CYTOTOXIC, ANTIMICROBIAL AND HEALING ACTIVITY OF THE *Jatropha gossypifolia* L EXTRACT.

ATIVIDADE CITOTÓXICA, ANTIMICROBIANA E CICATRIZANTE DO EXTRATO DA *Jatropha gossypifolia* L.

ACTIVIDAD CITOTÓXICA, ANTIMICROBIANA Y CICATRIZANTE DEL EXTRATO DE LA *Jatropha gossypifolia* L.

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ABSTRACT

Objective: to investigate the cytotoxic, antimicrobial and cicatrizing potential of extracts of leaves, branches and stem of *J. gossypifolia* L. **Method:** quantitative, experimental study. The extracts were obtained by maceration in ethanol, concentrated in a rotary evaporator and dried in a vacuum desiccator. In the analysis, phytochemical prospecting; cytotoxicity; microdilution in broth and *Scratch assay* tests were performed. Among the detected metabolites, were: tannins, steroids, flavonoids, flavones, xanthenes. **Results:** the stem extract presented cell viability above 80%. The leaves were moderately cytotoxic and the branches showed no cell viability. The extracts inhibited the growth of *S. aureus*, *S. epidermidis* and *P. aeruginosa* at different concentrations. *Scratch assay* showed that the methanolic fraction of the leaves allowed the cellular migration in 45% more than the control. **Conclusion:** studies with this plant species should be continued for isolation of the active principle, aiming at the production of a wound healing phytotherapeutic. **Descriptors:** Healing; Nursing; Cytotoxicity; Medicinal Plants.

RESUMO

Objetivo: investigar o potencial citotóxico, antimicrobiano e cicatrizante de extratos das folhas, galhos e caule da *J. gossypifolia* L. **Método:** estudo quantitativo, experimental. Os extratos foram obtidos por maceração em etanol, concentrados em evaporador rotatório e secos em dessecador a vácuo. Na análise, realizaram-se testes de prospecção fitoquímica; citotoxicidade; microdiluição em caldo e *Scratch assay*. Entre os metabólitos detectados, estiveram: taninos, esteroides, flavonoides, flavonas, xantonas. **Resultados:** o extrato do caule apresentou viabilidade celular acima de 80%. As folhas foram moderadamente citotóxicas e os galhos apresentaram ausência de viabilidade celular. Os extratos inibiram o crescimento de *S. aureus*, *S. epidermidis* e *P. aeruginosa* em diferentes concentrações. O *Scratch assay* evidenciou que a fração metanólica das folhas propiciou a migração celular em 45% a mais do que o controle. **Conclusão:** estudos com esta espécie vegetal devem ser continuados para isolamento do princípio ativo, visando à produção de um fitoterápico cicatrizante de feridas. **Descritores:** Cicatrização; Enfermagem; Citotoxicidade; Plantas Medicinais.

RESUMEN

Objetivo: investigar el potencial citotóxico, antimicrobiano y cicatrizante de los extractos de las hojas, ramas y el tallo de *J. gossypifolia* L. **Método:** estudio cuantitativo, experimental. Los extractos fueron obtenidos por maceración en etanol, concentrados en evaporador rotatorio y seco en desecador al vacuo. En el análisis, se realizaron pruebas de prospección fitoquímica; citotoxicidad; microdilución en caldo y *Scratch assay*. Entre los metabolitos detectados, estuvieron: taninos, esteroides, flavonoides, flavonas, xantonas. **Resultados:** el extracto del tallo presentó viabilidad celular por encima del 80%. Las hojas fueron moderadamente citotóxicas y las ramas presentaron ausencia de viabilidad celular. Los extractos inhibieron el crecimiento de *S. aureus*, *S. epidermidis* y *P. aeruginosa* en diferentes concentraciones. El *Scratch assay* evidenció que la fracción metanólica de las hojas propició la migración celular en un 45% más que el control. **Conclusión:** estudios con esta especie vegetal deben ser continuados para aislamiento del principio activo, visando la producción de un fitoterápico cicatrizante de heridas. **Descriptor:** La Curación; Enfermería; Citotoxicidad; Plantas Medicinales.

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INTRODUCTION

Throughout the ages, medicinal plants have been used to treat numerous diseases and also to heal wounds. This knowledge has passed from generation to generation, through empirical knowledge and, has now, increased the number of publications with the purpose of scientifically proving this use, since they represent an important source of metabolites that act as inhibitors of various biological activities through the action of their molecules bioactive.¹

The indiscriminate and inadequate use of antibiotics has contributed to the emergence of a microorganism resistant to several drugs, contributing to an increase in the number of colonized and/or infected wounds, thereby, delaying the healing process. According to data from the World Health Organization, 25% of the world's deaths are due to infectious diseases.² Wound care is part of the Nursing care attributions, with a large number of studies published in this area, demonstrating the important role and responsibility of nurses in this process and in the search for new ways of care, attentive to the innovations of care in this area, to improve the quality of care.³

In this way, the use of therapies, other than conventional therapies, such as the use of herbal medicines, extracts or, even medicinal plants *in natura*, for wound healing, has been increased, in recent years, by the search for active principles isolated from plants, that have an effective role in the healing process.⁴

The species chosen, *Jatropha gossypifolia* L., is popularly known as a purple top, teuton-potato, purgative herb, jalapon, mamoninha, teuton-root, purple peon and purple pinion. Several parts of the plant have been used, in folk medicine for the treatment of peptic ulcers, diabetes, neoplasias, diarrhea and, as a healer and diuretic. It is notable for its analgesic and anti-inflammatory potential, as well as being used for the treatment of eczema, abscesses, wound healing, dysentery, leprosy, arthritis, otitis, alopecia, venereal diseases, stomach pains, obstructions of the abdominal tract, rheumatism and bite of venomous animals⁵⁻⁶, becoming pertinent in the practice of folk medicine based on the specific knowledge of this plant.

It is a cut of a basic, experimental research that sought to generate new knowledge for the advancement of science. This type of study forms the foundation for clinical research and is recognized as a fundamental tool for the development of new therapies.⁷

This study aimed to investigate the cytotoxic, antimicrobial and healing potential of leaf, branch and stem extracts of *J. gossypifolia* L.

METHOD

♦ Plant material

The plant material was collected in the Cidade Universitária - Maceió-Alagoas complex, whose coordinates 9° 32'47.3 "S 35° 47'07.6" W, in the month of January 2016, and was identified at the Institute of the Environment (IMA) of State of Alagoas, the exsicata being deposited with the MAC registry 241.

♦ Obtaining Ethanolic Extract

The plant material of the leaves, branches and stem, of the plant species *J. gossypifolia* L., underwent drying, at room temperature, for five days. Then, the material was minced and was extracted by steeping with 98% ethanol. Subsequently, the extracts obtained were rotated at 40 ° C to concentrate them.

♦ Fractioning of the crude ethanolic extract

The fractionation of the extracts of the species in question was carried out with an aliquot of 9.9 grams of leaves and 9.6 grams of stem of the ethanolic extracts, in which they were partitioned in a vacuum filtering column, using, as a stationary phase, silica gel and, as mobile phase, hexane, chloroform (CHCl₃), ethyl acetate (EtOAc) and methanol (MeOH), following that order of polarity.⁸

The solutions obtained were concentrated in a rotary evaporator, resulting in four phases: hexane, chloroform, ethyl acetate and methanol. Thereafter, the wet materials were placed in suitable vials and dried at room temperature.

♦ Phytochemical Analysis

Phytochemical prospecting of *J. gossypifolia* L. was performed according to a methodology already used.⁸ Qualitative and semi-quantitative tests were carried out for phenols and tannins (reaction with ferric chloride), anthocyanins, anthocyanins, flavonoids, leucoanthocyanidins, catechin, flavanones, flavanones, xanthones (granulated magnesium efflorescence test and hydrochloric acid), steroids and triterpenes (Liebermann-Burchard's test), saponins (test of pH variation with sodium hydroxide and hydrochloric acid), flavonols, flavanones, foam and precipitation test), alkaloids (identification with Dragendorff), anthraquinones, anthrones and coumarins (UV light test).

♦ Evaluation of cell viability by the MTT technique

Cytotoxicity was evaluated by the use of the colorimetric method by the methyl-tetrazolium salt (MTT), in which macrophages of the J774 lineage were used. For the fractions from the ethanolic extracts of leaves and stems of the plant species under study, fibroblasts from the 3T3 lineage.⁹⁻¹⁰

The ethanolic extracts from leaves, stems and branches of *J. gossypifolia*, previously dissolved in Ethanol, were serially diluted in the RPMI medium to obtain the final concentrations and added in a 96 well plate (100 µL/well). Cells (1.5×10^5 cells/well) were cultured in a 96-well plate with culture medium (RPMI-1640 supplemented with 10% fetal bovine serum, 1% glutamine and 40 µg/mL gentamicin). Therefore, the cells were treated with extracts at concentrations ranging from 62.5; 125 or 250 µg/mL in culture medium. They were then incubated, for 24 hours, in a 5% CO₂ incubator at 37° C and, then 25 µL of the MTT solution were added, incubating the plates for a further three hours. After this time the absorbance reading was carried out in spectrophotometer at 595 nm.¹¹

♦ Microorganisms used

Gram-positive bacterial strains: *Staphylococcus aureus* (ATCC 25923) and *Staphylococcus epidermidis* (ATCC 31488) were used to evaluate the antimicrobial activity; Gram-negative: *Pseudomonas aeruginosa* (ATCC 27853) and *Escherichia coli* (ATCC 25922).

♦ Microdilution in broth for determination of Minimum Inhibitory Concentration (MIC)

CIM was performed according to the protocol based on the Clinical Laboratory Standards Institute, in which the Mueller-Hinton Broth (CMH) was distributed in the 96 well plates and 100 µL/well was placed.¹² The solubilization of the samples occurred by the association of 2000 µg of the sample in question, added to 20 µL of DMSO solution, being diluted in 980 µL of sterile saline, resulting in a stock concentration of 2000 µg/mL. In columns one to nine of line A, 100 µL of the solubilized samples were added, and columns ten, 11 and 12 were destined for the Growth Control, Negative Control and Plate Sterility Control, respectively.

After this process, 100 µL of the contents of each hole in line A were transferred to line B and, after homogenization, this process was repeated until line H, in which excess was discarded. Microbial inoculums were prepared based on an already used protocol, which used

the McFarland 0.5 scale (108 CFU/mL).¹² Bacteria were diluted in three mL of sterile saline and one mL of this was rediluted in the ratio of 1:10 sterile saline solution, then, five µL (104 CFU/mL) was deposited in the wells of columns one to 11. The plates were then incubated in a bacteriological oven at 35° C, for 16 to 20 hours. After this time, 20 µL of Triphenyl Tetrazolium Chloride (TTC) was added to each well and the plates were reincubated for another three hours. Then, the wells that changed from colorless to red indicated the presence of a living microorganism. However, absence of staining means positive result for inhibition of the samples on the evaluated bacteria.

♦ In vitro healing potential using Scratch assay

Fibroblast proliferation was studied in vitro using a 3T3 fibroblast assay of mouse embryo tissue.¹³ Initially, fibroblasts were cultured in DMEM culture medium supplemented with 10% fetal bovine serum (FBS) and incubated in a greenhouse at 5% CO₂ at 37° C, for 24 hours. Subcultures were performed using the trypsin-EDTA solution to peel off the adhered cells. After growing and being trypsinized, the fibroblasts were seeded in 12-well plates and kept in a 5% CO₂ oven, at 37° C. The culture medium was then removed and the Scratch assay was performed, in which a straight line was made in the median region of the plate with the tip of the 200 µL pipette, causing a rupture between the cells and causing a mechanical lesion. For the removal of debris resulting from the lesion, the wells were washed with phosphate buffered saline (PBS).

Then, the plates were given the extracts of *J. gossypifolia*, in concentration of 125 µg / mL. Established this concentration, after results of the MTT, with the fractions of leaves and stems of said plant, whose values in the concentration 125 µg/mL evidenced cell viability above 90% of the viability, using the migration times in hours, that were zero, 12 and 24 hours.¹³

The images were captured with a digital camera coupled to the inverted phase microscope using NIS Elements F 3.2 software, and obtained from the same field of view of the wound, creating reference points on the outside of the plate and on the microscope stage with fine-point markers. Thus, the migration of fibroblasts was evaluated by means of these photographs, in which the lesional area was measured at times zero, 12, 24 hours.

RESULTS

◆ Phytochemical prospecting

In the phytochemical prospection of the crude extracts of the leaves, stems and branches of the plant species *J. gossypiifolia*,

the presence of the tannin, steroid and flavonoid secondary compounds was evidenced in the three samples tested, and in the leaf extracts the constituents flavones and Xanthones were also found, as evidenced in Table 1.

Table 1. Phytochemical screening of extracts and fractions *J. gossypiifolia* L. Maceió (AL), Brazil, 2017.

Extracts/Fractions	Chemical groups					
	Tannins	Steroids	Flavonoids	Flavones	Alcaloids	Saponins
EEF	+	+	+	-	-	-
EEC	+	+	+	-	-	-
EEG	+	+	+	-	-	-
F1	+	+	+	-	-	-
F2	+	-	+	+	-	-
F3	+	+	-	-	-	-
F4	+	+	-	-	-	-
C1	-	+	+	+	-	-
C2	-	-	-	+	-	-
C3	+	+	+	+	-	-
C4	+	+	+	-	-	-

Caption: (-) absent, (+) present. EEF = leaves ethanolic extract; EEC = ethanolic extract of the stem; EEG = ethanolic extract of the branches; F = leaves; C = stem: 1 = methanolic fraction; 2 = fraction ethyl acetate; 3 = chloroform fraction; 4 = hexane fraction.

In relation to the chloroform and hexane fractions of the stem, the presence of tannins, flavonoids and steroids was verified, whereas in the methanolic fraction, the presence of flavonoids and steroids. In hexane only, flavones were found. For the leaf fractions, tannins and steroids were found in the four fractions samples, while in the methanolic and ethyl acetate fractions flavonoids, were identified. In the same manner as in the ethyl

acetate fraction, the flavones were evidenced. In none of the extracts and fractions were found saponins nor alkaloids.

◆ Cell Viability Assay by MTT Method

This assay was performed by the MTT method against J774 macrophages cells to evaluate possible cytotoxicity of the ethanolic extracts of leaves, stems and branches of *J. gossypiifolia* (Figure 1).

Figure 1. Cell viability of the ethanolic extracts of *J. gossypiifolia* L. Maceió (AL), Brazil, 2017.

Caption: The ethanolic extracts of the stem (C1), ethanolic extract of the leaf (F1) and ethanolic extract of the branches (G1).

The stem extracts, at the concentration of 62.5 µg/mL, had a percentage of cell viability of 77.09%, whereas, in the concentration of 125 µg/mL and 250 µg / mL, the percentage of viable cells was higher, with values of 87.00% and 82.00%, respectively.

Leaf extracts showed low percentages of cell viability at concentrations of 62.4 µg/mL (51.00%), 250 µg/mL (49.89%) and 250 µg/mL

(54.74%). The extracts from the branches, at the concentration of 62.5 µg/mL, showed a percentage of viable cells of 52.12% and reached 39.29% and 32.44%, respectively, at concentrations of 125 µg/mL and 250 µg/mL (Figure 2).

In the MTT fractions, 3T3 fibroblasts were used, since, for the MTT test of the fractions of the leaves and stems, the choice of 3T3

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fibroblasts rested on the need to perform

Scratch assay, which is done with fibroblasts

of this lineage. Figures 2 and 3 show these results.

Figure 2. Cell viability of stem fractions. Maceió (AL), Brazil, 2017.
Caption: F: Leaf; 1: Methanolic fraction; 2: Ethyl acetate fraction; 3: Chloroform fraction; 4: Hexanic fraction.

As shown in figure 2, only fractions of ethyl acetate and hexane in the concentration, of 250 µg / mL, showed lower percentages of cell viability, both with 67.02% and 67% 85%, respectively.

117.08% and 110.02%, respectively. While at the concentration of 62.5 µg / mL, the methanolic fractions (108%) and ethyl acetate (115.3%) were those that induced cell proliferation.

At the concentration of 125 µg / mL, the methanolic and chloroform fractions induced cell proliferation, presenting percentages of

Figure 3. Cell viability of leaf fractions. Maceió (AL), Brazil, 2017.
Caption: C: Leaf; 1: Methanolic fraction; 2: Ethyl acetate fraction; 3: Chloroform fraction; 4: Hexanic fraction.

Figure 3 shows that the methanolic fraction induced cell proliferation at all concentrations, especially the concentration of 250 µg/mL with a percentage of cell viability of 225.08%.

◆ **Determination of Minimal Inhibitory Concentration (MIC)**

The MIC was initially performed with the crude extracts and was repeated with the leaves and stem fractions from these extracts. All the ethanol extracts showed inhibitory activity against three microorganisms. The ethanolic extract of the leaves inhibited the

S. aureus, *S. epidermidis* and *P. aeruginosa* microorganisms, at the concentration of 500 µg/mL. The stem was resistant to *S. aureus* and *P. Aeruginosa*, at 1000 µg/mL. At the concentration of 500 µg / mL inhibited *S. epidermidis* growth. The ethanolic extract from the leaves inhibited *S. aureus* at the concentration of 1000 µg/mL, *P. aeruginosa* at 500 µg/mL and *S. epidermidis*, at a concentration of 250 µg/mL.

It is important to stress that the ethanolic extracts did not present antimicrobial activity for *E. coli* and reaffirm that the fractions

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showed no inhibitory activity for the tested microorganisms.

♦ In vitro healing potential using Scratch assay

In order to evaluate the cicatrization activity *in vitro*, the methanolic fraction from the leaves of *J. gossypifolia* was chosen

because it obtained the best results against MTT with 3T3 fibroblasts. In figure 4, fibroblasts treated with this fraction at 125 µg/mL, exhibited an increase in migration at 12 h and 24 h times, as compared to cells treated with culture media.

Figure 4. Analysis of fibroblast migration rate. Maceió (AL), Brazil, 2017.

The other fractions did not promote this migration. It was verified that the treatments with the fractions led to changes in these cells, inhibiting the migration process. The methanolic fraction may be a viable alternative to invest in further research that provides the necessary safety for the use of this plant species in the treatment of wounds.

The effect of the methanolic fraction of the leaves on the migration of the fibroblasts during the experiment at zero time, did not distinguish the control and the fraction. It was noticed that, in the time of 12h and 24h, the methanolic fraction surpassed in 45%, more than the control.

DISCUSSION

The following secondary metabolites: tannins, flavonoids, steroids and coumarins, were evidenced in the species *J. gossypifolia* of this study. These data corroborate with studies that carried out phytochemical prospecting with plants of the same genus, which identified the presence of alkaloids, tannins, flavonoids, steroids and saponins, these data are similar to those found in another study.¹⁴⁻¹⁵

Another study found secondary metabolites such as tannins, flavonoids, steroids and saponins in the different parts of *J. gossypifolia*, which corroborates this study.⁶ However, metabolites such as terpenoids, quinones, lactones and lignans reported by other studies were not found in the extracts studied in this study.^{6,15}

The *Jatropha* genus has several secondary metabolites such as organic acids, alkaloids,

carotenes, phenoesteroids, phytosteroids, flavonoids, glycosides, lactones (coumarins), lignans, mucilages, pectins, polysaccharides, quinones, saponins, tannins and terpenes, which characterize it as a genre that presents different biological activities, among them antimicrobial, antiinflammatory and adstringent.¹⁵ Other phytochemical studies showed the presence of sugars, alkaloids, amino acids, steroids, coumarins, lignans, flavonoids, proteins, saponins, tannins and terpenes in the gross extracts.¹⁶⁻¹⁷

The presence of the secondary metabolites, found in the plant species in question, serves as a possible basis, for the biological activities found in the tests performed with the *J. gossypifolia* samples.

As for cytotoxicity, the crude extracts were initially tested against macrophages, which presented slightly cytotoxic results, for extracts of the stems at concentrations 62.5; 125 and 250 µg/mL. For leaves, at the same concentrations, the results obtained were moderately cytotoxic, while the result of the branches, also in the same concentrations, showed severe cytotoxicity, with more than 50% of nonviability, which made it impossible to continue the study with this part of the plant.

In the *in vitro* cytotoxicity assay against HEK-293 cells, at concentrations of 62.5; 125; 250 and 500 µg/mL, from the crude extract of the leaves of *J. gossypifolia* obtained by decoction, no cytotoxic effect, was evidenced, therefore, being considered 100% cell viability.¹⁸ This data contradict those obtained in this research, since the

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cytotoxicity test of the crude extracts of the stem to the macrophages presented slightly cytotoxic results, the leaves were moderately cytotoxic; and the branches presented cell viability of less than 50%.

However, when cell viability tests were carried out, with fractions of leaf and stem extracts of *J. gossypifolia*, the methanolic and ethyl acetate fractions, in the three concentrations studied were not cytotoxic, with viability greater than 90%, while the chloroform fraction, in the concentration of 250 µg/mL, presented viability less than 50%. Virtually all the stem fractions did not present cytotoxicity except ethyl acetate and hexane at concentrations of 250 µg/mL considered to be moderately cytotoxic with a percentage of 50-79% viability.¹³ Increased chloroform fraction concentration induced a decline in number of viable cells, leading to a reduction of 50% ($p < 0.001$) in cell viability, at the concentration of 250 µg/mL. The hexane fraction decreased the viability of 3T3 fibroblasts at all concentrations tested.

In parallel, preclinical toxicological studies, in rats treated with the leaf and stem ethanolic extract of *J. gossypifolia* demonstrated low acute oral toxicity. However, their prolonged use in an animal model of chronic toxicity revealed severe liver, pulmonary and renal damage. Therefore, further studies should be performed to elucidate the cytotoxic potential of this plant.¹⁴

In relation to the minimal inhibitory concentration, the extracts of *J. gossypifolia* showed inhibition against the bacteria tested, while the ethanolic extract of the leaves presented a MIC of 500 µg / mL for *S. aureus*, *S. epidermidis* and *P.* while the ethanolic extract of the branches exhibited an inhibition of 1000, 500 and 250 µg/mL, and the ethanolic extract of the stem showed antimicrobial activity at 1000, 500 and 1000 µg/mL, respectively. It was also found that the extracts tested were not able to promote the inhibition of the *E. coli* microorganism.

J. gossypifolia had antimicrobial action against eight microorganisms tested (*Bacillus cereus* var. *Mycoides*, *B. pumilus*, *B. subtilis*, *Bordetella bronchiseptica*, *S. epidermidis*, *Klebsiella pneumoniae*, *Streptococcus faecalis* and *Candida albicans*).¹⁹ As well as extracts of *J. gossypifolia* presented activity against *P. aeruginosa* and *Bacillus subtilis*.¹⁵ These results corroborate with another study that states that extracts of the genus *Jatropha* have antibacterial action.¹⁷

Another study about the antimicrobial activity of the root, stem and leaf extracts of

Croton pulegioides Baill, belonging to the same *Jatropha* family, concluded that the methanolic extract of the stem showed antimicrobial activity against the strains of *S. aureus* and *S. epidermidis*, while the methanolic extract of the leaves showed no activity. On the other hand, all the extracts were inactive in the concentrations evaluated against the Gram-negative bacteria used in the study (*Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumoniae*), a result that was also observed in the in vitro antimicrobial assays in this study.²⁰

It is important to note that, the inhibition of the fractions in the extracts is probably due to the synergism of the secondary metabolites, that, when the fractions were separated, the antimicrobial action was inactivated. The actual efficacy of a medicinal plant may not be due to a major active component, but to the combined action of different compounds, which in many cases the synergism of the secondary metabolites potentiates the biological action of numerous plant species.²¹

A study carried out confirmed the antibacterial activity of extracts of leaves extracted in chloroform and methanol against *Salmonella typhi*, *P. aeruginosa*, *S. aureus* and *C. albicans*.⁶ A study carried out with methanolic and ethyl acetate extracts of *Jatropha multifida* latex active against the following microorganisms: *Bacillus subtilis*, *S. aureus*, *P. aeruginosa*, *E. coli*, *K. pneumoniae* and *Candida sp.*²⁰ This result, different from the one found in this study, when fractionating leaf and stem extracts, no antibacterial activity was found.

The migration of fibroblasts is a key step in the proper healing of wounds, since they play an important role in the production and deposition of components of the extracellular matrix, such as collagen.²²⁻²⁴

In this study the evaluation of cicatrization was performed using an in vitro bioassay using *Scratch assay*, which evaluates cell migration in the time intervals of zero, 12 and 24 hours. This technique is based on the creation of an interruption of continuity of a cellular monolayer, or wound, in which the follow-up of the closure of this lesion is performed by observation under an inverted phase microscope.²⁵ This method was used in order to observe the cicatrizing action *in vitro* of extracts fractions of leaves and stem, measured by the cellular migration of 3T3 fibroblasts.

Time zero is the time when the wound is performed and there is no presence of cells, fibroblasts. For stem fractions, the migration

of these cells was not observed at any of the times. For the leaf fractions, a significant migration occurred in the methanolic fraction (45%), observing this increase in the migration rate when used in the 125 µg / mL concentration, which demonstrates the possibility of this fraction being an alternative for the production of a phytotherapeutic for the wound care.

CONCLUSION

MTT results indicated that the stem extract presented cell viability from mildly cytotoxic to non-cytotoxic, while leaf extract was moderately cytotoxic. As for the branches, the result was severely cytotoxic, preventing the continuation of the study with this sample.

The CIM of the leaf extract was 500 µg/mL for the *S. aureus*, *S. epidermidis* and *P. aeruginosa* bacteria, while the ethanolic extract of the branches exposed an inhibition of 1000, 500 and 250 µg/mL and the ethanolic extract of the stem exhibited antimicrobial activity in 1000, 500 and 1000 µg/mL, respectively. It was also found that, the extracts tested were not able to promote inhibition of the *E. coli* microorganism. The CIM, performed with the fractions from the extracts in leaves and stem ethanol, resulted in the absence of inhibitory activity for these bacteria.

The healing evaluation, by the *Scratch Assay* method showed that the methanolic fraction of the leaves allowed a 45% increase in fibroblast migration, signaling the possibility of this fraction being an alternative for the production of a herbal medicine for the treatment of wounds. Studies with this plant species should be continued for isolation of the active principle aiming at the production of a wound healing phytotherapeutic.

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