

BIOLOGICAL ACTIVITIES OF *Solanum sessiliflorum* DUNAL

ATIVIDADES BIOLÓGICAS DE *Solanum sessiliflorum* DUNAL

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ABSTRACT: This study evaluated the antioxidant, antimicrobial and wound healing activities from the aqueous extract of cubiu (AEC - *Solanum sessiliflorum* Dunal). The antioxidant activity was evaluated by the scavenging method with 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) and, the antimicrobial activity, by the turbidimetric method. The dermal ulcers induced model in rabbits was used in order to study the wound healing activity. Creams containing 5% and 10% AEC and 5% AEC associated with 1% copaiba oil and 1% rosemary oil were tested. The wounds were macroscopically (ulcer aspect and ulcer area contraction) and microscopically examined. The histological study considered the number of inflammatory cells, fibroblasts, blood vessels, collagen and extracellular matrix areas. Analysis of variance (ANOVA) followed by *Tukey's post hoc* test was performed. The AEC showed $IC_{50}=65.12 \mu\text{g/mL}$ and no antimicrobial activity against all of the strains tested was verified. The formulation containing 5% AEC associated with 1% copaiba oil exhibited the greatest pharmacological potentiality in dermal wound healing due to the increased number of blood vessels ($p < 0.001$) and the largest extracellular matrix area ($p < 0.001$) presented. The results justify further studies about biological activities of cubiu in the search for bioactive compounds.

KEYWORDS: Antimicrobial activity, Antioxidant activity, Wound healing activity, Cubiu, *Solanum sessiliflorum* Dunal

INTRODUCTION

Phytotherapy is a form of health practice that has grown in recent years. Approximately 80% of the worldwide population believes in the therapeutic power of plants and, despite advances in drug synthesis, 25% of medical prescriptions still derive directly or indirectly from plants due to their diversity and chemical versatility (Fowler, 2006).

Cubiu (*Solanum sessiliflorum* Dunal), also known as topiro or Indian tomato, is a native solanaceous shrub from western Amazonia domesticated by indigenous populations (SILVA FILHO et al. 1999; LOPES; PEREIRA, 2005). In this region, it is used for different purposes such as medicine, food and cosmetic (PIRES et al. 2006). This plant has potentiality in the modern agricultural industry, due its rusticity and high fruit production (SILVA FILHO et al. 1999). In recent years, there has been an increase in the appreciation of fruit in the Brazilian market (SILVA FILHO et al. 2005), which has led cubiu to other places including Zona da Mata of Pernambuco and some locations in the southeast and southern regions (PIRES et al. 2006).

The cubiu fruits have high levels of niacin (MARX et al. 1998) and iron (SILVA FILHO et al.

2005) and they are being used *in natura* or in several ways such as in juices, candies, jams, ice creams, sauces, and in popular medicine such as a hypoglycemic and/or hypocholesterolemic agent (SILVA FILHO et al. 1999).

The high number of ethnic varieties of cubiu entails fruits in different shapes and sizes, which induce the search for knowledge, characterize the morphological variation and chemical composition of these fruits (YUYAMA et al. 2007). Over the past years there has been a growing appreciation for cubiu in the Brazilian market (SILVA FILHO et al. 2005). In the Amazon region, in particular, this fruit is used by native populations for food, medicine and cosmetics.

However, even considering its potentialities and large use in its origin region, pharmacological applications of cubiu are still rarely studied. In literature, no experimental work that contemplates such applications was observed. Studies on Brazilian biodiversity components (not yet explored nor totally known) has been increasingly valued because of their possible potential as raw material for medicine and industries sectors (pharmaceutical, food and chemical) and because of the capacity to produce socioeconomic benefits for local

population. Considering the lack of information, this study aimed to conduct an exploratory work around the biological activities from the aqueous extract of cubiu (AEC) through the antioxidant, antimicrobial and wound healing tests.

MATERIAL AND METHODS

The AEC was acquired commercially (lyophilized powder maná-cubiu fruit from Experiment Station Santa Luzia, Exotic Fruits, Brazil) and was used to perform all of the proposed tests. The supplier's report was followed, which shows 61.7% carbohydrates; 17.2% dietary fiber; 7.6% lipids; 7.0% protein; 0.095% calcium; 0.042% phosphorus; 0.007% iron; 0.004% sodium; 0.001% zinc; 0.00003% retinol (vitamin A); 0.00008% thiamine (vitamin B1); 0.00031% riboflavin (vitamin B2); 0.00501% niacin (vitamin B3); 0.00009% pyridoxine (vitamin B6); 0.00687% ascorbic acid (vitamin C) and 0,00007% α -tocopherol (vitamin E).

Antioxidant activity

The antioxidant activity was evaluated by the spectrophotometric method with a scavenging activity of 2,2-diphenyl-1,2-picrylhydrazyl radical (DPPH) (Sigma-Aldrich, USA) described by Sreejayan and Rao (1996), with minor modifications. This method is based on the reduction of DPPH radical in the presence of an antioxidant proton donor for a non-radical form (DPPH-H) (KOLEVA et al. 2002).

In this model, 150 μ L of 0.05 mM DPPH ethanolic solution were added to 50 μ L of the extract solution at concentrations from 250 to 0.97 μ g/mL, in 96-well microplate. Butylated hydroxytoluene (BHT) and ascorbic acid were used as a standard in the same concentrations. The reactions occurred at room temperature, in the dark, for 30 min. Then, the absorbance was read with the spectrophotometer (UV mini 1240, Shimadzu, Japan) (λ =510 nm). All tests were performed in triplicate.

Antimicrobial activity

Strains from American Type Culture Collection (ATCC): *Escherichia coli* (ATCC 8739), *Shigella flexneri* (ATCC 12022), *Shigella sonnei* (ATCC 25931), *Pseudomonas aeruginosa* (ATCC 9027), *Salmonella typhimurium* (ATCC 14028), *Staphylococcus aureus* (ATCC 25923) and *Candida albicans* (ATCC 10231) were used. It was utilized the microdilution technique, through which, the minimum inhibitory concentration (MIC), by

turbidimetric analysis, was determined. A bacterial suspension of each strain was prepared with sterile saline (sodium chloride - NaCl 9.0 g/L), to 25% transmittance, using the spectrophotometer (Libra S12, Biochrom, Denmark) (λ =580nm). The standardized microbial suspension was submitted to serial dilution with sterile saline. After the incubation period (24h for bacteria/48h for yeast), the colony-forming units (CFU's) were counted in Tryptone Soy Agar (TSA) for bacteria or Sabouraud Dextrose Agar (SDA) for yeast, in order to obtain 2×10^3 CFU/mL concentration in each microplate well (adapted from CANDAN et al. 2003).

For the negative control, 100 μ L of sterile broth were used while, for positive control, 100 μ L of inoculated broth were employed. Mueller Hinton Broth (MHB) was employed as the culture medium for bacterial strains and Sabouraud Dextrose Broth (SDB) for yeast, except for *Staphylococcus aureus* (ATCC 25923) for which Tryptone Soy Broth (TSB) was used. In order to prepare the test-groups, 100 μ L of inoculated broth and 100 μ L of different concentrations from the AEC (5.0 to 0.313 mg/mL) were used. Chloramphenicol (0.025 to 250 μ g/mL) was used for bacterial strains and nystatin (0.2 to 2000 U/mL) for yeast, as the standards. The microplate was incubated in time-temperature conditions above described. All procedures were carried out in triplicate.

Wound healing activity

New Zealand male rabbits (*Oryctolagus cuniculus*), weighing between 1.5 to 2.0 kg, from the Experimental Farm of UFMG (Igarapé/MG, Brazil) were used. The animals were kept at a controlled temperature ($22^\circ\text{C} \pm 2^\circ\text{C}$) and humidity (65 to 75%) and at light-dark cycle of 12 hours with water and food *ad libitum*. Temperature and humidity were monitored by a thermohygrometer (Thermo Hygro Meter J412CTH, China) and kept constant by the use of a 15000 Springer air conditioning (Mundial, Brazil) and Mechanical dehumidifier (Artel, Italy).

All procedures were carried out in accordance with the ethical principles of animal experimentation adopted by the Brazilian School of Animal Experimentation and approved by the Ethics Committee for Animal Experimentation of the Federal University of Juiz de Fora, under protocol numbers. 033/2011 and 034/2011.

In order to get a synergistic wound healing activity, we decided to combine cubiu with the essential oils due to their pharmacological activities. The essential oil of copaiba presents anti-inflammatory activity, whose responsible

components are sesquiterpene and hydrocarbons (especially β -bisabolene and β -caryophyllene); wound healing action; antiseptic, antibacterial and analgesic potential (Pieri et al., 2009). The essential oil of rosemary is used in cosmetics and drugs (Guerra et al., 2010). It is essentially composed of monoterpenes and is able to inhibit the growth of fungi and bacteria (especially gram-positive) (Probst, 2012), which can probably help in wound healing process by preventing infectious process.

Induction of dermal ulcers in rabbits

Trichotomy was performed in previously numbered animals (1 to 8), being the animals back divided into four quadrants (each quadrant received a different type of treatment). The local anesthesia with 3% prilocaine + felipressin (0.5 mL, dermal/quadrant) was carried out. The dermal ulcer inductions were made with a hot iron (5 cm²) for a period of 10 seconds/quadrant. The wounds were classified as grade II lesion (Moura, 2004). After this procedure, the animals were placed in cages with food and water *ad libitum*. Meloxicam was administered (0.2 mg/kg/day, subcutaneously) for pain relief for 4 days (DUTRA et al. 2009).

Cream containing 1% silver sulfadiazine was used as a positive control (quadrant 2) and a base cream (Lanette) as a negative control (quadrant 1). In the rabbits 1 to 4, quadrant 3 was treated with a cream containing 5% AEC and the quadrant 4 with a cream containing 10% AEC. In rabbits 5 to 8, the quadrant 3 was treated with a cream containing 5% AEC associated with 1% essential oil of copaiba (*Copaifera officinalis* L.) and quadrant 4 with a cream containing 5% AEC associated with 1% essential oil of rosemary (*Rosmarinus officinalis* L.). The formulations were applied twice daily (12/12h) for 10 consecutive days (total time of the experiment). At the end of the experiment, the rabbits were euthanized with sodium pentobarbital (200 mg/kg, intravenously) and the dermal ulcers were removed (50% of lesions – 2.5cm² - including the edges of lesions and excluding the muscular fascia) and fixed in 10% formalin buffered (pH=7.0), in a minimum 24h period, for histopathologic and histomorphometric analysis.

Macroscopic evaluation

Observations concerning the lesions appearance (presence or absence of open wound, red and white areas, purulent discharge, sore, swelling and bleeding) were evaluated daily during the 10 days of the experiment. Lesions were measured for further analysis about ulcer area contraction

(expressed in cm²) and photographed with digital camera (Cyber-Shot DSC-W35, Sony, USA).

Microscopic evaluation

After fixation, samples were dehydrated using high concentrations of ethanol (70% to 100%), diaphanized in xylene and embedded in paraffin by routine histopathological methods. The fragments included in paraffin were cut using a microtome (Spencer-820, American-Optical®, EUA) and 4 μ m thick sections were obtained. The slides were incubated and sections were stained by hematoxylin and eosin (HE) for further histopathological analysis.

The slides were analyzed with an optical microscope (BX51, Olympus, Japão). Images were captured with a video camera (Olympus, Japan) attached to it and scanned using Image-ProPlus software (Media Cybernetics). Images of five random fields from the inflammatory region, of each tissue sample (total magnification of 400x), were captured. The images were submitted to a count of inflammatory cells, fibroblasts and blood vessels and an evaluation of collagen and extracellular matrix areas. Due to collagen fibers acidophilia, they acquire a pink color when treated with HE staining enabling the analysis of collagen. The count was performed using the Image Tool software 3.0 and Image-ProPlus 6.0.

Statistical analysis

Inhibition of DPPH radical was calculated with the equation: $IC_{50}(\%) = 100 \times (A_0 - A_s) / A_0$, being A_0 negative control absorbance and A_s test-sample absorbance. The IC_{50} was calculated from the straight line equation of the linear dispersion graph and represents the extract concentration that inhibits 50% of DPPH radical.

The MIC was determined through turbidity observation in the cultivation media after the incubation period. No statistical analysis was applied.

The ulcer area contraction was evaluated by position and dispersion measures (mean and standard deviation) and compared with analysis of variance (ANOVA) followed by the Tukey *post hoc* test. A descriptive analysis for counts of inflammatory cells, fibroblasts, blood vessels and collagen and extracellular matrix areas was performed. To assess the difference significance between the results averages of study groups, ANOVA followed by the Tukey *post hoc* test were applied. The Statistical Software Package for the Social Sciences (SPSS) 14.0 was used. The limit of significance was $p < 0.05$.

RESULTS AND DISCUSSION

Antioxidant activity

The AEC showed $IC_{50}=65.12 \mu\text{g/mL}$ while the BHT and ascorbic acid standards showed, respectively, $IC_{50}=11.82 \mu\text{g/mL}$ and $IC_{50}=2.50 \mu\text{g/mL}$. The studied extract showed some scavenging ability against the radical DPPH. Rincón et al... (2011) evaluated the antioxidant activity of fruit pulp and seeds from three varieties of *Solanum sessiliflorum* Dunal fruit. The samples presented IC_{50} ranging from 2.20 to 5.49 g/g DPPH, and the authors considered them as being sources with high antioxidant capacity.

It is noteworthy that the cubiu fruit is rich in iron (219.8 grams) (SILVA FILHO et al. 2005), which can compromise its antioxidant activity. Pires et al... (2006) carried out the characterization of cubiu fruit pulp grown in Zona da Mata of Minas Gerais, where the contents were determined consisting of: moisture (91.51%), protein (0.82%), lipids (2.23%), ashes (0.77%), carbohydrates (4.66%), total caloric value (41.99 kcal/100g), concentration of minerals such as calcium (13.68 mg/100 g), iron (1.98 mg/100g), phosphorus (21.27 mg/100g), magnesium (17.49 mg/100g), potassium (359.75 mg/100 g), zinc (0.36 mg/100 g), pH (4.12), soluble solids (6.20°Brix), pectin (1.61%) and vitamin C (1.97 mg/100g). The sample used in our study has a higher amount of iron (7 mg/100g) than the sample characterized by Pires et al... (2006) and by Marx et al... (1998) (2.5mg/100g), which can explain the scavenging ability against the radical DPPH found.

The IC_{50} found for AEC can be also explained by the contents of hydroxycinnamic acids present in the cubiu fruit. The hydroxycinnamic acid derivatives are compounds (belonging to non-flavonoids class) directly related to the antioxidant activity (SILVA et al. 2010). Gonçalves et al. (2010) studied 16 native Brazilian fruits and detected cubiu as the one with the highest content of hydroxycinnamic acids (239 mg/100 g of sample dry weight). Among all fruits, cubiu presented the second best antioxidant activity, by means of the oxygen radical absorbance capacity (ORAC) assay, and the sixth best by the DPPH method.

Nogueira et al. (2007) determined the antioxidant activity of other species of *Solanum* methanolic extracts, by the Brand-Williams method, which is based on DPPH reduction. The leaf extract of *Solanum americanum* was the most active ($IC_{50}=24 \mu\text{g/mL}$) followed by leaf extract of *Solanum cernuum* ($IC_{50}=30 \mu\text{g/mL}$) and fruit extract of *Solanum palinacanthum* ($IC_{50}=88 \mu\text{g/mL}$). The

antioxidant activity of plant extracts may not be related to a single class of phytochemicals. It is known that flavonoids and their glycosidic derivatives are good antioxidants and the presence of these compounds certainly contributes to this activity. However, this activity may also be related to presence of coumarins and anthraquinones.

Coutinho (2009) evaluated the antioxidant activity in the *Solanum* genus species, one of the largest in the plant kingdom and the most representative of the Solanaceae family, by DPPH method. The methanolic extract from the *Solanum torvum* was more potent ($IC_{50}=7.36 \mu\text{g/mL}$) than the other extracts and no significant difference was found between methanolic extract and the standard (tebonin - $IC_{50}=7.30 \mu\text{g/mL}$) values. The methanolic extract of *Solanum mauritianum* proved be the least powerful ($IC_{50}=52.65 \mu\text{g/mL}$).

Considering results previously reported in literature, involving different antioxidant properties of various fractions from the same fruit (ROESLER et al. 2007; MELO et al. 2008) and, considering the results found in this work, further studies about other cubiu fractions in order to elucidate the best source of compounds with antioxidant activity are indicated. *In vitro* tests of antioxidant activity may not reflect the *in vivo* behavior, but can serve as a preliminary potential antioxidant indicator (NOGUEIRA et al. 2007). The continuation of this line of research becomes relevant since the search for natural antioxidants has increased considerably in recent years.

Antimicrobial activity

The AEC showed no antimicrobial activity against the strains tested. For bacterial strains, chloramphenicol, showed MIC ranging from 2.5 to 250 $\mu\text{g/mL}$ and, for *Candida albicans* ATCC 10231, the nystatin MIC was 20 U/mL.

Sandoval (2010) observed that 0.1 to 0.5 mL of cubiu fruit pure extract inhibited 100% of *Helicobacter pylori* growth *in vitro*. The inhibitory effect of the extract was higher than amoxicillin, tetracycline, clarithromycin and amikacin antibiotics. In the present study, the microorganisms were different from that one studied by Sandoval (2010).

Nogueira et al. (2007) evaluated the antimicrobial activity from other species of Solanaceae methanolic extracts, by measuring the diameter of the inhibition zone. For the active extracts, the susceptibility testing by broth microdilution was done. The extracts from *Solanum americanum* and *Solanum cernuum* leaves were active against *Pseudomonas aeruginosa* (MIC=2.5

mg/mL for both), *Bacillus cereus* (MIC=1.25 mg/mL and MIC=2.5 mg/mL, respectively) and *Shigella sonnei* / *Salmonella tythimurium* (MIC=5.0 mg/mL and MIC=2.5 mg/mL, respectively). The extract of *Solanum palinacanthum* fruit was active against *Staphylococcus aureus* / *Salmonella tythimurium* (MIC=5.0 mg/mL for both) and *Pseudomonas aeruginosa* / *Bacillus cereus* (MIC=2.5 mg/mL for both). None extract was active against *Escherichia coli* and *Klebsiella pneumoniae*.

Wound healing activity

Macroscopic evaluation

In the first day, after wound induction, the presence of whitish areas was observed, resulting from the induction of dermal ulcers, in all quadrants. In most cases, there was also the presence of reddened areas, especially at the edges.

In quadrants of positive control whitish and reddish areas were observed, with no pus and

bleeding. On the seventh day, scabs and healed edges were notice. In the negative control treatment, no healing processes were observed, with reddish areas during all the treatment.

In quadrants treated with 5% and 10% AEC, on the fifth to tenth day, a small decrease of whitish and reddish areas was noted. Healed edges appeared but few areas with bruises were still observed. There were no pus, discharge, bleeding and scabs. In quadrants treated with 5% AEC associated with 1% copaiba oil and 5% AEC associated with 1% rosemary oil, on the eighth day, a decrease in reddish areas and lesions starting to heal were observed. In all treatments, from the fifth day, the lesions looked "wet" due to possible emollient cream properties.

The contraction of the dermal ulcers was calculated as the difference between the fifth day and the tenth day areas. The ulcer area contraction was statistically similar in all treatments (Table 1).

Table 1. Evaluation of ulcer area contraction in rabbits submitted to the proposed treatments.

Treatments	N	Ulcer area contraction (cm ²)*	Ulcer area reduction (%)
Base cream (negative control)	4	1.07 ± 0.66	28.2
1% silver sulfadiazine (positive control)	4	1.49 ± 1.46	--
Cream containing 5% AEC	4	1.08 ± 0.72	27.5
Cream containing 10% AEC	4	1.45 ± 1.03	2.7
Cream containing 5% AEC + 1% copaiba oil	4	0.88 ± 1.20	40.9
Cream containing 5% AEC + 1% rosemary oil	4	1.46 ± 1.26	2.0

* Results are expressed as mean ± standard deviation. The ulcer area contraction was statistically similar in all treatments (p>0.05); AEC: aqueous extract of cubiu.

Microscopic evaluation

Figure 1 shows some of tissue sample microscopic fields containing the negative (A) and positive (B) controls and treatment groups with the extract of interest (C to F), where the numbers of inflammatory cells, fibroblasts, blood vessels and collagen and extracellular matrix areas could be evaluated.

The number of inflammatory cells in the positive control was lower than the number in treatments with 5% and 10% AEC (p<0.001 and p=0.019, respectively), and in comparison with negative control group (p<0.001) (Figure 2A). There was no statistically significant difference between

treatments of interest (AEC alone or in combination). The inflammatory cell number in all treatments was statistically similar to what was found in the negative control, demonstrating that the compounds tested did not have anti-inflammatory activity.

A product with wound healing activity should accelerate the migration and proliferation of fibroblasts. Treatments with 5% and 10% AEC (p=0.001 for both) and treatment with 5% AEC associated with 1% copaiba oil (p=0.020) showed a number of fibroblasts higher than the positive control. However, these values were not statistically superior to the cream base (Figure 2B).

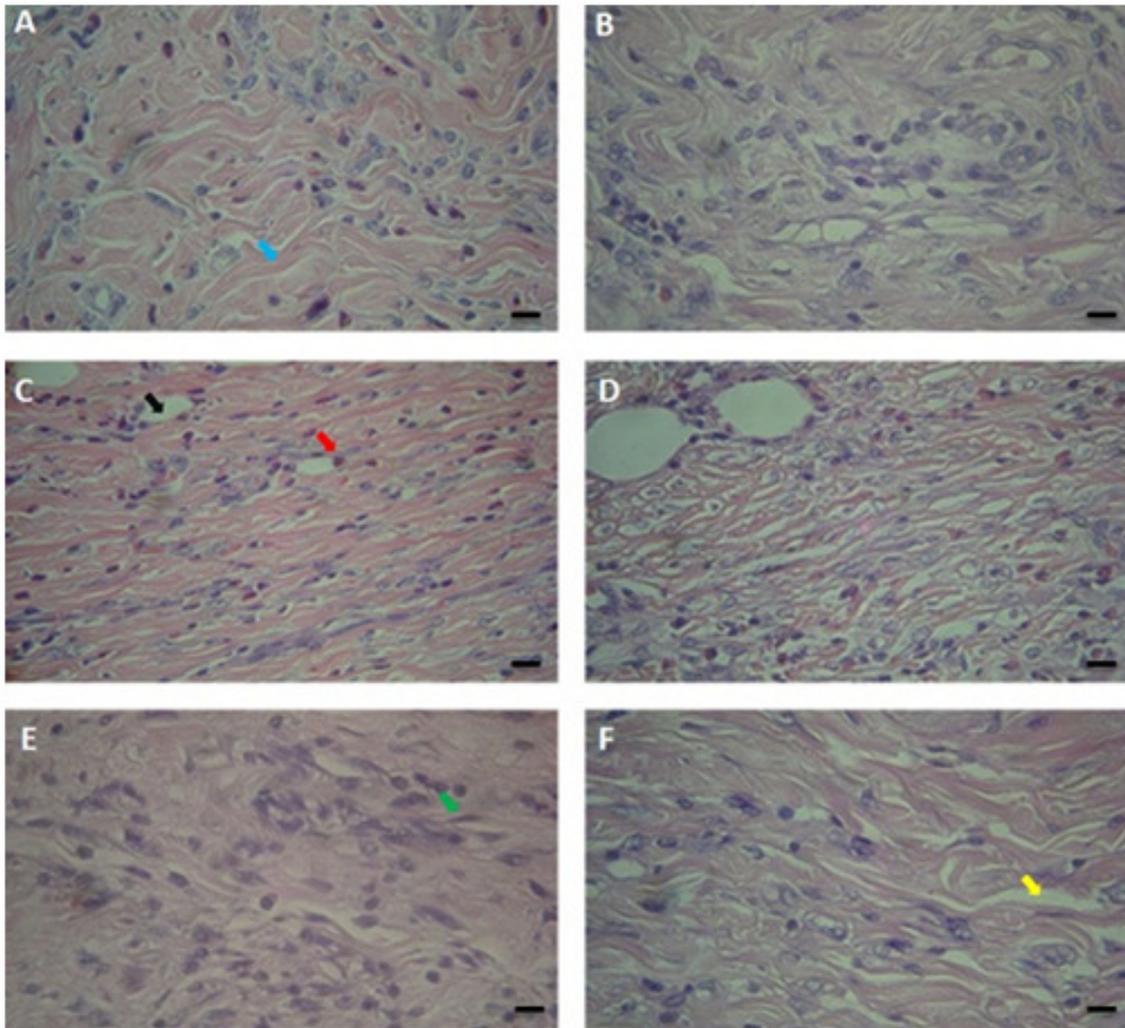


Figure 1. Microscopic analysis of skin ulcers treated with cream base (A), 1% silver sulfadiazine (B), cream containing 5% aqueous extract of cubiu (C), cream containing 10% aqueous extract of cubiu (D), cream containing 5% aqueous extract of cubiu associated with 1% copaiba essential oil (E) and cream containing 5% aqueous extract of cubiu associated with 1% rosemary essential oil (F). Cells inflammatory (red arrow), fibroblasts (green arrow), blood vessels (black arrow), collagen area (blue arrow) and extracellular matrix area (yellow arrow) were counted. Color with hematoxylin-eosin and analyze by optical microscope (total magnification of 400x). Bar equals 10 μ m.

Wound vascularization promotes more effective healing due to the transport of blood components and nutrients until the injured tissue (MANDELBAUM, 2003; BLANES, 2010). Treatment with 5% AEC associated with 1% copaiba oil showed the best result. It was statistically higher than the controls, the treatment with 10% AEC and the treatment with 5% AEC associated with 1% rosemary oil ($p < 0.001$, $p = 0.003$ and $p < 0.001$ respectively). It was also different from the negative control ($p = 0.024$), being this result consistent since the cream base has no apparent therapeutic activity (Figure 2C).

According to Carvalho (2002), the extracellular matrix is replaced by collagen fibers in fibroblastic phase of wound healing. Practically, all

treatments showed collagen area similar to controls. There was only a statistically significant difference between the positive control and treatment with 5% AEC associated with 1% copaiba oil ($p = 0.004$). Among the treatments, 5% AEC was superior to 5% AEC associated with 1% copaiba and rosemary oils ($p < 0.001$ and $p = 0.023$, respectively) (Figure 2D).

Treatment with 5% AEC associated with 1% copaiba oil presented an extracellular matrix greater than positive control ($p < 0.001$) and similar to negative control. Between treatments, there was a statistically significant difference among 5% AEC versus 5% AEC associated with 1% copaiba oil ($p < 0.001$) and 5% cubiu versus 5% AEC associated with 1% rosemary oil ($p = 0.022$) (Figure 2E).

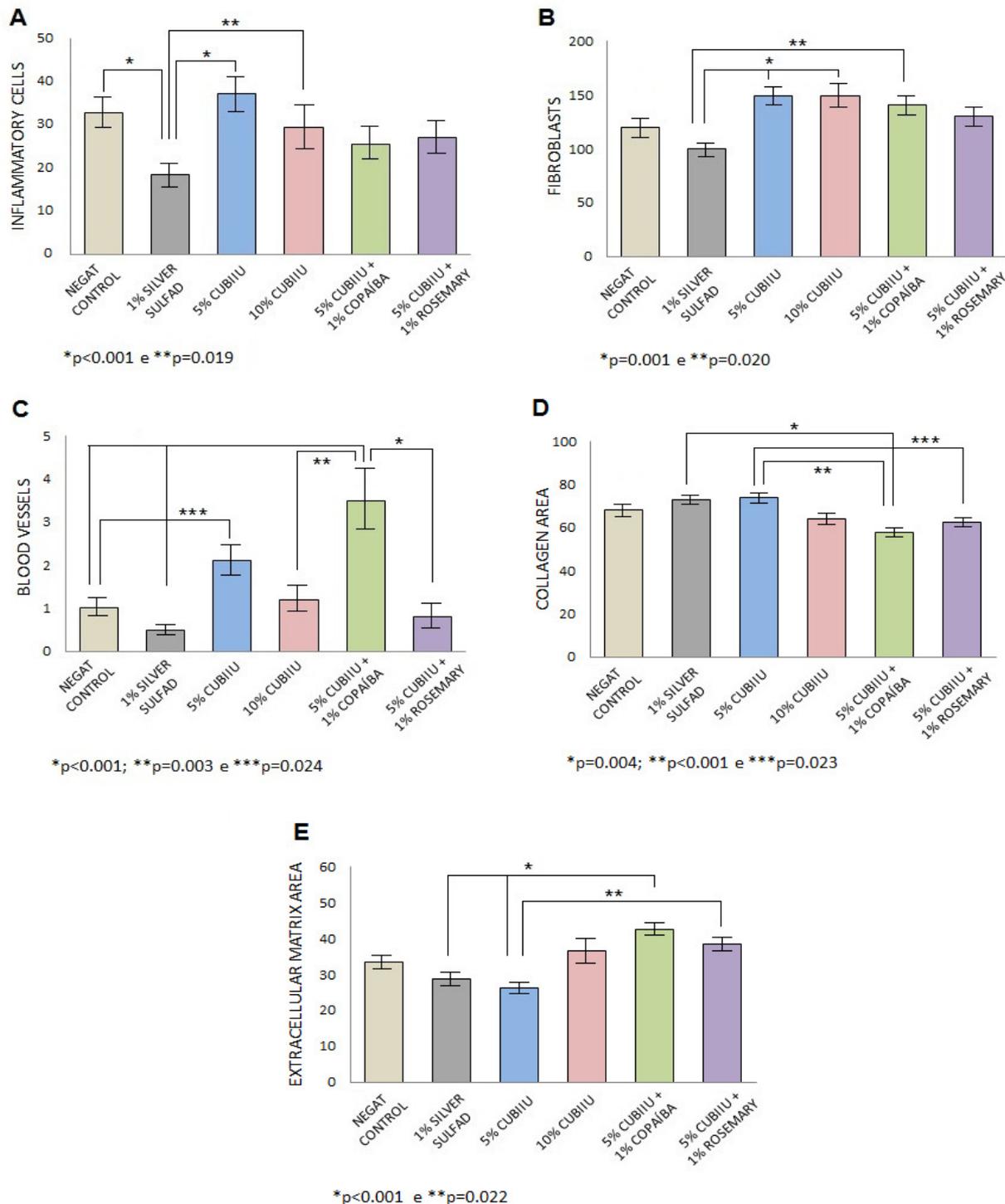


Figure 2. Number of inflammatory cells (A), fibroblasts (B), blood vessels (C) and percentage of microscopic field occupied by collagen (D) and extracellular matrix (E) areas, in tissue samples originating from animal dermal ulcers. Data were expressed as mean \pm standard error of mean. * Means were statistically different by ANOVA followed by Tukey *post hoc* test. Negat control=negative control; 1% silver sulfad=1% silver sulfadiazine (positive control); 5% cubiu=5% aqueous extract of cubiu; 10% cubiu=10% aqueous extract of cubiu; 5% cubiu + 1% copaiba=5% aqueous extract of cubiu associated with 1% copaiba oil and 5% cubiu + 1% rosemary=5% aqueous extract of cubiu associated with 1% rosemary oil.

A cream containing 1% silver sulfadiazine was used as positive control since it was described in several studies for treatment of burn wounds, with the purpose of removing necrotic tissue and combating local infection (FRANCO; GONÇALVES, 2008; COELHO et al. 2010). It is one of the most used topical agents in treating second and third degree burns (FERREIRA et al. 2003). Lanette cream, an anionic emulsifying wax widely accepted by doctors and consumers due to its giving softness and smoothness to skin, was employed as negative control (PRISTA, 2002).

Some studies have already evaluated the topical use of phytotherapeutic drugs in tissue healing process induction. Dutra et al. (2009) evaluated the healing potential of creams containing essential oil (EO) and hexane fraction (HF) from *Pterodon emarginatus* seeds. Wound healing activity of the creams was found to have a significant decrease in inflammatory cells number ($p < 0.01$ for all) and a significant increase in fibroblast numbers ($p < 0.01$ for 5% EO, 10% EO and 10% HF) and in blood vessel numbers ($p < 0.01$ for 10% EO and 20% HF), when compared to the Lanette cream base. Silva (2008) found *Centella asiatica* aqueous extracts (5 and 10%) were effective in healing process in rabbits, characterized by greater fibroblasts number of 5% aqueous extract cream ($p = 0.0001$), lowest inflammatory cells number ($p < 0.05$) and increased collagen area ($p < 0.05$) of both concentration, compared to Lanette cream base and silver sulfadiazine 1%.

The joint data analysis suggests that 5% AEC associated with 1% copaiba oil was the formulation which exhibited the greatest pharmacological potential in dermal ulcers healing. It is supported by the largest number of blood vessels, which facilitates the vascularization process, and the largest extracellular matrix area presented. However, the activity was modest and it is suggested a further deeper analysis like immunohistochemical tests or new healing activity studies using formulations including other associations.

CONCLUSIONS

The AEC showed antioxidant potential, being needed additional studies to use this property in pharmaceutical sector, and showed no antimicrobial activity against strains tested.

The use of AEC in association with copaiba oil showed promising results in wound healing study. It is important to continue this research line, about biological activities of cubiu, in the search for bioactive compounds.

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RESUMO: O presente estudo avaliou as atividades antioxidante, antimicrobiana e cicatrizante do extrato aquoso do cubiu (EAC - *Solanum sessiliflorum* Dunal). A atividade antioxidante foi avaliada pelo método sequestrante de radicais 2,2-difenil-1,2-picrilhidrazil (DPPH) e, a atividade antimicrobiana, por método turbidimétrico. Para o estudo da atividade cicatrizante foi utilizado o modelo de indução de úlceras dérmicas em coelhos, sendo testados cremes contendo 5% e 10% de EAC e cremes contendo 5% de EAC associado à 1% de óleo de copaíba e à 1% de óleo de alecrim. As lesões foram analisadas macro (aspecto da lesão e contração da área ferida) e microscopicamente. O estudo histológico considerou o número de células inflamatórias, fibroblastos, vasos sanguíneos, e as áreas de colágeno e de matriz extracelular. Foi realizada análise de variância (ANOVA) seguida do teste *post hoc* de Tukey. O EAC apresentou $IC_{50} = 65,12 \mu\text{g/mL}$ e não apresentou atividade antimicrobiana frente a nenhuma das cepas testadas. A formulação contendo 5% de EAC associado a 1% de óleo de copaíba foi a que exibiu maior potencialidade farmacológica na cicatrização de lesões dérmicas, devido ao maior número de vasos sanguíneos ($p < 0,001$) e a maior área de matriz extracelular ($p < 0,001$) apresentados. Os resultados encontrados justificam novos estudos sobre as atividades biológicas do cubiu na busca de compostos bioativos.

PALAVRAS-CHAVE: Atividade antimicrobiana. Atividade antioxidante. Atividade cicatrizante. Cubiu. *Solanum sessiliflorum* Dunal.

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