



Topical anti-inflammatory activity in TPA-induced mouse ear edema model and in vitro antibacterial properties of *Cordia alba* flowers

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Received: 18 July 2018 / Accepted: 22 November 2018 / Published online: 27 November 2018
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Abstract

Cordia is a genus of trees and bushes widely distributed in warm regions around the world. *Cordia alba* is used in the Colombia Caribbean Coast to treat inflammatory and infectious health issues. The aim of this work was to evaluate the anti-inflammatory and antibacterial activity of the total ethanolic extract obtained from the flowers of *Cordia alba*. The plant material was extracted by maceration with ethanol and the anti-inflammatory effect was evaluated using the in vivo model of TPA-induced mouse ear edema and MPO enzyme activity. Additionally, the scavenging activity of the DPPH and ABTS free radicals by the extract was determined. The antibacterial activity was assessed using the broth microdilution method. The total ethanolic extract of *Cordia alba* flowers decreased the edema and the MPO activity in the ear tissue and showed scavenging effects of the DPPH and ABTS free radicals. This extract also presented antibacterial activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

Keywords Anti-inflammatory · Antibacterial · Ear edema · *Cordia alba*

Introduction

For a long time, humanity has used plants for the treatment of diseases, which continue to be one of the most important therapeutic alternatives, estimating that between 70–80% of the world's population relies on traditional medicine to deal with their primary health issues, a tendency acknowledged and approved by the World Health Organization. Natural products have always contributed to the development of modern-day medicine, bringing the discovery of antibiotics, anticancer agents, analgesics and anti-inflammatory compounds (Fabricant and Farnsworth 2001; Kingston 2010; Sen and Samanta 2015). About half of the FDA approved drugs between 1980 and 2010, were natural products, their derivatives, or synthetic mimetics related to natural products (Chen et al. 2015; Ovadje et al. 2015). In this sense, drug discovery based on the study of medicinal plants remains as

an important area in the search for compounds with potential pharmacological activity.

Cordia alba (Jacq.) Roem. and Schult. is native to South and Central America (Cervantes Ceballos et al. 2017). The flowers of this species are widely used in the Caribbean Colombian Coast to treat inflammatory and infectious ailments. Taking into account the use of *Cordia alba* in the folk medicine, this species constitute a promising source of metabolites that could be used for the development of new anti-inflammatory and antibacterial drugs, which are necessary to the medical community, as bacterial resistance to antibiotics has become a public health problem (Fair and Tor 2014) and the anti-inflammatory agents currently used, especially the ones used chronically, have low efficacy and multiple side effects (Joshi et al. 2007; Makris et al. 2010; Harirforoosh et al. 2014). In this work we evaluated the in vivo anti-inflammatory effect, in vitro free radical scavenger capacity and in vitro antibacterial activity of the total ethanolic extract obtained from the flowers of *Cordia alba*.

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Materials and methods

Animals

6–7 weeks old, female ICR mice (25–30 g) were provided by the Instituto Nacional de Salud (Bogotá—Colombia) and kept at the animal house of the University of Cartagena. They were housed in standard cages under standard conditions (22 ± 3 °C and 65–75% of relative humidity) with a 12-h dark/light cycle and standard food and water ad libitum. All experiments were designed and conducted in accordance with the guidelines of the Ethics Committee of University of Cartagena (Minute No. 32 of August 4 of 2011) and the European Union regulations (CEC council 86/809).

Collection and identification of plant material

Flowers of *Cordia alba* were collected at Pueblo Nuevo, Bolivar, Colombia ($10^{\circ}44'26''\text{N}$; $75^{\circ}15'43''\text{W}$; elevation 5 m.a.s.l.), identified in the herbarium of the University of Antioquia (Medellin—Colombia) by the biologist Felipe Alfonso Cardona Naranjo and deposited with the identification code HUA 175,329.

Extract preparation

Dried and powdered flowers of *Cordia alba* (176.4 g) were exhaustively extracted with ethanol by maceration at room temperature (25 ± 3 °C). The extract was then filtered and concentrated using a rotary evaporator (Hei-Vap Value, Hei-dolph, Germany) under controlled temperature (45 – 50 °C) to obtain 20.45 g of total extract.

Phytochemical screening, flavonoids and phenolic compounds quantification

The total extract was screened for the presence of alkaloids, coumarins, flavonoids, cardiotoxic glycosides, leucoanthocyanidins, phenolic compounds, quinones, saponins, tannins, and triterpenoids/steroids (Sanabria-Galindo et al. 1997; Thirupathi et al. 2008). Phenolic compounds and flavonoids were quantified using the Folin-Ciocalteu and the aluminum trichloride method respectively (Rivera et al. 2018).

TPA-induced ear edema and myeloperoxidase assay

The anti-inflammatory activity was determined using the *in vivo* murine model of 12-O-tetradecanoyl-phorbol-13-acetate (TPA)-induced ear edema and MPO activity was determined in the tissue supernatants obtained from the ear edema. Assays were performed as previously described

(Rivera et al. 2018). Briefly, a TPA/acetone solution (0.125 µg/µL) was applied topically in both the internal and external side of the mice ear (10 µL/side) to induce inflammation. Immediately after, acetone solutions of *Cordia alba* extract (1000 µg/ear) and indomethacin (500 µg/ear) were applied on the inner and outer surface of the ear. The left ear was treated with just acetone as a control. Four hours after the treatment application, the animals were sacrificed by cervical dislocation and circular sections from both ears (7 mm) were taken and weighted to calculate the edema as weight difference between treated and non-treated ear. The inhibition of inflammation in the treated animals was determined in comparison to the TPA group.

MPO enzyme activity was determined in the tissue supernatants obtained from the ear edema assay, as an indirect measure of neutrophil infiltration. Ear tissue homogenates were prepared with phosphate buffer (pH 7.4). Samples were centrifuged at $10,000$ rpm at 4 °C, the pellet obtained was suspended in a solution of HTAB 0.5% and EDTA 0.3% on phosphate buffer (pH 6.0). The homogenate obtained underwent two fast freeze–thaw cycles, sonication for 10 s and lastly it was centrifuged for 10 min at $10,000$ rpm at 4 °C. The recovered supernatant was used to evaluate the MPO activity, 50 µL of the supernatant were mixed with 50 µL of O-Dianisidine (0.067%) and 50 µL of hydrogen peroxide (H_2O_2 0.003%). OD_{450} was determined using a Multiskan EX Thermo. The results are presented as enzymatic activity units, where an activity unit is defined as the amount of enzyme capable of degrading 1 µmol of hydrogen peroxide in a minute at 25 °C.

DPPH and ABTS radicals scavenging activity

DPPH (1,1-diphenyl-2-picrylhydrazyl) scavenging activity was determined through the method by Brand-Williams et al. (1995) with modifications, 75 µL of the different concentrations of the extract were added to 150 µL of a methanolic solution of DPPH (100 µg/mL). The mix was incubated at room temperature (25 ± 3 °C) for 30 min; after incubation, the absence of DPPH radical was measured spectrophotometrically at OD_{517} in a Multiskan EX Thermo. Trolox (50 µM) was used as a positive control and the vehicle as negative control.

Scavenging of the ABTS 2,2'-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) radical was determined with the method by Pellegrini et al. (1999), with modifications (Rivera 2018). The ABTS radical was produced by the reaction of ABTS (7 mM) with potassium persulfate (2.45 mM), incubated in the darkness for 16 h at room temperature. The ABTS radical solution was diluted with ethanol until it had an absorbance of 0.8 ± 0.3 at 734 nm, 180 µL of the diluted solution were mixed with 20 µL of different concentrations of the tested extract. This mix was incubated at room

temperature (25 ± 3 °C) for 30 min, and then the absence of ABTS radical was measured spectrophotometrically at OD₇₃₄ in a Multiskan EX Thermo. Trolox (50 µM) was used as a positive control and the vehicle as negative control.

In vitro antibacterial activity

The evaluation of the *C. alba* extract was performed following the protocol by Rivera et al. 2015. A bacterial suspension correspondent to 0.5 of the McFarland scale (1 × 10⁸ UFC/mL) was prepared and diluted to obtain a working suspension (5 × 10⁵ UFC/mL). Equal volumes of the extract (1000 µg/mL) and the bacterial suspension (*Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Staphylococcus aureus*), were incubated during 12 h at 37 ± 2 °C. Gentamicin (16 µg/mL) was employed as positive control. The OD₆₂₀ was determined after the incubation period and bacterial proliferation was estimated according to the maximum growth control. The IC₅₀ was determined when the extract it inhibited over 50% of bacterial growth. The *C. alba* extract was dissolved in dimethyl sulfoxide.

Statistical analysis

Results were expressed as mean ± standard error of the mean (S.E.M) and analysed using one-way analysis of variance (ANOVA), followed by Dunnett’s or Tukey’s post hoc test, to determine the differences between groups. Values of p < 0.05 were considered significant. IC₅₀ value was calculated using nonlinear regression analysis.

Results and discussion

Cordia is a genus of trees and bushes widely distributed in warm regions. Around 300 species have been identified all over the world. The plants of this genus are used in the traditional medicine as scaring, astringent, anthelmintic, anti-malarial, diuretic, antibacterial and anti-inflammatory. Some species in the genus, like *Cordia cylindrostachya* Roem. & schult, *Cordia myxa* L., and *Cordia verbenacea* DC, possess scientifically proven traditional uses as anti-inflammatory and antibacterial agents (Thirupathi et al. 2008; Matias et al. 2015). However, for the flowers of *Cordia alba*, this would

constitute the first scientific report supporting the traditional use for these biological activities.

The TPA application produced a strong inflammatory response in the mice ear, with massive edema formation and influx neutrophils, which were significantly decreased by extract of the *Cordia alba* flowers, with inhibition values similar to those shown by the positive control, indomethacin (Table 1; Fig. 1). These results show the potential anti-inflammatory activity of this species. Neutrophils are cells that participate in the immune defense of the host against pathogens, they have been considered as the first line of defense for the organism, although they are also involved in the adaptive immunity. These cells constantly circulate the blood stream, until recruitment to the peripheral tissues in case of pathogen invasion or tissue damage, like the one caused by the TPA in the ear (Sadik et al. 2011; Móc-sai 2013). During the inflammatory response, neutrophils release enzymes like myeloperoxidase (MPO) and free radicals like ROS which contribute to host defense. MPO is one of the most abundant proteins in neutrophils; it is stored in the azurophil granules and is released after the activation of cells (Lee et al. 2003; Metzler et al. 2011; Prokopowicz et al. 2012). Topical application of TPA activates protein kinase

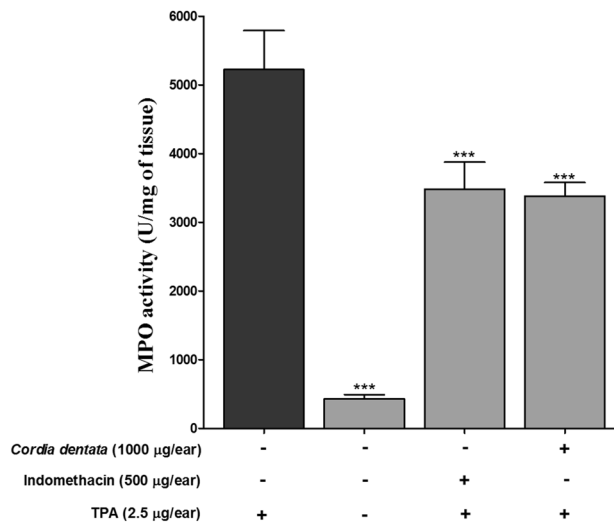


Fig. 1 Effect of the total extract from *Cordia alba* flowers on MPO activity in ear tissue. Results are expressed as mean ± SEM. (n=6) (***)p < 0.001 ANOVA, statistically significant vs TPA group)

Table 1 Effect of the total extract from *Cordia alba* flowers on TPA-induced ear edema model

Groups	Dose (mg/ear)	Ear weight increase (mg)	Inhibition %
Control (TPA + acetone)	–	13 ± 0.5	–
Indomethacin	0.5	5.8 ± 0.7 ***	55 ± 5.8
<i>Cordia alba</i>	1.0	7.5 ± 0.4***	42 ± 2.8

Results are expressed as mean ± SEM. (n=6)

***p < 0.001 ANOVA, statistically significant vs control group (TPA + acetone)

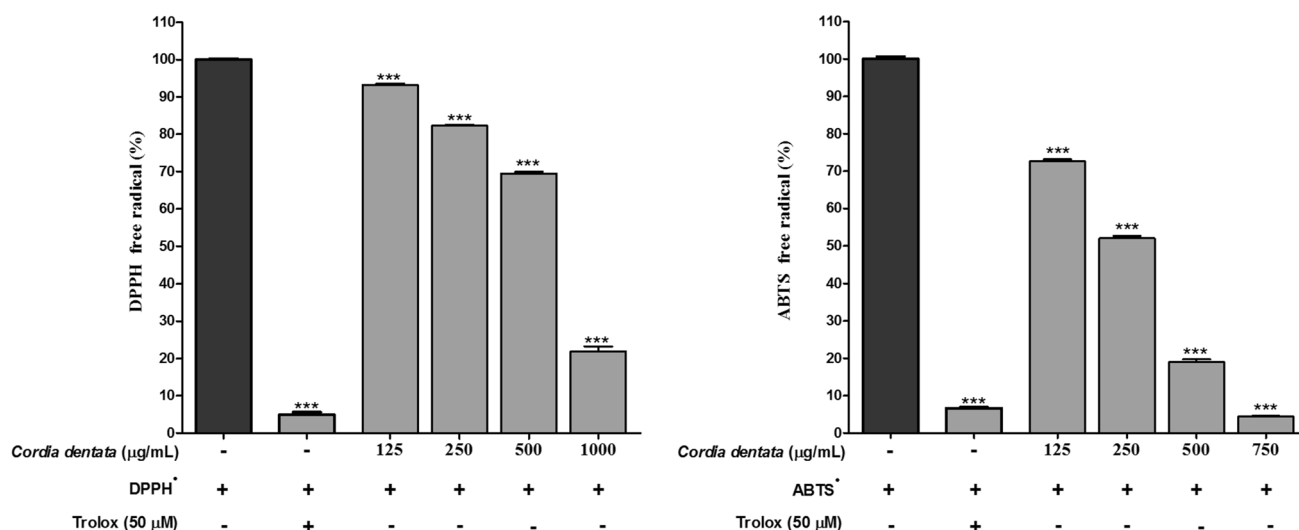


Fig. 2 Scavenging effect of DPPH and ABTS free radicals by the total extract from *Cordia alba* flowers. Results are expressed as mean \pm SEM. (n = 8) (***) $p < 0.001$ ANOVA, statistically significant vs control group)

Table 2 Effect of the total extract from *Cordia alba* flowers against *P. aeruginosa*, *K. pneumoniae* and *S. aureus* growth

Strains	Inhibition %	IC ₅₀ (µg/mL)
<i>P. aeruginosa</i>	85.30 \pm 3.51	107.04 (68.73–140.93)
<i>S. aureus</i>	39.71 \pm 3.42	n.d
<i>K. pneumoniae</i>	9.14 \pm 7.23	n.d
Gentamicin	74.16 \pm 0.56	n.d

Results are expressed as mean \pm SEM. (n = 8)

n.d. not determined

C, promoting the synthesis of chemokines by epidermal cells and therefore neutrophil infiltration (Nakadate 1989). *Cordia alba* is presented as a species with potential value in the treatment of chronic inflammatory diseases, since the total extract obtained from *Cordia alba* flowers reduced the neutrophil infiltration caused by the TPA, decreasing inflammation and minimizing the damage caused by the ROS produced by neutrophils in the tissue. Additionally, the extract may also do it directly by scavenging of ROS, as it showed a scavenging activity of the tested radicals in a concentration-dependent manner, with IC₅₀ values of 673.6 \pm 1.5 µg/mL for DPPH and 276.7 \pm 1.3 µg/mL for ABTS (Fig. 2).

P. aeruginosa, *K. pneumoniae* and *S. aureus* affect mostly immunocompromised hosts, being responsible a high percentage of the development of nosocomial infections. Antibiotic resistance in these strains is now a really important issue that restricts the therapeutic alternatives (Shon et al. 2013; Sedighi et al. 2014). In this study, we demonstrated that the *Cordia alba* flowers extract has an inhibitory effect on the growth of *P. aeruginosa* and *S. aureus*, similar to other *Cordia* species (Table 2) (Okusa et al. 2007; Moura-Costa

Table 3 Phytochemical constituents of *Cordia alba* flowers

Metabolite	<i>Cordia alba</i> extract
Alkaloids	–
Coumarins	+++
Flavonoids	+++
Cardiotonic glycosides	++
Leucoanthocyanidins	–
Phenolic compounds	+++
Quinones	–
Saponins	–
Tannins	+++
Triterpenoid/steroids	+++

– not detected, ++ indicates moderate presence, +++ indicates high presence

et al. 2012) and in accordance with reports from Cervantes et al. (2017), who evaluated different polarity extracts from the leaves of *C. alba*, finding that the extracts were able to inhibit the growth of *S. aureus*, *S. epidermidis*, *K. pneumoniae* and *P. aeruginosa*. Similarly, Molina-Salinas et al. (2007) and Rodrigues et al. (2012) demonstrate the capacity of other species of the *Cordia* genus like *C. verbenacea* and *C. boissieri*, to inhibit the growth of *S. aureus*.

The anti-inflammatory and antibacterial activity showed by the extract of the flowers of *Cordia alba* might be related to the synergic action of flavonoids, triterpenoids, phenolic compounds, tannins and coumarins; metabolites reported by their anti-inflammatory, antioxidant and antibacterial properties, that have also been found in other species of the *Cordia* genus (Table 3) (Cowan 1999; Thirupathi et al. 2008;

Kurek et al. 2011; Kumar and Pandey 2013; Venugopala et al. 2013).

The anti-inflammatory activity of the flowers of *Cordia alba* was demonstrated, as well as its capacity to inhibit the growth of *P. aeruginosa* and *S. aureus*, establishing the first scientific report that support the traditional use of this species for these biological activities.

Funding This work was supported by the University of Cartagena, Colombia (Grant 054-2013 and 097-2015).

Compliance with ethical standards

Conflict of interest All authors (Jenny Castro, David Rivera and Luis Franco) declare that they have no conflicts of interests.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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