inflammatory activity, as confirmed by the fact that all the extracts decrease edema and neutrophil migration to the inflammatory site. The edema was induced by topical application of the croton oil, whose main inflammatory compound is the 12-*O*-tetracanoylphorbol-3-acetate (TPA) (Hecker et al., 1968; Rao et al., 1993). Croton oil or purified TPA application in mouse skin has been used by several authors to access the topical anti-inflammatory effects of non-steroidal and steroidal agents (Inoue et al., 1989). The TPA in contact with the plasmatic membrane release arachidonic acid that under action of the enzymes cyclooxygenase or lipoxygenase results in formation of cytokines or leukotrienes, respectively (Hecker et al., 1968; Rao et al., 1993).

In the present study, AE was the least effective extract in decreased the edema induced by croton oil application, since it is only able to reduce the edema at a higher dose, while PE and RLE displays the anti-edematous effect and decreases neutrophil migration at all the tested doses. The *in vitro* experiments provided some evidence about the action mechanisms involved in the anti-inflammatory activity observed for the three extracts. AE and PE are not able to inhibit the NO production induced by LPS in macrophages, while LRE is a potent inhibitor. This suggests that STL has an important role in this effect. Indeed, several STL, *e.g.*, parthenolide, isohelenin, and dehydrocostus lactone, were shown to inhibit the expression of inducible nitric oxide synthase (iNOS) in various cell systems (Dirsch et al., 2000). NO is over-produced endogenously by iNOS in response to pro-inflammatory cytokines and LPS. There some evidence that STL inhibit iNOS expression via inhibition of the NF-κB. This is an interesting point, since it was demonstrated that enhydrin and uvedalin, two LST isolated from yacon leaves, were able to inhibit the NF-κB, a transcriptional factor that has a central role in the transcription of the genes related to the inflammatory process (Ghosh et al., 1998; Schorr et al., 2007).

Additionally, all the three yacon extracts are able to inhibit the *in vitro* PGE_2 production stimulated by LPS. However, these extracts display low activity regarding TNF-α production, which is indication that neither STL nor CGA have significant activity on this inflammatory mediator. These findings can be useful to explain the *in vivo* anti-inflammatory effect of the extracts. Recently it has been demonstrated that TNF-α and PGE_2 levels increased with edema formation after TPA application in mouse skin (Fürstenberger & Marks, 1980; Inoue et al., 1989, Murakawa et al., 2006). The increase of TNF-α peaked at ~5 h after TPA application, whereas the increase of PGE_2 is biphasic with peaks at ~3 and 24 h after edema induction (Murakawa et al., 2006). AE and LRE treatment culminates in a more potent inhibition PGE_2, indicating that STL plays an important role in this activity. The effect of these extracts in decreased PGE_2 levels can be related with their effect on inhibition of leukocyte migration as demonstrated in the MPO assay, since it was demonstrated that PGE_2 production at the late phase in the TPA edema model depends on the infiltrated leukocytes, while TNF-α levels are not related to cell infiltration in this model (Murakawa et al., 2006).

Thus, our results have demonstrated that all the three tested yacon leaf extracts exhibit topical anti-edematous activity *in vivo*. This activity may be a consequence of an anti-inflammatory action, as evidenced by the fact that all the assayed extracts exert some effect on inflammatory mediators. This suggests that both STL and CGA contribute to the observed responses. However, the LRE extract, which is rich in STL, has a more pronounced effect on the anti-inflammatory mediators. In conclusion, our observations suggest that yacon leaf extracts, especially the one rich in STL, has potential application as a topical anti-inflammatory agent.

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Authors' Contributions

RBO (Ph.D. student) contributed in collecting plant material, taxonomic identification, confection of herbarium vouchers, running the laboratory work, data analysis and drafting the manuscript. DACP, THG and AS contributed to the biological studies. LHF, APC and FBDC designed the study, supervised the laboratory work and contributed to critical reading of the manuscript. All the authors have read the final manuscript and approved the submission.

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