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RESEARCH ARTICLE

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HEALING PROPERTIES OF A OINTMENT FORMULATION CONTAINING ETHANOLIC EXTRACT OF CNIDOSCOLUS QUERCIFOLIUS POHL BARK

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ABSTRACT

The skin is responsible for protecting the system against external agents and, in the event of injury, its integrity is compromised and it needs repair. The use of *Cnidoscopus quercifolius*, known as faveleira, a plant native to the Brazilian caatinga, in the treatment of wounds by traditional populations is well known, due to the presence of tannins which act as a physical, antiradical and chelating agent during the healing process. The aim of this study was to evaluate the use of an ointment based on the extract of faveleira bark, rich in tannins, as a model for treating wounds. For this purpose, samples of the faveleira tree were collected in the municipality of Serra dos Bois (Pernambuco - Brazil), for extraction with Soxhlet in increasing order of polarity with the choice of hexane and ethanol solvents. Based on the *in vitro* results, the ethanolic extract was chosen after evaluating the yield, phytochemistry and quantification of tannins, showing antioxidant capacity. From the *in vivo* evaluation of topical toxicity (negative result), healing potential was observed, with a 94 % reduction of the wound, and also demonstrating anti-inflammatory activity. These results help to provide understanding on *Cnidoscopus quercifolius* therapeutic potential for wound healing.

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INTRODUCTION

The integumentary tissue is the first protective barrier to pathogens, such as bacteria, viruses and fungi, and it needs to be intact in order to carry out its biological activities (Olczyk *et al.*, 2014; Júnior 2016). In situations of damage, the healing process signals cell renewal mechanisms that are mediated by chemical agents of metabolism (Dragicevic; Lau, 2015; Gomes; Carmo, 2015). Through healing, if performed satisfactorily, it is possible to obtain a shorter repair time, better appearance, coloration and absence of fibrosis in cases of injury, adapting the treatment according to the type, size, depth, etiological agent and specific shape (Than *et al.*, 2017; Rodrigues *et al.*, 2020). Due to the increase in the number of cases of injuries and complications caused by ineffective pharmacological therapy, costs and susceptibility to infection, there is an increase for means to stimulate healing, with the use of medicinal plants being the most widely used by the traditional population in contrast to entirely synthetic solutions (Gomes *et al.*, 2014; Takeo *et al.*, 2015; Paredes *et al.*, 2016). The caatinga, one of the six Brazilian biomes that is still little covered in research due to its erroneous historical background as a "land without life", is one of the largest possessors of plant groups that are unknown in terms of their biotechnological and medicinal potential (Amaral *et al.*, 2015). It is possible to note that the local population, which already uses various plants to heal wounds, in turn reveals a great intangible wealth through orality, with *Cnidoscopus quercifolius* being widely used (Paredes *et al.*, 2016; Ferreira *et al.*, 2019).

Cnidoscopus quercifolius Pohl is a plant belonging to the Euphorbiaceae family and popularly known as "faveleira", "urtiga branca" or "unha de gato" in the Caatinga. This plant is native and endemic to Brazil, distributed in the northeastern regions of the country (Maya-Lastra *et al.*, 2021; Paredes *et al.*, 2016). Its use is seen in the processing of leather and in the reforestation of degraded areas (Melo; Sale, 2008; Coelho *et al.*, 2012). However, *C. quercifolius* is also used in traditional medicine, being used by the local population to treat dysentery, dental pain, urinary infections, peptic ulcers and superficial wounds (Novaes *et al.*, 2021; Paredes *et al.*, 2016). This particular use for the treatment of superficial wounds was first reported at the beginning of the 20th century, when its bark and exudate were used as a poultice to be applied or as a tea to drink to help restore the skin (Melo; Sale, 2008). *C. quercifolius* medicinal action on the skin is due to its secondary metabolites, mainly the presence of tannins (Vizzotto *et al.*, 2010; Paredes *et al.*, 2016). These polyphenols have a healing effect by forming a polysaccharide layer on the skin, helping cell replication without interference of physical, chemical and infectious agents (Macakova *et al.*, 2014). Other pathways also appear to be responsible for accelerating re-epithelialization, namely antioxidant, chelating, anti-inflammatory and antimicrobial (Lima *et al.*, 2015; Amaral *et al.*, 2015; Nogueira *et al.*, 2021; Lai *et al.*, 2011). Despite the extensive ethnobotanical knowledge about faveleira and its properties, it is still necessary to prove this therapeutic potential topically in cases of abrasions in living models in order to fully understand its healing potential (Rosa *et al.*, 2014). Thus, the aim of this study was to investigate the healing potential of the tannin-rich extract of faveleira in an ointment formulation.

METHODOLOGY

Collection and extraction: *Cnidioscolus quercifolius* was collected in the town of Taquaritinga do Norte in the rural area of Sítio dos Bois in the state of Pernambuco - Brazil (7° 53' 17" South, 36° 5' 33" West). An exsiccate was produced in the Geraldo Mariz herbarium in the botany department of the Federal University of Pernambuco (UFPE), identified by Dr. Marlene Carvalho de Alencar Barbosa and deposited under registration number 85312 and registered in the National System for the Management of Genetic Heritage - SISGEN under number A191B7E. After removing the bark with a blade, the moisture content was determined by gravimetry in an oven at 40° C, obtaining 32.31% during the drying process. It was ground in a forage grinder to a powdery state. Extraction was carried out according to Madhura *et al.* (2003) using hexane and ethanol solvents, obtaining yields of 3.077% and 7.797%, respectively.

Phytochemical analysis: The extracts were screened according to the methodology of Kloss (2016), where using specific reagents the presence or absence of color in flavonoids, tannins, fatty acids, terpenes, carbohydrates or precipitate with particle formation as in alkaloids and quinone was seen, being a qualitative indication as to the presence of these components.

Quantification of Phenols and Tannins: 20 µL of the Hexanolic and Ethanolic extracts diluted in methanol (1mg/mL) was added to 100 µL of the Folin-Ciocalteu reagent (1:10 in water). After 3 min, 80 µL of sodium carbonate solution (75 g/L) was added. After 2 hours of incubation at room temperature, the absorbance of total phenols was measured at 735 nm and 725 nm for tannins (BIOTEC). A gallic acid calibration curve (25-500 mg/mL) was used for the determination of total phenols in milligrams of gallic acid equivalents (mgEAG) per gram of material and a tannic acid curve (25-500 mg/mL) for tannins which were expressed in milligrams of tannic acid equivalence (mg EAT/g) (Li *et al.*, 2008).

Flavonoid dosage: The methodology proposed by Woisky and Salatino (1998) was used, where 100 µl of the already diluted hexanolic and ethanolic extract (1 mg/mL) was added to a 2% ethanol (w/v) AlCl₃ solution. After 1 hour of incubation protected from light at room temperature, the absorbance was measured at 420 nm and the results expressed in milligrams quercetin equivalent (mg EQ/g dry weight of plant extract).

2,20-Diphenyl-1-picrylhydrazyl (DPPH) activity: The DPPH radical was prepared in methanol with an absorbance between 0.600-0.700 at 517 nm. The hexanic and ethanolic extracts were also diluted in methanol and a serial dilution was made at concentrations of 1000-31.25 µg/ mL. 250 µl of DPPH reagent was added to 200 µl of each extract dilution. After 30 minutes of incubation in a dark area, the results were read at 517 nm using gallic acid as a control (BLOIS 1958). The results were expressed as free radical scavenging activity (SRL):

$$\text{SRL (\%)} = \frac{\text{abs control} - \text{abs sample}}{\text{Abs control}} \times 100$$

Total antioxidant capacity (Phosphomolybdenum): The methodology proposed by Pietro *et al.* (1999) was followed. 100 µl of the hexanic and ethanolic extracts diluted in methanol (1 mg/mL) were used, after which 1 mL of phosphomolybdenum reagent (sodium phosphate, ammonium molybdate and distilled water) was added to obtain their antioxidant value, with ascorbic acid as a control. It was placed in a dry bath at 95°C for 1 hour and 30 minutes and then read using a spectrophotometer with absorbance measured at 695 nm against a blank (1 mL of reagent and 0.1 mL of solvent). The result was expressed as total antioxidant capacity (% TAC):

$$\text{AA(\%)} = \frac{\text{Ab sample} - \text{Ab control}}{\text{Ab.Aa} - \text{Ab control}} \times 100$$

Iron ion reduction test (FRAP): A ferrous sulphate curve was made at concentrations of 0, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 mg/ml dissolved in water. 27 µl of the hexanolic and ethanolic extracts diluted with 200 µl of the FRAP solution were mixed and left in the absence of light for 30 minutes for subsequent reading at 539 nm, with the result expressed in µg Fe 2+ /g, where a linear equation was created after a Fe 2+ standard curve (Benzie; Strain, 1996).

3-ethylbenzothiazoline-6-sulfonic acid (ABTS) method: Following the methodology of Rasera *et al.* (2019), 1 mg/mL of the hexanolic and ethanolic extracts was diluted in methanol and 2 µL was removed and added to 200 µL of the ABTS reagent prepared using distilled water and sodium persulfate at an

absorbance of 0.700 at a length of 734 nm. The negative control consisted of adding 2 µL of the diluent to 200 µL of ABTS solution. The positive control was Trolox. Trolox was diluted to make the reference curve (10-1000 µg/mL). The plate was read using a BIOMETIL ELISA at a length of 734 nm over a period of 6, 15, 30, 35, 45, 60, 80 and 120 minutes respectively, with the result expressed according to the percentage of inhibition described in the calculation below and expressed as antioxidant capacity equivalent to Trolox (CAET)/ mg of material.

$$\text{Inhibition (\%)} = \frac{(\text{ABTS control} - \text{ABTS sample})}{(\text{ABTS control})} \times 100$$

Anti-hemolytic evaluation: The methodology proposed by Tenore *et al.* (2015) was used with some modifications. After blood collection in an EDTA tube, centrifugation was carried out at 2,500 rpm for 3 min, and the red blood cell precipitate was washed three times in PBS (0.2M, pH 7.4) at 2,500 rpm for 10 minutes. A 20% red blood cell suspension was prepared in PBS. Then 0.2 mL of the diluted sample (2-0.062 mg/ml) was added to 0.2 mL of the 20% erythrocyte suspension. This mixture was pre-incubated for 30 min at 37°C and then 0.2 mL of 3% oxygen peroxide (H₂O₂) (commercial solution) was added and incubated for 2 h at 37°C. After this time, 3.2 ml of PBS was added and centrifuged at 2,500 rpm for 10 min. The reading was carried out using a spectrophotometer at 540 nm and the absorbances used to determine the inhibition of hemolysis according to the formula below, with 3% H₂O₂ as the positive control and PBS as the negative control.

$$\% \text{ Inhibition} = \frac{1 - \text{Abs sample}}{\text{Abs hemolysis}} \times 100$$

Animals and ethical aspects: Animal experimentation under the responsibility of Professor Dr. Vera Lúcia de Menezes Lima was approved by the ethics committee of the Federal University of Pernambuco - CEUA, under process number 0013/2021. Vera Lúcia de Menezes Lima, using male *Mus musculus albino swiss* mice, aged from 2 months, which were kept in the Biotherium of the Biochemistry Department of the Federal University of Pernambuco (UFPE), with a minimum adaptation time of one week, having access to food and drink in an environment with a temperature of 22°C and a 12h-on/12h-off lighting cycle.

Cream development: A Polawax emulsion (Farmácia A Fórmula - Recife/PE) was used to formulate the cream, following preliminary physical and chemical tests to determine its short- and medium-term stability using the calculation of the ideal combination of emulsifiers (EHL) described below, for the subsequent addition of the extract diluted in ethanol according to the specifications of the Brazilian Health Surveillance Agency (ANVISA) (Chorioli *et al.*, 2006; Isaac *et al.*, 2008; ANVISA, 2012). The Polawax base with the addition of a 5% tannin-rich extract had a galenic appearance, a semi-solid appearance and good compatibility with the extract from the emulsion, making it ideal for use (Coutinho; Santos, 2014; Lima *et al.*, 2020).

$$\text{EHL of the emulsion} = \frac{(\% \text{ contribution} \times \text{Individual E.H.L.})}{100}$$

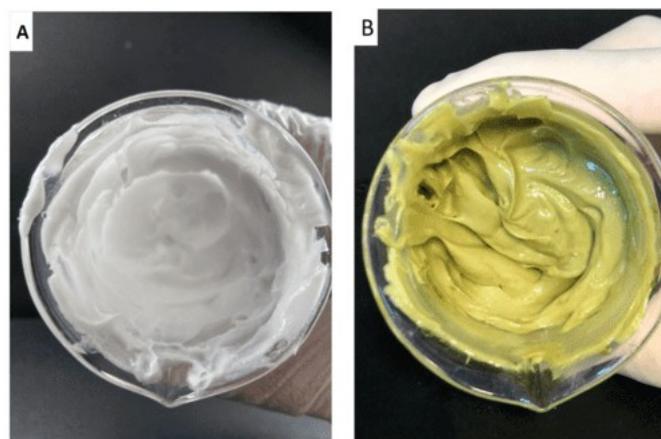


Figure 1. A. Polawax emulsion without added extract; B, Polawax emulsion with 5% ethanolic extract of *Cnidioscolus*

Topical toxicity test: For the experiment, 4 groups of 3 animals each were separated, with 2 controls (positive and negative) corresponding to 100% ethyl alcohol (used for its similarity to the process of skin irritation with hyperemia,

burning and itching) and saline solution respectively. Two solutions of 500 mg/kg and 2000 mg/kg of the ethanolic extract were also prepared for application to the dorsal region, which was trichotomized for better absorption. The solutions were added for 14 days to observe changes such as irritation, the presence of exudate, inflammation, signs of infection, lack of hair growth and death. After 14 days, the animals were euthanized using intramuscular xylazine (10mg/kg) and ketamine (115 mg/kg) (Kim *et al.*, 2012).

Evaluation of wound healing activity: Six groups were divided, each containing a total of six adult animals. The experimental groups were: Negative control without the use of drugs, vehicle group containing saline solution; base cream group containing polawax alone; Positive control with Dexpanthenol (Bepantol® Derma, a drug already on the market); Treatment group with topical use of cream containing polawax emulsion and 5% extract rich in CqEE tannins; Treatment group with topical addition of 2.5%; topical group with addition of 5% extract. The animals were anaesthetized using xylazine (10mg/kg) and ketamine (115 mg/kg) intramuscularly. First, the dorsal region was trichotomized and the skin was properly aseptically with 70% alcohol and a 1 cm diameter delimitation. The skin was then removed/excised, based on a standardized experimental model using a punch. The treated animals received topical applications immediately after the skin excision, which was considered to be the first day, with the application continuing until the 14th day, when the characteristics of the lesion were monitored and at the end their vital organs were removed after euthanasia.

Vancouver scale evaluation: The Vancouver model adapted for mice in common wounds was used as an analytical criterion, establishing scores of 0-2 for each test group, highlighting appearance, coat, hemorrhage and coverage, with 0 as no alterations and 2 as highly present. At the end, each phase is summed up, showing the qualitative evolution over the days (Barysa *et al.*, 1995).

Analysis of reduction by scar area: To analyze the results, photographs were taken of the cuts on the mice on their first and last days. With the aid of the image J program, the extent of the lesion per area was counted and compared in a table using the following calculation:

$$X = 100 - \frac{\text{End area}}{\text{Starting area}} \times 100$$

Assessment of inflammatory modulation: The methodology was carried out according to Souza and Ferreira (1985) with some modifications. The mice were randomly divided into five groups, each group containing five animals. Each group received a different treatment: Extract (50 mg/kg, 100 mg/kg and 200 mg/kg), dexamethasone as a positive control group (5 mg/kg in water), negative control group treated with 100 µL of the vehicle, all via gavage p.o. After 1 hour, 1% carrageenan in 0.9% NaCl solution was used to induce peritonitis. After 4h, the animals were euthanized by cervical dislocation and then 3mL of sterile saline solution (containing 5U/mL heparin) was injected into the peritoneal cavity. After massaging the peritoneal cavity, a longitudinal incision was made to open the peritoneum and allow the exudate to be collected under aseptic conditions for subsequent quantification of IFN-gamma and TNF-alpha by ELISA (GE Healthcare, USA) and nitric oxide (NO) by the Griess methodology using a commercial kit and expressed in µM of NO.

Statistical analysis: The test results were expressed as mean± standard deviation, and analyzed using ANOVA, followed by Bonferroni's test (post hoc test). The data was analyzed using *GraphPad Prism 5.0*, USA.

RESULTS AND DISCUSSION

To obtain the extract, Soxhlet was chosen because of its high extraction potential due to the multiple passes of the solvent through the sample, avoiding the displacement of masses that slow down extraction, as in other methods, and also due to its easy technical applicability, not requiring filtration during extraction and being able to control the temperature (Lopes-Bascón, 2020). The phytochemical screening analysis with the ethanolic and hexane extracts of *C. quercifolius* bark indicated the presence of compounds such as phenols, flavonoids, anthocyanins, saponins, alkaloids, coumarins, triterpenes with their derivatives and tannins, as shown in Table 1, which corroborates the findings published elsewhere (Gomes *et al.*, 2014; Paredes *et al.*, 2016; Oliveira-Junior, *et al.*, 2020). This analysis shows that, as it is an ethanolic solution, solubility does not affect the solute-solvent ratio as it does with aqueous and methanolic extracts, which does not influence the yield and dosage of its constituents (Lira *et al.*, 2017). In the additional findings using the thin layer chromatography (DLC) technique, it was possible to identify the presence of alkaloids and the absence of coumarin in the ethanolic extract.

Table 1. Phytochemical analysis of *Cnidoscopus* bark extracts

Classes of Compounds	EWC	CqHE
Carbohydrates	Present	Absent
Terpenes	Present	Present
Fatty acids	Absent	Present
Tannins	Present	Present
Saponin	Absent	Absent
Quinone	Absent	Absent
Flavonoids	Present	Present
Alkaloid	Present	Present

CqHE, hexanic extract; CqEE, ethanolic extract

When measuring total phenols and tannins, a high percentage of these was observed in the ethanolic extract, in contrast to the hexane extract, as described in Table 2, with these results being expressed as total tannins per gram of weight of the sample used. This statement corroborates the preliminary studies by Madhura *et al.*, 2003 and Su *et al.*, 2016, in which the main property of these compounds was related to factors such as wound healing through the use of the plant, being the main reason for choosing the extract. In order to elucidate the choice of extract with the highest percentage of tannins, in addition to the ethanolic and hexanic extracts, liquid-liquid partitioning was carried out, obtaining fractions with chloroform, ethyl acetate and a hydroalcoholic solution. Analysis of the partitions (results not shown) showed few phenolic compounds and tannins, since the use of these solvents affects the reading kinetics in the interaction with methanolic solution, which is also reflected in other tests such as DPPH (Dawidowicz *et al.*, 2012).

Table 2. Phytochemical quantification of ethanolic and hexanic extracts of *Cnidoscopus quercifolius* bark

Samples	Total phenols (mg EAG/g)	Tannins (mg EAT/g)	Flavonoids (mg EQ/g)
CqHE	27.56 ± 0.04*	22.533 ± 0.01*	43.320 ± 0.03*
CqEE	125.91 ± 0.01	99.422 ± 0.05	17.705 ± 0.01

CqHE, hexanolic extract; CqEE, ethanolic extract; mgEAT/g, tannic acid equivalent; mg EAG/g, gallic acid equivalent; mg EQ/g, quercetin equivalent. *p < 0.01 vs CqEE, not paired t Test.

The extracts were analyzed using different methods to assess their antioxidant activity, including DPPH, phosphomolybdenum, ABTS and FRAP, which work by combating free radicals, sometimes simulating them, chelating metals and inhibiting pro-oxidative enzymes (Macakova *et al.*, 2014; Pessuto *et al.*, 2009). The antioxidant action of the extract was evident in the CqHE and CqEE, although it was more significant in the hexanic extract by different routes. The use of the ethanolic extract is also encouraged, with its activity being evident through ABTS.

Table 3. Antioxidant analysis by different routes in extracts of *Cnidoscopus quercifolius*

Samples (1000 µg/mL)	%SRL DPPH (IC 50)	FRAP (µg Fe (II)/g)	ABTS (TEAC)	CAT (%)
CqEE	18.04 (>1000)	114.186 ± 0.02	0.822 ± 0.03	31.12 ± 0.01
CqHE	36.70 (239.1)*	381.475 ± 0.03*	0.694 ± 0.04*	80.99 ± 0.01*

CqHE, hexanolic extract; CqEE, ethanolic extract; %SRL DPPH, 2,2-Diphenyl-1-picrylhydrazyl expressed as inhibitory concentration; FRAP, Reduction by iron ion; ABTS, inhibition percentage; CAT, total antioxidant capacity expressed as a percentage. *p < 0.01 vs CqEE, not paired t Test.

In the evaluation of anti-haemolytic activity, i.e. the inhibition or not of haemolysis by lipid peroxidation using hydrogen peroxide, it was observed that, at all concentrations tested (1 - 0.125 mg/mL), there was inhibition of haemolysis, thus indicating that the hexanic and ethanolic extracts are also antioxidant agents via lipid peroxidation inhibition activity in red blood cells. The topical ethanolic extract did not cause death in the animals or the appearance of lesions according to the notes reported by the OECD guidelines (2001), nor did it cause any behavioral alterations such as agitation, motor alterations, abdominal writhing, lethargy, piloerection, respiratory alterations, bleeding, alterations in urine and feces during follow-up, as shown in Tables 4 and 5 for all the groups in the experiment.

Table 4. Analysis of topical toxicity using the ethanolic extract of *Cnidoscopus* bark

	Groups (mg/kg)			
	Vehicle	Ethanol	500	2000
Abnormalities	0/3	0/3	0/3	0/3
Deaths	0/3	0/3	0/3	0/3

Vehicle, Saline solution; Ethanol, 100% ethyl alcohol; 500 mg/kg and 2000 mg/kg, Solution containing extract diluted in ethanol and saline solution

Table 5. Analysis of healing using the cream with ethanolic extract of *Cnidoscopus bark*.

	Products						
	Control -	Vehicle	Control +	2,5%	5%	Base	Cream 5%
Abnormalities	0/7	0/7	0/7	0/7	0/7	0/7	0/7
Deaths	0/7	0/7	0/7	0/7	0/7	0/7	0/7

Control (-)* Group without drug application; Vehicle*Solution containing tween 80 and distilled water; Control (+) Commercial drug bepantol; 2.5%, Solution containing extract diluted in vehicle; 5%, Solution containing extract diluted in vehicle; Base, Polawax emulsion without additives; Cream 5%, Polawax with addition of 5% ethanolic extract of *Cnidoscopus quercifolius* barks

Table 6. Healing property of ointment with ethanolic extract of *Cnidoscopus quercifolius bark* by criteria from the adapted *Vancouver scale*

	Groups																				
	Negative control			Vehicle solution			Positive control			2.5% CqEE solution			5% CqEE solution			Base			CQ cream 5%		
Days	2°	8°	14°	2°	8°	14°	2°	8°	14°	2°	8°	14°	2°	8°	14°	2°	8°	14°	2°	8°	14°
Coverage	1	1	0	1	1	0	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0
Coat	2	2	1	2	2	1	2	2	1	2	2	1	2	2	1	2	2	1	2	2	0
Bleeding	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aspect	2	1	0	2	1	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0
Total	6	4	1	5	4	1	3	2	1	4	2	1	4	2	1	3	2	1	3	2	0

Negative control* Group without application of any drug; Vehicle solution* Solution containing tween 80 and distilled water; Positive control* Bepantol (Commercial drug); 2.5% CqEE solution, Solution containing extract diluted in vehicle; 5% CqEE solution, Solution containing extract diluted in vehicle; Base, Polawax (Commercial); 5% CQ cream, Polawax base with addition of 5% ethanolic extract of *Cnidoscopus quercifolius bark*.

Table 7. Analysis of the percentage reduction in wound area of mice after treatment with ointment containing ethanolic extract of *Cnidoscopus quercifolius bark*

Groups	Percentage reduction by area (%)
Negative Control	78.60 ± 0.31
Vehicle solution	71.63 ± 0.25
Positive Control	82.54 ± 0.35*
2.5% CqEE solution	60.37 ± 0.47*
5% CqEE solution	79.00 ± 0.36
Base	70.76 ± 0.45
CQ cream 5%	94.83 ± 0.67*

Negative control, Nothing administered; Vehicle, Saline solution + tween 80; Positive control, Bepantol; 2.5% solution, solution containing extract diluted in vehicle; 5% solution, extract diluted in vehicle; Base, Polawax (commercial); 5% CQ cream, Polawax base with the addition of 5% ethanolic extract of *Cnidoscopus quercifolius bark*. * p < 0.01 vs Negative control, one-way ANOVA followed by Bonferroni's post-test.

No changes were observed in the characteristics of the organs (heart, spleen, kidneys and liver) during euthanasia of the topical toxicity group in terms of coloration, cysts, structural changes or size, in line with the standard for the male group obtained in accordance with OECD standards (2001) and by Junior *et al.* (2012), following the description of the necropsy guide by Vicenzo Covelli (2019). There were no significant changes in the weight of the mice or in the development of their limbs as a result of the topical application, and the same results were also shown in the groups for assessing scarring. All mice followed normal developmental patterns with age-appropriate mass gain, and there were no dietary changes rich in carbohydrates or lipids that could have influenced the results. The Vancouver healing scale, adapted to the rodent group for superficial wounds, was used as a means of analyzing the best response time over the 14 days of experimentation, following 4 scales: layer coverage, hair growth, presence of bleeding and appearance of the lesion (Table 6). The results showed that the 5% extract cream had a greater healing effect than the standardized commercial drug, influencing better hair growth, with this peak being more evident from the 9th day after application compared to the other groups. With regard to the analysis of the reduction in the area after the cut, during the follow-up, a high reduction was seen in the use of the cream with the addition of the extract, totaling 94.83% in relation to the control and other solutions (Table 7), which in turn shows the therapeutic quality after treatment, which is superior to many drugs that are commercially available and is driven by different mechanisms of action with the use of tannins, such as antioxidant, mechanical barrier, anti-inflammatory and anti-microbiological activity (Li *et al.*, 2011; Barros *et al.*, 2022). Figure 2 shows the topical application of different solutions containing the tannin-rich extract of *Cnidoscopus quercifolius* for tissue repair, which was injured using surgical scissors in the dorsal region. A 14-day follow-up was carried out with photographic records, emphasizing the comparison between the commercial cream (Bepantol derma creme - Derma, Brazil) and the

ointment containing the tannin-rich extract derived from *Cnidoscopus quercifolius* produced for this study. Groups included 2.5% and 5% solutions of the extract to assess its effect on its own, without additives, a polawax base used individually to emulsify the extract, a vehicle containing saline solution and a diluent (tween 80) and a group with no application. Due to the formation of a polypeptide layer (Li *et al.*, 2011) and the presence of local granulation tissue with the absence of marked hyperemia in the groups containing the extract, it can be seen that there was a greater response in the repair, which was demonstrated more markedly on day 11 with the fall of the overlying crust which was larger than the others. In the end, the commercial ointment, as well as the one rich in tannins, showed macroscopically significant results, with the absence of a bleeding point, hair growth around and overlapping the replacement tissue, coloration, absence of exudate, shine, edema and a reduction in the edges (Figure 2). This action of the tannins in the ointment is due to the longer residence time/conservation in the skin compared to the topical use in the preservatives of the formulation developed compared to the individual extract solutions of 2.5% and 5% in which, due to a greater tendency to interact with other molecules and low stability on the part of the tannins, the results were inferior (Liang *et al.*, 2021). It is well known that inflammatory markers are indicators of biological status and are used in the diagnosis, prognosis and monitoring of patients in various conditions, one of which is wound healing due to oxidative stress. Markers, such as tumor necrosis factor (TNF- γ) and interleukins, as part of cytokines, myeloperoxidases and reactive oxygen species are present in persistent injuries and are also associated with high bacterial growth and low venous supply. It is in healing, during the inflammatory phase, that the action of proteases such as collagenase, if overstimulated, reduces the action of fibroblasts which subsequently release collagen in the proliferative phase, thus requiring control by protease inhibitors (PIMs) which must be stimulated under coordination so as not to damage growth factors such as epidermal

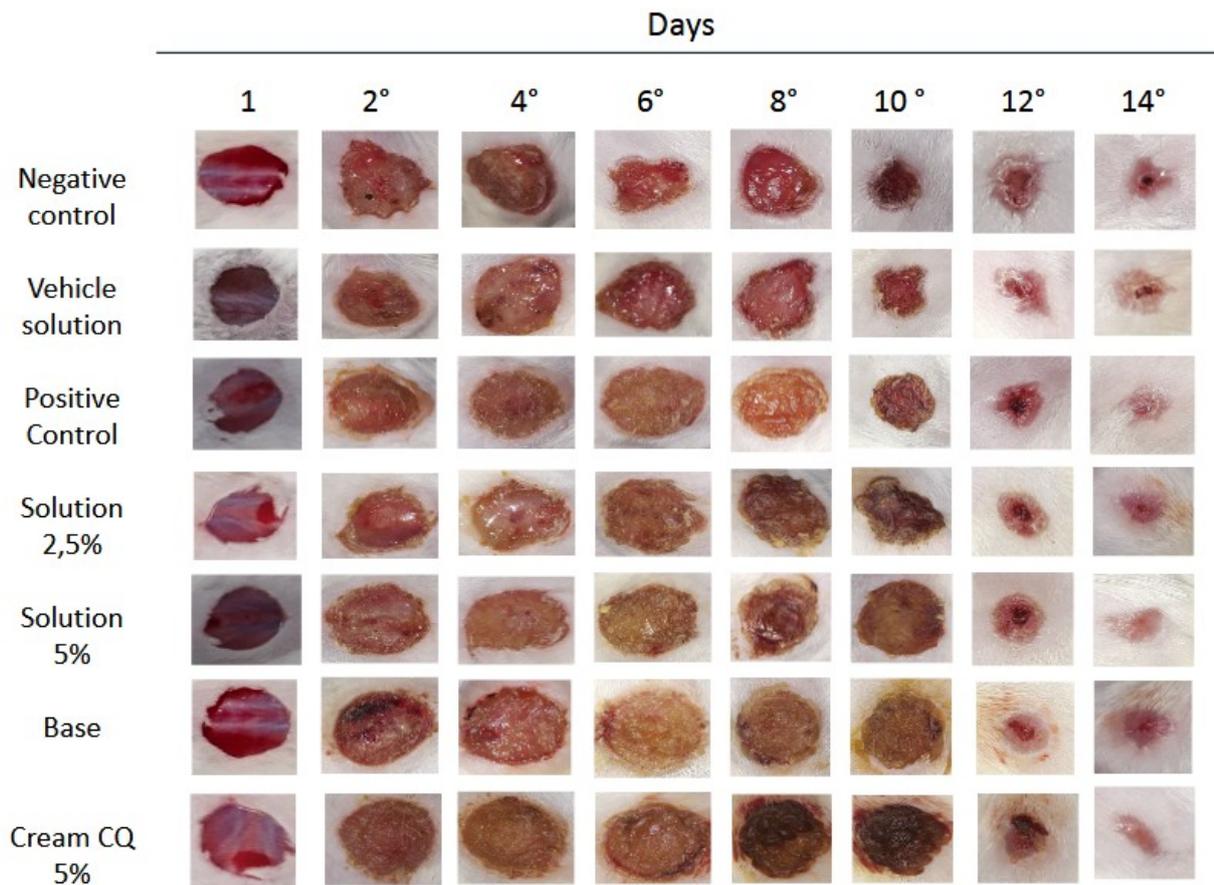


Figure 2. Macroscopic observation of the lesions during the healing process. Negative control, nothing administered; Vehicle, saline solution + tween 80; Positive control, Bepantol; 2.5% solution, solution containing extract diluted in vehicle; 5% solution, extract diluted in vehicle; Base, Polawax (commercial); 5% CQ cream, Polawax base with the addition of 5% ethanolic extract of *Cnidocolus quercifolius* bark

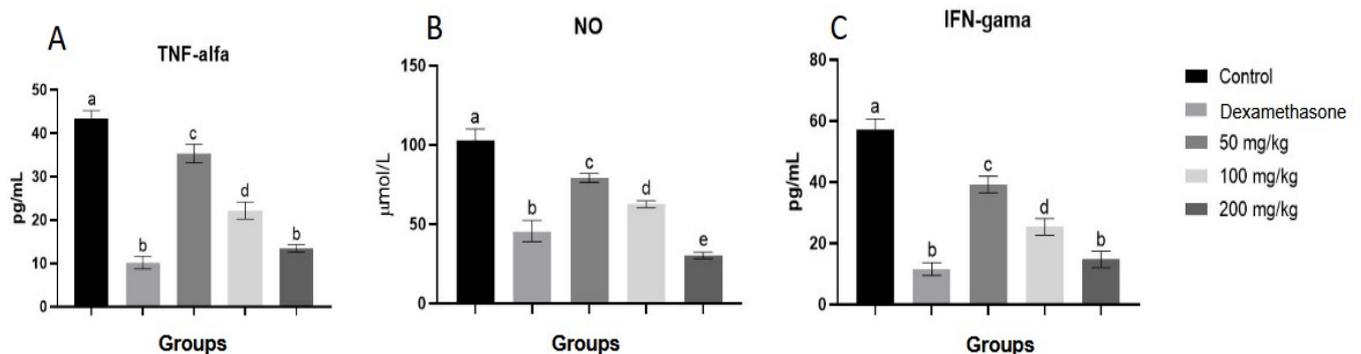


Figure 3. Evaluation of inflammatory modulation by carrageenan induction using the peritonitis test

growth factor (EGF) and platelet-derived growth factor (PDGF), which can destroy the extracellular matrix (ECM) (Patel *et al.*, 2016). Proteases can be divided into 4 main classes: serine, metalloproteinases, cysteine and aspartic (Hahm *et al.*, 2011). They are the inflammatory markers responsible for mediating the duration of the stimulus inducing this phase and if there is a high demand, they can ultimately demonstrate a direct action in the breakdown of collagen, releasing free hydroxyproline and also preventing the arrival of immune system agents at the site (Murthy *et al.*, 2013). According to the results obtained with the ethanolic extract of *Cnidocolus quercifolius* using the peritonitis test, it was observed that the markers used, namely tumor necrosis factor (TNF- α) and gamma interferon (IFN- γ) showed dose dependence, which is an indication of their

potential for action being directly related to the amount of dexamethasone, however nitric oxide (NO) showed a similar effect, but the higher dose was better than dexamethasone as shown in Figure 3. Nitric oxide, like other markers of inflammation, can cause an imbalance in the plasmin activator system, which leads to an imbalance in fibrinolysis, increasing the degree of inflammation and preventing healing (Teixeira *et al.*, 2014, 2014), it is susceptible to oxidation by reduction in the radical that gives rise to the nitroxyl anion, which has an extremely short half-life (Vizotto, 2017), as well as the calling of adhesion molecules by increasing tumor necrosis factor (Sproston; Ashworth, 2018), where it has already been elucidated that high doses cause an antagonistic effect at the molecular level (Ashcroft *et al.*, 2011). Interleukin and interferon

gamma in calling phagocytic agents such as neutrophils and macrophages (Oliveira et al., 2017). Despite the large amount used in the tests, it is possible to observe that it can be more beneficial in clinical use compared to the control because it does not cause adverse effects, respecting the healing cascades in its 3 phases (Fagundes et al., 2020), such as raising the inflammatory degree more prominently and also avoiding the generation of oxidative stress that encourages the overproduction of reactive species by encouraging lipoperoxidation, protein carbonylation and DNA damage by enzymatic action (Sulzbacher; Heck, 2020).

CONCLUSION

This study highlights the action of the ethanolic extract of the bark of *Cnidoscopus quercifolius* emulsified in an ointment as a potential healing agent, with antioxidant and anti-inflammatory capacity. In addition to its lack of topical allergic reactions, its efficacy stands out when compared to established commercial products such as commercial ointments. This benefit is mainly associated with the presence of tannins, making it a more accessible alternative for the traditional population.

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