academicJournals

Vol. 11(2), pp. 22-33, 10 January, 2017
DOI: 10.5897/JMPR2015.6294
Article Number: AA00B8F62306
ISSN 1996-0875

http://www.academicjournals.org/JMPR

Journal of Medicinal Plants Research

Full Length Research Paper

Chemical composition, antioxidant and topical anti-inflammatory activities of *Croton cordiifolius* Baill. (Euphorbiaceae)

Iasmine Andreza Basilio dos Santos Alves[1], Simone Maria dos Santos[1], Raudiney Frankilin Vasconcelos Mendes[1], José Wellinton da Silva[1], Maria de Fátima Rodrigues[1], Bárbara de Azevedo Ramos[2], Márcia Vanusa da Silva[2], Maria Tereza dos Santos Correia[2], Fernanda das Chagas Angelo Mendes Tenório[3], Roberta Jeane Bezerra Jorge[4], René Duarte Martins[4], Julianna Ferreira Cavalcanti de Albuquerque[1], Karina Perrelli Randau[5] and Rafael Matos Ximenes[1*]

[1]Departamento de Antibióticos, Universidade Federal de Pernambuco, Recife, Brazil.
[2]Departamento de Bioquímica, Universidade Federal de Pernambuco, Recife, Brazil.
[3]Departamento de Histologia e Embriologia, Universidade Federal de Pernambuco, Recife, Brazil.
[4]Centro Acadêmico de Vitória, Universidade Federal de Pernambuco, Vitória de Santo Antão, Brazil.
[5]Departamento de Ciências Farmacêuticas, Universidade Federal de Pernambuco, Recife, Brazil.

Received 8 November, 2016; Accepted 23 December, 2016

Croton cordiifolius **is widely used in Brazilian Caatinga folk medicine to treat general inflammation, pain, and gastrointestinal disturbances. Currently, its medicinal properties are not well understood, owing to the absence of chemical and pharmacological studies. The aims of this work were to analyze the chemical composition of *C. cordiifolius* stem bark and evaluate its *in vitro* antioxidant and *in vivo* anti-inflammatory activities. *C. cordiifolius* ethanolic extract (CcEE) was obtained by maceration, while essential oil (CcEO) was extracted by hydrodistillation in a Clevenger-type apparatus. The chemical composition was evaluated by thin-layer chromatography and GCMS. Total phenolics, flavonoids, and antioxidant activity were quantitated by spectrophotometry. Topical anti-inflammatory activity was evaluated by different ear edema models in mice. The major compounds in CcEO were α-pinene (51.76%) and β-pinene (19.08%). CcEE analysis indicated the presence of alkaloids, mono- and sesquiterpenes, flavonoids, phenylpropanoids, triterpenes, steroids, and coumarins. CcEE showed antioxidant activity *in vitro*. In a topical anti-inflammatory assay, CcEO showed no activity. On the contrary, CcEE inhibited ear edema induced by phorbol 12-myristate 13-acetate (PMA), arachidonic acid (AA), ethyl phenylpropriolate (EPP), and phenol. Probable mechanisms include inhibition of AA metabolite biosynthesis, vasoactive amine activity, and cytokine release/activity. These results corroborate the popular reputation of *C. cordiifolius* as an anti-inflammatory remedy**.

Key words: *Croton cordiifolius*, Euphorbiaceae, caatinga, topical anti-inflammatory activity, ear edema.

INTRODUCTION

The Euphorbiaceae family comprises 228 genera and more than 6,500 species, including trees, shrubs, herbs and creepers (The Plant List, 2013). This family is, quantitatively, the second most representative of Brazilian Caatinga, with about 60 species only of *Croton* genus (Souza et al., 2012). This genus, the second

largest of the family, consists of about 1,200 species, of which 350 are distributed in Brazil (Silva et al., 2010). Species of *Croton*, the predominant genus in the Caatinga, attract interest owing to their diverse array of chemical compounds, ethnopharmacological uses, and proven biological activities. Various compounds have been isolated from many species of the genus *Croton*. These include mono- and sesquiterpenes, diterpenes, flavonoids, tannins, and alkaloids, suggesting that this genus can be a promising source of bioactive molecules with remarkable research potential (Randau et al. 2004). Compounds extracted from several species to date display anti-inflammatory, antinociceptive (Falcão et al., 2005; Lima et al., 2015; Rocha et al., 2008), antioxidant (Shukla et al., 2009; Rocha et al., 2008), antiulcer (Almeida et al, 2003), antidiabetic (Torrico et al, 2007), healing, anticancer, antimycotic, antibacterial, and antiviral activity (Pieters and de Bruyne, 1995; Melo et al., 2013; Rodrigues et al., 2012).

Croton cordiifolius, popularly known as “quebra-faca” in the Brazilian Northeast, is used to treat general inflammation, pain, and gastrointestinal disturbances (Cartaxo et al., 2010; Monteiro et al., 2011). Nogueira et al. (2015) described the antinociceptive activity and chemical composition of its leaf’s essential oil.

Although the *Croton* genus is widely studied, including some species found in the caatinga, no studies characterizing the chemistry and pharmacology of *C. cordiifolius* stem bark have been published. The present study analyzed the chemical composition of the extract and essential oil of the stem bark of *C. cordiifolius* and evaluated its *in vitro* antioxidant and *in vivo* topical anti-inflammatory activities.

MATERIALS AND METHODS

Chemicals and drugs

Ethanol, phorbol 12-myristate 13-acetate (PMA), arachidonic acid (AA), ethyl phenylpropriolate (EPP), phenol, capsaicin, Folin-Ciocalteu reagent, phosphomolibdenium, DPPH, and ABTS were purchased from Sigma-Aldrich (USA, St. Louis). All other reagents and substances were of analytical grade.

Plant material and extraction

Stem bark fragments from *C. cordiifolius* were collected (April/2014) in the morning in the rural area of Salgueiro, Pernambuco, Brazil (-8º 04' 27" S, -39º 07' 09" W, 420 m). The botanical material was authenticated by Maria Olívia de Oliveira Cano. A voucher specimen was deposited in the Herbarium of the Instituto Agronômico de Pernambuco (IPA 89,210).

For the essential oil extraction, fresh stem bark fragments were immediately submitted to hydrodistillation for 2 h in a Clevenger-type apparatus, yielding 1.56% of *C. cordiifolius* essential oil (CcEO). The essential oil was subsequently dried over anhydrous sodium sulfate, protected from light and frozen under -20ºC until use. Fresh plant material was also extracted with ethanol (1:10, w/v) by dynamic maceration for 3 h. After filtration, the solvent was evaporated using a rotary evaporator (RV10, IKA, Germany), yielding 6.1% of *C. cordiifolius* ethanolic extract (CcEE).

Essential oil composition

Gas chromatography and mass spectrometry were carried out using a Shimadzu™ model 7A/QP 5050A equipped with a capillary non-polar DB-5 column (30 m x 0.25 mm x 0.25 µm). The oven temperature was programmed at 70ºC with an increase of 4ºC/min until 280ºC, and maintained for 15 min. The carrier gas was helium, with a constant flow of 1.4 ml/min. The temperature of the ionization source was maintained at 280ºC, the ionization energy at 70 eV, and the ionization current at 0.7 kV. Mass spectra were recorded from 30 to 450 m/z. Individual components were identified by matching their 70 eV mass spectra with those of the spectrometer data base using the Wiley L-Built and NIST libraries and by comparing their retention indices with those reported in the literature (Adams, 2001). The retention indices were compared with those of obtained by Craveiro et al. (1981) for other Euphorbiaceae species.

Phytochemical screening, total phenolic and flavonoid content

The ethanolic extract of *C. cordiifolius* stem back was analyzed by thin-layer chromatography (TLC) using Si gel F_{254} plates (ALUGRAM® 818131, Macherey-Nagel, Germany) and different solvent systems. It was verified the presence or absence of flavonoids, phenylpropanoids, terpenoids, steroids, saponins, alkaloids, coumarins, proanthocyanidins and quinones as described in Table 1.

Total phenolic content was determined by the Folin-Ciocalteu method (Li et al., 2008). Briefly, an aliquot of 200 µL of the extracts diluted in methanol (1 mg/mL) was added to 1 mL of the 10-fold diluted Folin-Ciocalteu reagent. After 4 min, 2.5 mL of sodium carbonate solution (0.7 M) was added. The test tubes were incubated for 30 min at room temperature, protected from light, after which the absorbance was read at 765 nm. Gallic acid was used for standard curve calibration (0 – 100 mg/L). The results were obtained from the regression equation: $y = 0.0121x + 0.032$, $R^2 = 0.9967$ and expressed as milligram of gallic acid equivalent (mgGAE)/g of extract. The experiments were performed in triplicate.

The determination of flavonoids was made by the method described by Woisky and Salatino (1998). An aliquot of 0.5 mL of the extracts diluted in methanol (1 mg/mL) was added to 0.5 mL of 2% $AlCl_3$ in methanol. The test tubes were incubated for 30 min at room temperature, protected from light, after which the absorbance was read at 420 nm. Quercetin was used for standard curve calibration (0 – 100 mg/L). The results were obtained from the regression equation: $y = 0.0262x - 0.0892$, $R^2 = 0.9982$ and expressed as milligram of quercetin equivalent (mgQE)/g of extract. The experiments were performed in triplicate.

Total antioxidant activity by phosphomolybdate assay (P-Mo)

Total antioxidant capacity of CcEE was determined by the

*Corresponding author. E-mail: rafael.ximenes@ufpe.br Tel: +55 81 2126-8866.

phosphomolybdate method using ascorbic acid as standard (PIETRO et al., 1999). A 0.1 mL aliquot of extract diluted to 1 mg/mL in methanol was added to 1 mL of solution containing 600 mM sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate. Test tubes were capped and incubated in a boiling water bath at 95ºC for 90 min. Samples were then cooled to 25ºC at room temperature and absorbance relative to a blank (1 ml of reagent solution and 0.1 mL of methanol) was measured at 695 nm. Total antioxidant activity (TAC) was expressed relative to ascorbic acid and calculated by the following formula,

$$\%TAC = \frac{Sabs - Babs}{AAabs - Babs} x\ 100$$

Where Babs is the absorbance of the blank, Sabs is the absorbance of the sample (extracts) and AAabs is the absorbance of ascorbic acid.

1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging activity

DPPH free radical-scavenging activity of CcEE was determined using 96-well microplates as described by Brand-Williams et al. (1995). Briefly, 0.04 mL of CcEE or gallic acid diluted in methanol (31.25, 62.5, 125, 250, 500, 1000 µg/ml) and 0.25 ml of 1 mM DPPH in methanol was added to each well. Microplate was kept for 30 min at 25ºC, protected from light. After this time, absorbance was measured at 517 nm. The free radical-scavenging activity of CcEE was expressed as percentage of inhibition of DPPH, calculated by the following formula,

$$DPPH\ inhibition\ (\%) = \frac{(Sabs - Babs)}{Babs}\ x\ 100$$

Where Babs is the absorbance of the blank (0.04 mL of methanol and 0.25 mL of 1 mM DPPH) and Sabs is the absorbance of the sample (CcEE or gallic acid).

2,2-azino-bis-(3 ethylbenzothiazoline)-6-sufonic acid (ABTS) radical cation scavenging activity

ABTS (7 mM) was oxidized by the addition of 140 Mm potassium persulphate. The solution was kept at 25ºC for 16 h, in the dark. Before use, the ABTS+ solution was diluted with ethanol to an absorbance of 0.70 ± 0.02 units at 734 nm. For the determination of ABTS+ radical scavenging activity of CcEE, 0.01 mL of CcEE (31.25, 62.5, 125, 250, 500, 1000 µg/ml) diluted in methanol was added to 1.0 mL of ABTS+ solution. After 6 min, the absorbance was measured at 734 nm. ABTS radical cation scavenging activity of CcEE was expressed as percentage of inhibition of ABTS+, calculated by the following formula,

$$ABTS\ inhibition\ (\%) = \frac{(Sabs - Babs)}{Babs}\ x\ 100$$

Where Babs is the absorbance of the blank (0.01 mL of methanol and 1.0 mL of ABTS+) and Sabs is the absorbance of CcEE.

Animals

Male Swiss or BALB/c mice (20–30, n = 6) were provided by the Animal Facility of the Universidade Federal de Pernambuco. The animals were housed in a room with controlled temperature (23 ± 2°C) under a 12/12 h light/dark cycle with food and water *ad libitum*. Experiments were carried out according to the Guide for the Care and Use of Laboratory Animals of the US Department of Health and Human Services (NIH publication number 85-23, revised in 1985). The project received prior approval from the Animal Ethics Committee of Universidade Federal de Pernambuco (protocol number 23076.016724/2016-62).

Topical anti-inflammatory activity

Ear edema measurement

For evaluation of ear weight, animals were euthanized and samples of 6 mm diameter were taken from both ears using a biopsy punch (Richter®, Brazil). Each biopsy was weighed on a semi-micro analytical balance (AUW-D 220, Shimadzu, Japan). Ear edema (EE) was expressed as the increase in ear sample weight, using the following formula:

$$EE\ (mg) = wRE - wLE$$

Where wRE is the weight obtained from the right ear sample (inflamed ear) and wLE is the weight obtained from the left ear sample (noninflamed ear).

For all treatments, animals were anesthetized with 1% halothane. Right ears were then challenged with different phlogistic agents diluted in acetone (20 µL). CcEE and CcEO were applied topically in 20 µL acetone. Dexamethasone or indomethacin (0.1 and 0.5 mg/ear, respectively) was used topically as a positive control. Ruthenium red (3 mg/kg, s.c.) was used as a positive control for capsaicin-induced ear edema.

PMA-induced ear edema

Ear edema was induced by topical application of 20 µL of PMA (2.5 µg/ear) in acetone on both sides of the right ear. Immediately after, CcEE (0.1, 0.3, and 1.0 mg/ear), CcEO (1, 3 and 10 µL/ear) or dexamethasone (0.1 mg/ear) was topically applied on both sides of the right ear, while the left ear received 20 µL of acetone. After 6 h, animals were euthanized for ear edema measurement (Carlson et al., 1985).

Histological analysis

After weighing, ear samples were fixed in buffered formalin for 24 h. Fixed tissued were then dehydrated by adding increasing concentrations of ethanol (70-100%), and processed for embeddging in paraffin. The resulting blocks were sliced into 4 µm thick sections, stained with hematoxylin and eosin and observed under a light microscope (Nikon, Japan).

Investigation of the mode of action of CcEE on ear edema

The mechanisms of topical anti-inflammatory activity of CcEE on PMA-induced ear edema were evaluated using different phlogistic agents to induce ear edema: arachidonic acid (2 mg/ear), ethyl phenylpropriolate 5% (20 µL/ear), phenol 10% (20 µL/ear), and capsaicin (0.25 mg/ear). Immediately, CcEE (1.0 mg/ear) or indomethacin (0.5 mg/ear)/dexamethasone (0.1 mg/ear) was topically applied on both sides of the right ear, while the left ear received 20 µL of the vehicle. Ruthenium red (3 mg/kg, s.c.) was used as positive control for capsaicin-induced ear edema. After 1 h, animals were euthanized for ear edema measurement. Animals challenged with capsaicin were euthanized after 30 min of exposure (Brattsand et al., 1982; Carlson et al., 1985; Gábor and Rázga, 1992).

Table 1. Solvent systems and spray reagents used for thin-layer chromatography analysis of *Croton cordiifolius* Baill. (Euphorbiaceae) stem bark ethanolic extract.

Secondary metabolite classes	Standard	Solvent system	Spray reagent	References
Flavonoids and phenylpropanoids	Quercetin, rutin and chlorogenic acid	EtOAc-HCOOH-AcOH-H_2O (100:11:11:26 v/v)	Natural products-polyethylene glycol reagent	Wagner and Bladt (1996); Brasseur and Angenot (1986)
Triterpenes and steroids	β-sitosterol	Toluene:EtOAc (90:10 v/v)	Lieberman-Burchard reagent	Harborne (1998)
Saponins	-	EtOAc-HCOOH-AcOH-H_2O (100:11:11:26 v/v)	Lieberman-Burchard reagent	Harborne (1998)
Mono and sesquiterpenes	Thymol	Toluene: EtOAc (97:3 v/v)	Anilsaldehyde-sulphuric acid reagent	Harborne (1998)
Coumarins and quinones	Coumarin and lapachol	$CHCl_3$-MeOH (98:2 v/v)	Potassium hydroxide reagent	Wagner and Bladt (1996)
Alkaloids	Pilocarpine	EtOAc-HCOOH-AcOH-H_2O (100:11:11:26 v/v)	Dragendorff reagent	Wagner and Bladt (1996)
Proanthocyanidins	Catechin	EtOAc-HCOOH-AcOH-H_2O (100:11:11:26 v/v)	Vanillin-hydrochloric acid reagent	Roberts et al. (1957)
Hydrolysable tannins	Gallic acid	n-BuOH-H_2O-AcOH (40:50:10 v/v)	Ferric ammonium sulfate 1%	Sena Filho et al. (2008)

Statistical analysis

Data are expressed as mean ± S.E.M. and analyzed by ANOVA followed by Bonferroni post-test using GraphPad Prism 5.0 with significance * set at $p < 0.05$.

RESULTS

Chemical composition and antioxidant activity

Phytochemical screening of CcEE showed the presence of flavonoids (aglycones, mono and diglycosides of 3',4'-OH flavonoids), phenylpropanoids, mono- and sesquiterpenes, triterpenes and steroids, alkaloids, and coumarins. The predominant compounds were terpenes and steroids. There were no hydrolyzable tannins, quinones, saponins, proanthocyanidins, or leucoanthocyanidins. The total phenolic and flavonoid contents of CcEE were determined by spectrophotometry to be 135.8 ± 7.62 EAG/g extract and 21.24 ± 0.37 EQ/g extract. GCMS analysis of CcEO identified 97.92% of the compounds, revealing a monoterpenoid rich essential oil (91.13%), with only 6.79% of sesquiterpenoids. The major compounds identified were α-pinene (51.76%), β-pinene (19.08%), camphene (9.71%), borneol (4.52%), camphor (4.37%), and β-caryophyllene (4.31%) are listed in Table 2.

Total antioxidant activity was determined with the phosphomolybdenum method, which is based on reduction of molybdenum VI to molybdenum V, in the presence of antioxidants. This reduction results in the formation of a green complex. According to this assay, total antioxidant activity in CcEE was 26.88 ± 2.83% of the activity of the ascorbic acid control. Free radical scavenging activity by DPPH and ABTS+ assays were also measured, with EC_{50} values > 1,000 µg/mL of CcEE for both assays.

Anti-inflammatory activity

PMA-induced ear edema

CcEE (0.1, 0.3 and 1 mg/ear) significantly inhibited PMA-induced ear edema at all tested doses as seen in Figure 1A. By contrast, CcEO (1, 3 and 10 µL/ear) showed less promising results, with significant activity only at the highest dose (Figure 1B). Histological analysis of ear samples aimed to evaluate the following parameters: congested vessels (CV); polymorphonuclear cells infiltration (PCI) and/or edema (E). Total ear and epidermis thickness was also evaluated relative to the negative control group. PMA-induced ear edema produced CV filled with white blood cells, intense PCI and edema. Dexamethasone (0.1 mg/ear) and CcEE at 0.3 and 1 mg/ear yielded similar results. CV were present and filled with red blood cells and discrete PCI and E. Ears treated with CcEE at 0.1 mg/ear and CcEO at all doses tested (1, 3 and 10 µL/ear) had CV filled with red and white blood cells and moderate PCI and E (Figure 2A-H). Based on these results, CcEE at 1.0 mg/ear was chosen for elucidation of CcEE's mechanism of topical anti-inflammatory activity using other phlogist agents.

Investigation of the anti-inflammatory mechanism of CcEE

CcEE (1 mg/ear) and indomethacin (0.5 mg/ear) demonstrated significant reductions of 57.7 and 53.2% respectively in AA-induced ear edema relative to the negative control (Figure 3A). In EPP-induced ear edema, CcEE (1.0 mg/ear) inhibited ear edema by 42%. This was less effective than the positive control drug dexamethasone (0.1 mg/ear), which inhibited ear edema by 75.2% (Figure 3B). CcEE (1.0 mg/ear) reduced phenol-

Table 2. Chemical composition of the essential oil of *Croton cordiifolius* Baill. (Euphorbiaceae) stem bark.

Peak	Compound	Retention time (min)	RI[a]	RI[b]	Relative area (%)
1	NI	5.092	-	-	0.51
2	α-pinene	5.405	938	932	51.76
3	Camphene	5.781	943	946	9.71
4	β-pinene	6.573	968	974	19.08
5	Myrcene	6.917	978	988	0.39
6	Cymene	7.992	1011	1020	0.29
7	Limonene	8.158	1022	1024	1.01
8	NI	8.242	-	-	0.21
9	NI	10.192	-	-	0.23
10	NI	10.583	-	-	0.58
11	Camphor	12.203	1121	1141	4.37
12	Borneol	13.017	1148	1165	4.52
13	NI	13.425	-	-	0.26
14	NI	13.908	-	-	0.28
15	β-caryophyllene	22.075	1434	1417	4.31
16	β-selinene	23.175	1479	1489	0.50
17	α-muurolene	24.675	1480	1500	1.08
18	Caryophyllene oxide	27.267	1567	1582	0.90
	Total identified				**97.92**
	Monoterpene hydrocarbons				82.24
	Oxygenated monoterpenes				8.89
	Sesquiterpene hydrocarbons				5.89
	Oxygenated sesquiterpenes				0.90

[a]Kovats retention index according to n-alkanes (C8–C26); [b]According to Adams (2009).

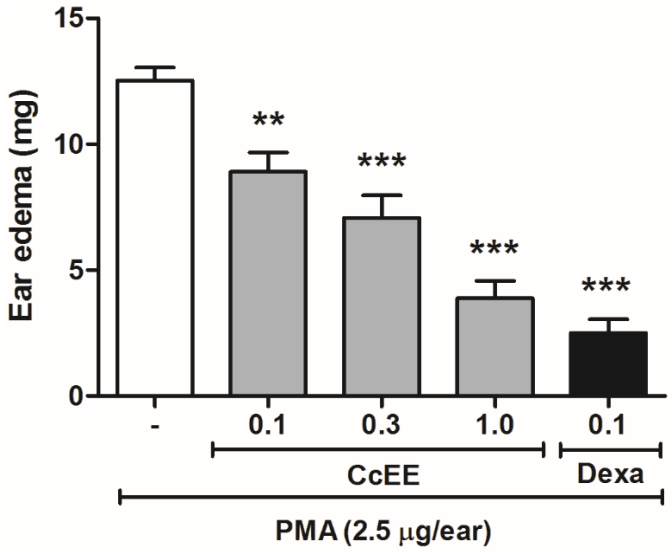
induced ear edema by 33.9%, while dexamethasone (0.1 mg/ear) reduced this edema by 91.6% (Figure 3C). In capsaicin-induced ear edema, CcEE (1.0 mg/ear) was not able to inhibit ear edema. Ruthenium red (3 mg/kg), a standard positive control drug, when given 30 min prior to capsaicin administration, inhibited ear edema by 73.5% (results not shown).

DISCUSSION

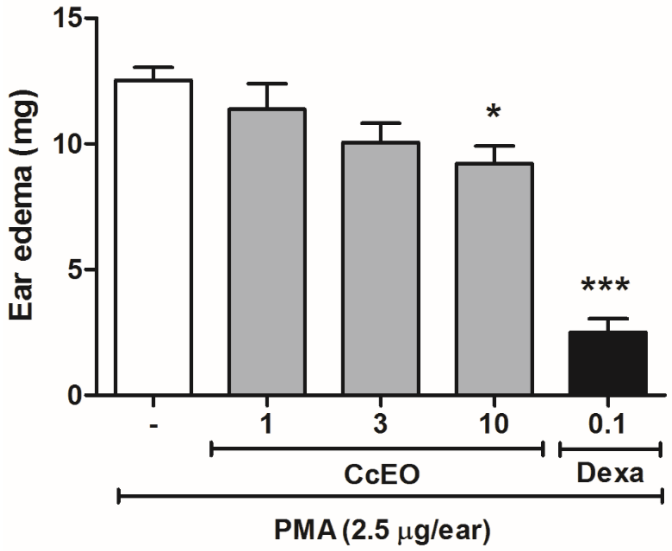
The *Croton* genus is characterized by a large chemical diversity. Based on the existing descriptions of chemical composition of many *Croton* species, this genus can be characterized as a promising source of bioactive molecules with a remarkable potential for discovery of novel biological activities. Growing interest in studying *Croton* species has resulted in isolation and identification of more than 100 currently known chemical compounds in Brazil (Angélico, 2011; Song et al., 2015).

Although not common in the Euphorbiaceae family, some *Croton* species should be highlighted for the production of different alkaloid classes (Salatino et al., 2007). Identification of this group of secondary metabolites by TLC in *C. cordiifolius* corroborates several studies in other species of the same genus. Salatino et al. (2007) described 35 alkaloid types from 14 *Croton* species. Among the species present in Brazilian caatinga, β-carboline alkaloids 2-ethoxycarbonyltetrahydroharman and 6-hydroxy-2-methyltetradydroharman have been isolated from *C. moritibensis* (Araújo-Júnior et al., 2004); anabasine and the novel guaiane-type alkaloids muscicapines A, B and C were isolated from roots of *C. muscicapa* (Araújo-Júnior et al., 2005). Alkaloids were also identified in *C. campestris* (Brito-Júnior et al., 2014) and *C. rhamnifolius* roots, and *C. rhamnifolioides* leaves, stem and roots (Randau et al., 2004); however, no compounds have been isolated.

Flavonoids are a class of polyphenols widely distributed in plants, including the genus *Croton*. Different studies have reported antioxidant, analgesic, antibacterial, antifungal, anti-inflammatory, antiviral, antitumor, antiallergic, and antiparasitic activities for these metabolites (Coellho et al., 2016). Among *Croton* species, it has not yet been possible to establish a standard for flavonoids. Although some compounds have already been isolated from hexanic, ethanolic and methanolic extracts of *Croton* spp., most investigations are not directed at the characterization of the flavonoid



A.



B.

Figure 1. A. Effect of *C. cordiifolius* ethanolic extract (CcEE, 0.1, 0.3 and 1 mg/ear), and **B.** essential oil (CcEO, 1, 3 and 10 µL/ear) on PMA-induced ear edema in mice. Dexamethasone (0.1 mg/ear) was used as positive control. Results are expressed as mean ± S.E.M. (n = 6) and analyzed by ANOVA with Bonferroni post test. * p <0.05, ** P <0.01, and *** p <0.001 compared to negative control group.

profile presented by the plant. A standardized analysis of the flavonoids present in different species of *Croton* may be an auxiliary tool in the differentiation of this genus subgroups.

In this context, the presence of flavonoids and phenylpropanoids in *C. cordiifolius* stem bark is consistent with the findings of Palmeira Júnior and collaborators (2005, 2006) for *C. sellowii* leaves and stem, containing the flavonoids artemetin, crysosplenetin, casticin, penduletin, tiliroside, and the phenylpropanoid threo-7-ethoxy-8-hydroxy-dihydroanol; and *C. brasiliensis* leaves and stem, containing casticin, penduletin,

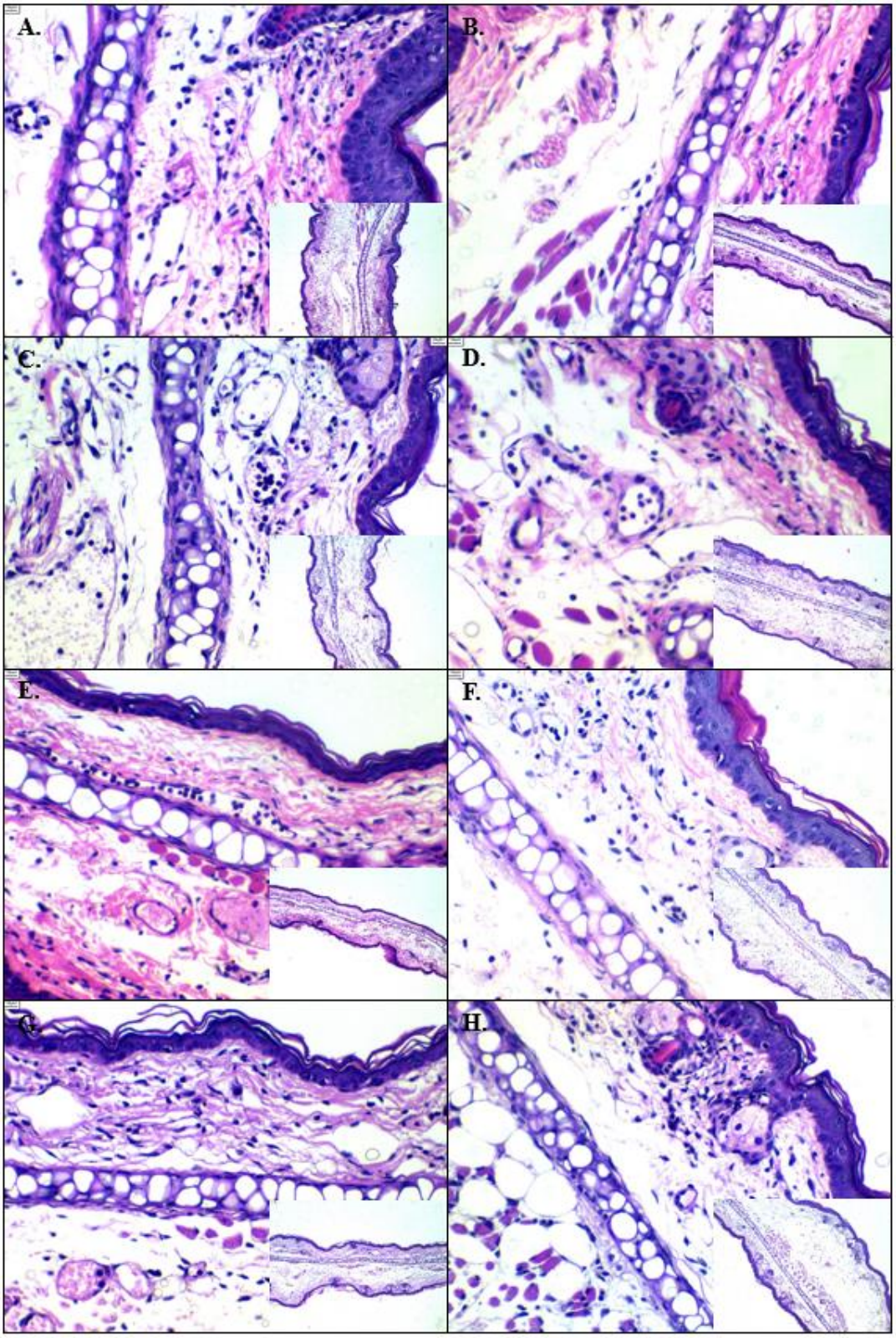


Figure 2. Histogical analyses of PMA-induced ear edema samples. **A.** Negative control group, **B.** Dexamethasone (0.1 mg/ear); **C.**, **E.**, and **G.** *C. cordiifolius* ethanolic extract (CcEE, 0.1, 0.3 and 1 mg/ear, respectively). **D.**, **F.**, and **H.** *C. cordiifolius* essential oil (CcEO, 1, 3 and 10 µL/ear, respectively). The ears were stained with hematoxylin and eosin. The tissue sections were observed under light microscope at 400x and 100x (detail). Scale bars represent 10 µm.

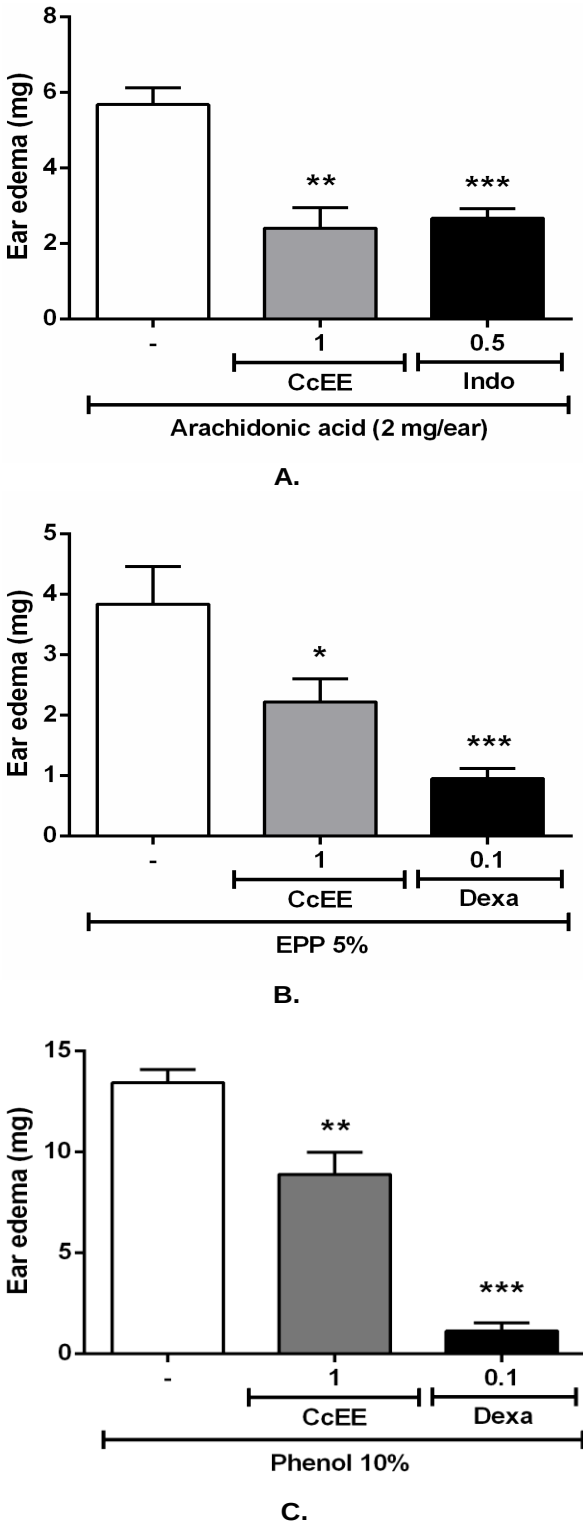


Figure 3. Effects of *C. cordiifolius* ethanolic extract (CcEE, 1 mg/ear) on **A.** arachidonic acid-, **B.** ethyl phenylpropriolate, and **C.** phenol-induced ear edema in mice. Indomethacin (Indo, 0.5 mg/ear) and dexamethasone (Dexa, 0.1 mg/ear) were used as positive controls. Results are expressed as mean ± S.E.M. (n = 6) and analyzed by ANOVA with Bonferroni post test. * $p < 0.05$, ** $P < 0.01$, and *** $p < 0.001$ compared to negative control group.

chrysosplenol-D and artemetin; of Morais and collaborators (1988) for *C. mucronifolius* aerial parts, who found found polyhydroxylated flavonoids; of Barreto and collaborators (2013) for *C. muscicarpa* leaves, stem and roots, which contains the flavonoids retusin, 3,7,4'-trimethoxy kaempferol, ombuine, pachipodol, kaempferol, casticin, 5-hydroxy-3,6,7,4'-tetramethoxyflavone and artemetin; of Coelho and collaborators (2016) for *C. betulaster* leaves, which isolated 5-hydroxy-7,4'-dimethoxyflavone, casticin, and penduletin and of Lopes and collaborators (2012) for *C. pedicellatus* leaves, who found seven flavonoids: tiliroside, 6"-*O*-*p*-coumaroyl-β-galactopyranosyl-kaempferol, 6"-*O*-*p*-coumaroyl-β-glucopyranosyl-3"-methoxy- kaempferol, kaempferol, 3-glucopyranosyl-quercetin and alpinumisoflavone, as well as 4-hydroxy-3,5-dimethoxybenzoic acid.

Although tannins are ubiquitous polyphenols in plants, only proanthocyanidins have been reported for *Croton* species, specifically those containing red latex such as *C. urucurana, C. lechleri* and *C. panamensis*. These metabolites were not found in Caatinga species (Salatino et al., 2007).

Terpenes and steroids are chemical constituents of interest owing to their anti-inflammatory, analgesic, antimicrobial, antiviral, hepatoprotective, and hormonal activities (Gupta et al., 1969; Mahato et al., 1988). Clerodane diterpenes are frequently found in the stem bark of *Croton* species, such as *C. cajucara, C. urucurana, C. lechleri*, but few were found in caatinga species, such as *C. brasiliensis*, from which clerodanes diterpenes crotobrasilins A and B were isolated from leaves and stem bark (Palmeira-Júnior et al., 2005). Diterpenes bearing the novel skeleton sarcopetalane, besides labdane, trachylobane, cembranoid, and halimane skeletons are not found in species from Caatinga.

The presence of triterpenes and steroids in *C. cordiifolius* agrees with the findings for *C. muscicarpa* leaves, stem, and roots, from which were isolated 6α-methoxy-cyperene, dammaradienol, squalene, acetyl aleuritolic acid and spathulenol (Barreto et al., 2013); *C. regelianus* leaves and stem, from which were found the bisnorditerpene rel-(5b,8a,10a)-8-hydroxy-13-methylpodocarpa-9(11),13-diene-3,12-dione and the guaiane sesquiterpene rel-(1R,4S,6R,7S,8aR)-decahydro-1-(hydroxymethyl)-4,9,9-trimethyl-4,7-(epoxymethano)azulen-6-ol (Torres et al., 2010); and *C. sellowii* leaves and stem, from which were isolated sitosterol, stigmasterol, cycloart-25-ene-3β,24β-diol, cycloart-25-ene-3β,24α-diol and betulonic acid (Palmeira-Júnior et al., 2006).

Coumarins, which are rare in the Euphorbiaceae family and *Croton* genus, were found in *C. cordiifolius* in this study. This result concurs with those reported by Lima and collaborators (2010), who related the presence of a coumarin in *C.adenocalyx*, representing an important role in the chemotaxonomy differentiation of these species. Essential oils have been described for various species of this genus (Almeida et al., 2015; LIMA et al., 2010; LEITE et al., 2015; Melo et al., 2013; Neves and Camara, 2012; Ramos et al., 2013; Turiel et al., 2016). Nogueira et al. (2015) described the constituents of the essential oil of *C. cordiifolius* leaves, which is rich in mono- and sesquiterpenes and whose main constituents were the monoterpenes 1,8-cineole and α-phellandrene. By contrast, the major constituents of the essential oil of *Croton cordiifolius* stem bark were the monoterpenes α- and β-pinene. α-pinene was also a major compound of the essential oil of *C. argyrophylloides* aerial parts (Morais et al., 2006), *C. conduplicatus* stem bark (Almeida et al., 2015), and *C. adenocalyx* leaves (Lima et al., 2010). Some authors have speculated that the co-occurrence of α- and β-pinene is a characteristic of the genus *Croton.* Others claim that β-caryophyllene and linalool are equally frequent major constituents (Almeida et al., 2015).

In addition to differences in essential oil constitution among distinct species of the genus *Croton*, several authors have demonstrated distinct essential oil compositions of different tissues from the same species (Brasil et al., 2009; Lima et al., 2010; Morais et al., 2006; Neves and Camara, 2012). In general, the essential oils of Brazilian *Croton* species present mono and sesquiterpenes as major components, with the exception of *C. zehntneri*, in which the phenylpropanoids eugenol and anetol predominate (Craveiro et al., 1981).

Most studies of the chemical constitution of the essential oil of *Croton* species refer to the leaves or aerial parts. Among the studies that report the constitution of the stem essential oil, it can be highlighted the one realized by Neves and Camara (2012) for *C. pulegioides*, with α-calacorene as the major compound, and for *C. heliotropiifolius*, with guaiol as the major compound; by Almeida et al. (2015) for *C. conduplicatus*, with α-pinene as the major compound; and by Suarez et al. (2005) for *C. molambo* with methyleugenol as the major compound.

The chemical composition of *Croton* species may be correlated with their geographical distribution. An example of this is the labdane and caurane diterpenes, which have not yet been reported for New World species; alkaloids, which were only found in American species; and the aromatic species, which are also prevalent in America (Salatino et al., 2007). Moreover, the presence of compounds found only in Brazilian caatinga species could indicate the existence of unique chemotypes within species of *Croton* found only in that biome (Angélico, 2011).

C. cordiifolius, C. heliotropiiflolius, and *C. conduplicatus* are popularly known as "quebra-faca" due to the rigidity of their stem (Randau et al., 2004). These species also present similar popular uses: *C. cordiifolius* is used to treat general inflammation, pain, and gastrointestinal disturbances (Nogueira et al., 2015); *C. heliotropiiflolius* is used as folk remedy for the treatment of wounds,

inflammation, fever, cancer, stomach pain, vomiting, bloody diarrhea and to alleviate fever (Neves and Camara, 2012; Randau et al., 2004); the decoction of *C. conduplicatus* leaves and stem bark is used in folk medicine to treat influenza, headache, indigestion, stomach problems and stomachache (Almeida et al., 2015).

In this context, the differentiation of their chemical composition and pharmacological activities, together with their botanical characterization, constitute a diagnostic tool for these species. Neves and Camara (2012) studied the chemical composition of the essential oil of *C. heliotropiiflolius* stem bark, observing a characteristic profile of the genus by the presence of β-caryophyllene as one of the major constituents. It differs from *C. cordiifolius* stem bark essential oil by the presence of sesquiterpenes and phenylpropanoid derivatives. Almeida et al. (2015) described for the first time the chemical constitution of the essential oil of *C. conduplicatus* stem bark, whose main compounds were α-pinene, β-pinene, camphor and (E)-caryophyllene. The chemical composition of *C. cordiifolius* stem bark essential oil is very similar, with α- and β-pinene as major constituents. The major difference is the presence of the monoterpene camphene as the third major constituent in *C. cordiifolius*, which can be converted into camphor in vivo. Randau et al. (2004) performed a phytochemical study of the extract of different parts of *C. rhamnifilolioides*. It was observed the presence of alkaloids, triterpenes and steroids, sugars, flavonoids and phenylpropanoglycosides. Therefore, the presence of coumarins in *C. cordiifolius* bark extract can be considered a useful marker in the differentiation of these species. Despite the existence of some studies that point to chemical patterns in the genus, given its variability and the small sampling of species chemically studied, definitive conclusions can not be drawn about chemical relationships among *Croton* species (Salatino et al., 2007).

Many *Croton* species studies have reported outstanding antioxidant activities, which set a precedent for the results of this study of *C. cordiifolius*. Morais and collaborators (2006) evaluated the antioxidant activities of *C. zenhtneri*, *C. nepeatefolius,* and *C. argyrophylloides* essential oils. This activity is probably due to the presence of E-anethole in *C. zenhtneri*, methyleugenol in *C. nepetaefolius*, and α-pinene, E-caryophyllene, and 1,8-cineole in *C. aryrophylloides*. Angélico (2011) reported antioxidant activities in *C. blanchetianus* and *C. heliotropiifolius* extracts, attributing it to the possible presence of phenolic compounds belonging to the tannin and flavonoid classes.

Chronic inflammation mediates the development of various diseases, and increased levels of free radicals have been found in many pathological conditions other than inflammation, including cancer, ischemic disorders, and dementia (Ramos et al., 2013). The search for medicinal plants with anti-inflammatory and antioxidant activities is fundamental to direct the development of pharmaceutical products designed to treat major diseases.

Mechanisms of anti-inflammatory activity can be elucidated through research with animal models in which edema is induced by different phlogistic agents with different well-understood mechanisms. To identify topical anti-inflammatory mechanisms of *C. cordiifolius*, we used five phlogistic agents with distinct mechanisms in a mouse model of topical inflammation.

Ear edema induced by PMA is primarily mediated by prostaglandin E2 and protein kinase C. This model is useful for screening topical active extracts and/or compounds, and anti-inflammatory efficacy implies the presence of prostaglandin and leukotriene biosynthesis inhibitors (Silva et al., 2005). In this model, CcEE probably inhibited edema by directly interfering with prostaglandins' action or by inhibiting enzymes such as cyclooxygenase or phospholipase A2 (PLA2). According to Nair and collaborators (2006), the flavonoid quercetin decreased production and expression of proinflammatory cytokine TNF-α in human mononuclear cell cultures stimulated by phorbol myristate acetate (PMA). This effect was associated with inhibition of NF-kB. Other flavonoids, such as santin and ermanin, also inhibited PMA-induced ear edema in mice, being more active than the positive controlindomethacin at identifcal concentrations (3 mg/ear) (Martinez et al., 1997). Thus, the presence of flavonoids demonstrated in phytochemical screening and quantified by spectrophotometry could contribute to this activity.

The inflammatory process instigated by AA is mediated by production of metabolites such as prostaglandin E2 (PGE2), leukotriene C4 (LTC4), and leukotriene D4 (LTD4) (Humes et al., 1986). The ear edema induced by AA is characterized by sensitivity to inhibitors of cyclooxygenase and lipoxygenase enzymes. Therefore, AA is commonly used to validate substances capable of inhibiting the action of mediators of edema (Crummey et al., 1987; Opas et al., 1986; Humes et al., 1986). This model is not sensitive to PLA2 inhibitors such as glucocorticoids. CcEE probably inhibit edema in this model by preventing the metabolism of arachidonic acid to prostaglandins and leukotrienes or by inhibiting the action of its metabolites. In the literature, there is no evidence of topical anti-inflammatory activity in that model from species of the genus *Croton*.

Because ethyl phenylpropiolate (EPP) is responsible for release of inflammatory mediators such as histamine and serotonin (Nualkaew et al., 2009), CcEE may interfere with the release of these inflammatory mediators. Phenol-induced ear edema mimics contact dermatitis processes visualized in humans (Lim et al., 2004). The intense irritation resulting from application of this phlogistic agent is a consequence of disruption of keratinocytes plasma membranes, with release of

preformed IL-1α, IL-8, TNF-α, free radicals, and inflammatory mediators such as AA downstream metabolites.

Although this process occurs independently of AA metabolites, the mentioned cytokines (IL-1α, IL-8, TNF-α) can induce production and release of AA and reactive oxygen species (Lim et al., 2004; Murray et al., 2007; Wilmer et al. 1994). In contrast to PMA, phenol activates nuclear transcription factors through protein kinase C dependent pathways to induce inflammatory cytokines. Despite using different routes, both models produce arachidonic acid metabolites and reactive oxygen species in the inflammatory response (Wilmer et al., 1994). In this context, the performance of CcEE in reducing production of inflammatory mediators and cytokines through protein kinase C dependent pathways can be highlighted.

Capsaicin, an alkaloid obtained from species of the genus *Capsicum*, mediates a neurogenic inflammatory response characterized by vasodilation-induced edema with increased blood flow, and plasma extravasation, in addition to sensitized nociceptors responsible for contact induced-pain sensation in skin or mucous membranes (Zegarska et al., 2006). In this process, TRPV-1 receptors are activated, resulting in the release of neuropeptides, such as substance P, which are responsible for erythema and papule formation and macrophage degranulation, followed by release of substances such as histamine and serotonin (Inoue et al., 1993).

Topical application of capsaicin promotes the release of pro-inflammatory mediators which result in an immediate vasodilation and erythema response followed by edema, which peaks within 30 minutes after administration (Gabor, 2000; Zegarska et al., 2006). CcEE did not reduce capsaicin-induced edema, suggesting that it does not act on TRPV-1 receptors, and therefore does not inhibit mast cell degranulation or release of histamine and serotonin, mediators responsible for increasing cell permeability and edema (Inoue et al., 1993).

The anti-inflammatory activity of *C. cordiifolius* found in this study corroborates the ethnobotanical survey conducted by Nogueira and collaborators (2015), which showed the highest number of “quebra-faca” citations for inflammation and pain treatment.

Conclusions

The first of its kind phytochemical survey of *Croton cordiifolius* stem bark extract and the characterization of its essential oil provide important information for directing future research aimed at isolating chemical constituents of this species, which is widely used in Northeast Brazilian folk medicine.

Our *in vivo* anti-inflammatory activity tests showed that *C. cordiifolius* essential oil is not effective at the doses tested. In contrast, the extract contained anti-inflammatory compounds with different mechanisms of action because it showed significant activity in four of the five assays. Our results suggest that the extract can interfere with AA metabolism and prostaglandin biosynthesis, release of inflammatory mediators such as histamine and serotonin, and inhibition of preformed cytokine release. No inhibition of TRPV-1 receptors or mast cells degranulation was observed. Additionally, the observed antioxidant activity of *C. cordiifolius* stem bark extract could be, in part, responsible for its anti-inflammatory effects.

Conflict of interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

Authors are grateful to Propesq/UFPE for research grants.

REFERENCES

Adams RP (2001). Identification of essential oil components by gas chromatography/quadrupole mass spectroscopy. 3. Ed. Allured Publishing Corporation, Carol Stream.

Almeida ABA, Melo PS, Hiruma-Lima CA, Gracioso JS, Carli L, Nunes DS, Haun M, Brito ARMS (2003). Antiulcerogenic Effect and Cytotoxic Activity of Semi-synthetic Croton in Obtained from *Croton cajucara* Benth. Eur. J. Pharmacol. 472(11):205-212.

Almeida JRGS, Souza AVV, Oliveira AP, Santos US, Souza MD, Bispo LP, Turatti ICC, Lopes NP (2015). Chemical Composition of Essential Oils from the Stem Barks of *Croton conduplicatus* (Euphorbiaceae) native to the Caatinga Biome. African J. Pharm. Pharmacol. 9(4):98-101.

Angélico EC (2011). Avaliação das Atividades Antibacteriana e Antioxidante de *Croton Heliotropiifolius* Kunte e *Croton Blanchetianus* Baill. Dissertação (Mestrado em Zootecnia), Universidade Federal de Campina Grande, Patos.

Araújo-Júnior VT, Silva MS, Cunha EVL, Agra MF, Athayde-Filho PF, Vieira IJC, Braz-Filho R, Barbosa-Filho JM (2005). Muscicapines, a New Class of Guaiane-Type Sesquiterpene Alkaloids from *Croton muscicapa*. J. Braz. Chem. Soc. 16(3b):553-557.

Araújo-Júnior VT, Silva MS, Cunha EVL, Agra MF, Silva-Filho RN, Barbosa-Filho JM, Braz-Filho R (2004). Alkaloids and Diterpenes From *Croton moritibensis.* Pharm. Biol.42(1):62-67.

Barreto MB, Gomes CL, Freitas JVB, Pinto FCL, Silveira ER, Gramosa NV (2013). Flavonoides e terpenoides de *Croton muscicarpa* (Euphorbiaceae). Quim. Nova. 36(5):675-679.

Brasil DS, Muller AH, Guilhon GMSP, Alves CN, Andrade EHA, Silva JKR, Maia JGS (2009). Essential Oil Composition of *Croton palanostigma* Klotzsch from North Brazil. J. Braz. Chem. Soc. 20(6):1188-1192.

Brito-Júnior FE, Oliveira DR, Boligon AA, Athayde ML, Kamdem JP, Macedo GE, Silva GF, Menezes IRA, Costa JGM, Coutinho HDM, Kerntopf MR, Posser T (2014). Protective effects of Croton campestris A. St-Hill in different ulcer models in rodents: Evidence for the involvement of nitric oxide and prostaglandins. J. Ethnopharmacol. 153:469-477.

Carlson RP, O’Neyll-Davis L, Chang J, Lewis AJ (1985). Modulation of mouse ear edema by cyclooxygenase and fipoxygenase inhibitors and other pharmacologic agents. Ag. Actions. 17(2):197-204.

Coelho PLC, Freitas SRVB, Pitanga BPS, silva VDA, Oliveira MN, Grangeiro MS, Souza CS, El-Bachá RS, Costa MFD, Barbosa PR,

Craveiro AA, Rodrigues AS, Andrade CHS, Matos FJA, Alencar JW, Machado MIL (1981). Volatile constituents of Brazilian Euphorbiaceae genus *Croton*. J. Nat. Prod. 44:602-608.

Crummey A, Harper GP, Boyle EA, Mangan FR (1987); Inhibition of arachidonic acid-induced ear oedema as a model for assessing topical anti-inflammatory compounds. Ag. Actions 20:69-76.

Gabor M (2000). Mouse Ear Inflammation Models and their Pharmacological Applications. Budapeste: Akadémiai Kiadó.
Humes JL, Opas EE, Galavage M, Soderman D, Bonney RJ (1986). Regulation of macrophage eicosanoid production by hydroperoxy and hydroxi-eicosatetraenoic acids. Biochem. J. 233:199-206.
Inoue H, Nagata N, Koshihara Y (1993). Profile of capsaicin-induced mouse ear oedema as neurogenic inflammatory model: comparison with arachidonic acid-induced ear edema. Braz. J. Pharmacol. 110:1614-1620.
Leite TR. Silva MAP, Santos MAF, Santos ACB, Costa NC (2015). Allelopathic Activity and Chemical Analysis of the Essential Oil of *Croton limae* A. P. S. Gomes, M. F. Sales & P. E. Berryc (Euphorbiaceae). J. Agr. Sci. 7(11):90.
Lim H, Park H, Kim HP (2004). Inhibition of contact dermatitis in animal models and suppression of proinflammatory gene expression by topically applied flavonoid, wogonin. Arch. Pharmacol. Res. 27:442-448.
Lima GS, Castro-Pinto DB, Machado GC, Maciel MAM, Echevarria A (2015). Antileishmanial Activity and Trypanothione Reductase Effects of Terpenes from the Amazonian Species *Croton cajucara* Benth (Euphorbiaceae). Phytomedicine. 22:1133-1137.
Lima SG, Citó AMGL, Lopes JAD, Neto JMM, Chaves MH, Silveira ER (2010). Fixed and volatile constituents of genus *Croton* plants: *C. Adenocalyx* baill – Euphorbiaceae. Rev. Latinoam. Quim. 38(3):133-144.
Lopes EL, Andrade-Neto M, Silveira ER, Pessoal ODL (2012). Flavonoides de sesquiterpenos de *Croton pedicellatus* Kunth. Quim. Nova. 35(11): 2169-2172.
Mahato SB, Sakar SK, Poddar G (1988). Triterpenoid saponins. Phytochemistry. 37:3037-3067.
Martinez J, Silván AM, Abad MJ, Bermeio P, Villar A, Sölhuber M (1997). Isolation of two flavonoids from Tanacetum microphyllum as PMA-induced ear edema inhibitors. J. Nat. Prod. 60(2):142-144.
Melo GFA, Costa ACV, Carino-Junior F, Medeiros RS, Madruga MS, Queiroga-Neto V (2013). The sensitivity of bacterial foodborne pathogens to *Croton blanchetianus* Baill essential oil. Braz. J. Microbiol. 44(4):1189-1194.
Morais SM, Catunda-Júnior FEA, Silva ARA, Martins-Neto JS (2006). Atividade Antioxidante de Óleos Essenciais de *Croton* no Nordeste do Brasil. *Quim Nova* 29(5):907-910.
Morais SM, Matos JÁ, Braz-Filho R (1988). Estudo químico de *Croton mucronifolius* Muell. Arg. (Velaminho). Supl. Acta Amazonica 18(1-2):471-472.
Murray AR, Kisin E, Castranova V, Kommineni C, Gunther MR, Shyedova AA (2007). Phenol-induced in vivo oxidative stress in skin: evidence for enhanced free radical generation, thiol oxidation, and antioxidant depletion. Chem. Res. Toxicol. 20:77-1769.
Nair MP, Mahaian S, Reynolds JL, Aalinkeel , Nair H, Shwarts AS, Kandaswami C (2006). The flavonoid quercetin inhibits proinflammatory cytokine (tumor necrosis factor alpha) gene expression in normal peripheral blood mononuclear cells via modulation of the NF-κβ system. Clin. Vacc. Immunol. 13:319-328.
Neves IA, Camara CAG (2012). Volatile Constituents of Two *Croton* Species from Caatinga Biome of Pernambuco – Brasil. Rec. Nat. Prod. 6(2):161-165.
Nogueira LM, Silva MR, Santos SM, Albuquerque JFC, Ferraz IC, Albuquerque TT, Mota CRFC, Araújo RM, Viana GSB, Martins RD, Havt A, Ximenes RM (2015). Antinociceptive Effect of the Essential Oil Obtained from the Leaves of *Croton cordiifolius* Baill. (Euphorbiaceae) in Mice. Evid. Based Complement. Alternat. Med. 1-7.
Nualkaew S, Rattanamanee K, Thongpraditchote S, Wongkrajang Y, Narstedt A (2009). Anti-inflammatory, analgesic and wound healing activities of the leaves of *Memecylon edule* Roxb. J. Ethnopharmacol.121: 278-281.
Opas EE, Bonney RJ, Humes JL (1985). Prostaglandin and Leukotriene Synthesis in Mouse Ears Inflamed by Arachidonic Acid; J. Invest. Dermatol. 84:253-256.
Palmeira-Jpunior SF, Alves VL, Moura FS, Veira LFA, Conserva LM, Lemos RPL (2006). Constituintes químicos das folhas e caule de *Croton sellowii* (Euphorbiaceae). Rev. Bras. Farmacogn. 16(3):397-402.
Palmeira-Júnior SF, Conserva LM, Silveira ER (2005). Two clerodane diterpenes and flavonoids from *Croton brasiliensis*. J. Braz. Chem. Soc. 16: 1420-1424.
Pietro P, Pineda M, Aguilar M (1999) Spectrophotometric quantification of antioxidante capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Analyt. Biochem. 269(2):337-341.
Ramos JMO, Santos CA, Santana DG, Santos DA, Alves PB, Thomazzi SM (2013). Produção de matéria seca e óleo essencial de menta sob diferentes doses de fósforo. Rev. Bras. Plant. Med. 8(1):9-12.
Randau KP, Florêncio FC, Ferreira CP, Xavier HS (2004). Estudo farmacognóstico de *Croton rhamnifolius* H.B.K. e *Croton rhamnifolioides* Pax & Hoffm. (Euphorbiaceae). Rev. Bras. Farmacognos. 14(2):89-96.
Rocha FF, Neves EMN, Costa EA, Matos LG, Müller AH, Guilhon GMSP, Cortes WS, Vanderlinde FA (2008). Evaluation of Antinociceptive and Anti-Inflamatory Effects of *Croton pullei* var. Glabrior Larnj. (Euphorbiaceae). Rev. Bras. Farmacogn.18(3):344-349.
Rodrigues GR, Di Naso FC, Porawski M, Marcolin E, Kretzmann NA, Ferraz AB, Richter MF, Marroni CA, Marroni MP (2012). Treatment with Aqueous Extract from *Croton cajucara* Benth Reduces Hepatic Oxidative Stress in Streptozotocin-Diabetic Rats. J. Biomed. Biotechnol. 2012:1-7.
Salatino A, Salatino MLF, Negri G (2007). Tradicional Uses, Chemistry and Pharmacology of *Croton* Species (Euphorbiaceae). J. Braz. Chem. Soc. 18(1):11-33.
Shukla S, Mehta A, John J, Singh S, Mehta P, Vyas SP (2009). Antioxidant Activity and Total Phenolic Content of Ethanolic Extract of *Caesalpinia bonducella* Seeds. Food Chem. Toxicol. 47:1848-1851.
Silva JKR, Silva JC, Guilhon JMSP, Muller AH, Santos LS, Arruda MSP, Secco RS (2005). Flavonoides das folhas de *Croton lobatus* e das partes aéreas de *Croton trinitatis* (Euphorbiaceae). 28ª Reunião Anual da Sociedade Brasileira de Química, Poços de Caldas.
Silva MA, Pessotti BMS, Zanini SF, Colnago GL, Nunes LC, Rodrigues MRA, Ferreira L (2010). Uso de óleo de aroeira-vermelha sobre o desempenho e a morfometria intestinal de frangos de corte. Cienc. Rural 40(10):2151-2156.
Song JT, Han Y, Wang XL, Shen T, Lou HX, Wang XN (2015). Diterpenoids from the Twigs and Leaves of *Croton caudatus*. Fitoterapia, 107:54-59.
Souza RC, Kill LHP, Araújo JLP (2012). Fenologia de Espécies Nativas da Caatinga de Potencial Medicinal na Região de Petrolina, PE. In: Anais da VII Jornada de Iniciação Científica da Embrapa Semiárido e I Jornada de Iniciação Científica Da Facepe/Univasf, Petrolina. Disponível em Available at: http://ainfo.cnptia.embrapa.br/digital/bitstream/item/74884/1/KIILL-2012-1.pdf
Suarez AI, Alirica I, Vásques LJ, Manzano MA, Compagnone RS (2005). Essential oil composition of *Croton cuneatus* and *Croton molambo* growing in Venezuela. Flavour Frag. J. 20:611-614.
Torres MCM, Braz-Filho R, Silveira ER, Diniz JC, Viana FA, Pessoa ODL (2010). Terpenoids from Croton regelianus. Helvet. Chim. Acta. 93:375-381.
Torrico F, Cepeda M, Guerrero G, Melendez F, Blanco Z, Canelón DJ, Diaz B, Compagnone RS, Suárez AI (2007). Hypoglycaemic Effect of *Croton cuneatus* in Streptozotocin-induced Diabetic Rats. Rev. Bras. Farmacogn. 17(2):166-169.
Turiel NA, Ribeiro AF, Carvalho NCC, Monteiro OS, Lucas FCA, Carreira LMM, Andrade EHA, Maia JGS (2016). Variability in Essential Oil Composition of *Croton* Species with Occurrence in the Eastern Brazilian Amazon. Rec. Nat. Prod.10(3):380-384.
Wilmer JL, Burleson FG, Kayama F, Kanno J, Luster MI (1994). Cytokine induction in human epidermal keratinocytes exposed to contact irritants and its relation to chemical – induced inflammation in mouse skin. J. Invest. Dermatol. 102 915-922.
Woisky RG, Salatino A (1998). Analysis of propolis: some parameters and procedures for chemical quality control. J. Apicult. Res. 37(2):99-105.
Zegarska B, Leliñska A, Tyrakowsk T (2006). Clinical and experimental aspects of cutaneous neurogenic imflammation. Pharmacol. Rep. 58:13-21.