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Antimicrobial, antioxidant and cytotoxic activity of extracts of *Tabebuia impetiginosa* (Mart. ex DC.) Standl.

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ABSTRACT

Infection is one of the factors that most influence the wound healing retardation. The allocation of alternative resources to overcome the infection is essential for nurses, since they are responsible for the healing. This is an in vitro experimental study that investigated the antimicrobial, antioxidant and cytotoxic potential of crude extracts of plant species Tabebuia impetiginosa. The ethanolic leaf extract showed promising antimicrobial activity against Gram-positive strains of S. epidermidis (MIC ≥ 625 mg / mL) and S. aureus (MIC ≥ 312.5 mg / mL), demonstrating weak and moderate inhibitory activity, respectively. These same ethanol extracts of the leaves and twigs showed no cytotoxicity. These results provide evidence of safety in the therapeutic use of plant species so that future tests may be performed in vivo. This study highlights the expanded perspective on health, from the nursing context, as a way to promote technological innovation in infected wounds therapy. It is based on the achievement of experimental research, promoting the incorporation of new skills, knowledge and procedures for nursing science.

Keywords: *Tabebuia impetiginosa*; Anti-Infective Agents; Medicinal Plants; Nursing;

INTRODUCTION

The use of plants for medicinal purposes is as old as the emergence of human species on earth. Historically, first civilizations realized that some plants contained active ingredients in their essences, which empirically revealed their healing power when they were tested in disease [1].

Medicinal plants have been studied as alternatives to the treatment of different dermatological diseases, especially in those with complex healing process of resolution [2].

This complexity involves some factors that can affect the tissue repair process, which are systemic and local. Among the systemic factors, stand out: age, immobility, nutritional status, associated diseases and the use of continuous medication, especially immunosuppressants. These factors often cannot be eliminated, but they should be controlled. Among local factors, there are wound´s anatomic location, presence of devitalized tissue, and especially the presence of infection [3].

Infection can be understood as the invasion, growth and multiplication of microorganisms in body tissue [4]. Thus, presence of infection prolongs the inflammatory phase, consisting in one of the biggest barriers to the healing process. In determining major tissue damage and in retarding the healing process, it increases the healed tissue production, which can affect the function, aesthetics or both [5].

Among thousands Brazilian medicinal plants, Ipês are the most important. These species are grouped according to the color of the flower that can be yellow, purple or white. In Spanish speaking countries, some of these are called lapacho or Tabebuia [6].

Purple "Ipê" (*Tabebuia impetiginosa*) belongs to the Angiospermae's category, Bignoniaceae´s family, and genus *Tabebuia*. This plant is a native tree of Brazil, but it can be found in all Latin America [7].

Plants of the genus *Tabebuia* have lapachol as the main active component, a quinone compound, which is widely studied in various parts of the world [8]. Besides lapachol, the *Tabeuia impetiginosa* is extensively recognized for its biological activities due to contain other active components, such as quinones, tannins and flavonoids. The presence of naphthoquinones in species of this genus could justify its medicinal use [9].

Searches discovered anti-inflammatory and antinociceptive properties in this plant, and also a hypocholesterolemic effect in hyperlipidemic mice [10, 11].

Corroborating with these research, more recent studies have shown different biological properties in their full review of lapachol and its derivatives. These features are antiulcer, leishmanicidal, anticarcinogenic, antiedematogenic, anti-inflammatory, antimalarial, antiseptic, antiviral, bactericide, fungicide, pesticide schistosomicide, which have been associated with the lapachol [12].

The interest in finding new and safe antioxidants from natural sources has increased, especially to prevent oxidative damage to the living cells, since the use of synthetic antioxidants has declined due to a suspected activity as promoters of carcinogenesis [13].

However, conducted surveys show that there are still few studies that describe the active principles of *Tabebuia impetiginosa* when related to the antimicrobial activity in skin wounds.

Being the assistance in wound a key role of nurses, wound care requires that nursing professionals hold technical-scientific knowledge, and insight to the professional critical thinking. Thus, it is of high importance that nursing professionals are increasingly encouraged to contribute to research that aimed the use of medicinal plants for the treatment of wounds.

From what was foregoing, the present study aimed to investigate the *in vitro* antimicrobial and cytotoxic potential of *Tabebuia impetiginosa´*s crude extracts in order to support future *in vivo* studies.

EXPERIMENTAL SECTION

Basic research, along the lines of quantitative and experimental research, which aims to develop new knowledge.

Obtaining plant material

Samples of leaves, stem bark and branches of trees were collected in September 2012, in Maceió Municipal Park, located in the neighborhood (geographical coordinates S 9°37'01" and O 35° 48'30").

The plant material, after drying at ambient temperature and milling, was individually extracted by maceration with ethanol (EtOH) 98%. Crude extract was obtained after concentrating the solution on a rotary evaporator and drying at surrounding temperature. It was performed at the Laboratory of Technology and Drug Control, located at the Federal University of Alagoas. Tests for antimicrobial activity are undertaken at the Research Laboratory of Wound Care (LPTF/ESENFAR/UFAL). To determine the cytotoxicity, the tests were performed at the Laboratory of Pharmacology and Immunology (LAFI/ICBS/UFAL). Tests for the assessment of antioxidant capacity were performed at the Research Laboratory of Chemistry of Natural Products (LPQPN/IQB/UFAL).

Microbial strains

Extracts were tested against bacterial strains: *Staphylococcus aureus* ATCC 25923, *Salmonella entérica* ATCC 1307, *Acinetobacter baumannii* ATCC 17978, *Enteroccocus faecalis* ATCC 29212, *Pseudomonas aeruginosa*

ATCC 27853, *Escherichia coli* ATCC 25922. Microbial strains used in this study were standardized by the American Cell Type Cellection - ATCC/Manassas-VA/USA.

Broth dilution technique

Antibacterial activity of the ethanol extract was determined using the Minimum Inhibitory Concentration (MIC), adapted from the protocol of Clinical and Laboratory Standard Institute (CLSI) [14]. Ethanolic samples were solubilized in dimethylsulfoxide (DMSO) and the hexane sample was solubilized in Cromophor at concentrations 10,000 µg/mL (0.4g sample/400 µL DMSO of 2% or Cremophor + 19.6 mL of Saline 0.9%), thereby obtaining a stock sample solution extracted from plant species.

MIC was carried out in sterile polystyrene microplates of 96 wells 12 columns (1 to 12) and 8 rows (A to H). A volume of 200 µL of the stock solution at a concentration of 10,000 µg /mL of various plant samples was inoculated in triplicate in columns 1-9 of row A. All openings from line A were filled with 100 µL of broth Mueller Hinton (MH). A volume of 100 µL of the stock solution, at a concentration of 10,000 µg/mL of plant sample, was inoculated in triplicate in columns 1-9 from the line A. The wells in columns 10, 11 and 12 are intended to the control tests of the experiment, which are respectively growth control (GC), negative control (NC) Control and sterility plate (EC).

Subsequently, an aliquot of 100 µL of the contents of each well from the line A was transferred to the line B orifices. After the homogenization, the same volume was transferred to the line C, repeating this procedure until the line H, and ignoring the excess dilution after homogenization, thereby obtaining decreasing concentrations of the extract (5,000 µg/mL - line B; 2,500 µg/mL - line C; 1,250 µg/mL - line D; 625 µg/mL - line E; 312.5 µg/mL - line F; 156.25 µg/mL - line G; 78.125 µg/mL - line H).

Then, microbial inoculum was prepared at a concentration of 0.5 McFarland (1.5 x 10^8 CFU/ mL) and subsequently 1/10 was diluted in sterile SST and, thus, a volume of 5µL dilution (10^4 CFU/mL) was deposited in all the holes from the A-H lines.

Holes in columns 10, 11 and 12 were used for testing the control experiment. The holes in column 10 received only MH broth and microbial inoculum (5 µL), allowing the positive control of bacterial viability (GC). The column 11 was reserved for negative control of the diluent DMSO inhibitory activity, used in the preparation of extracts. In this column, the solution of 2% DMSO was diluted in MH broth until the line H and the same bacterial inoculum was added. Holes in the column 12 received only MH broth to check the sterility of the plate.

Microplates were incubated in a bacteriological incubator at 35°C for 24h. After this time interval, 20 µL of an aqueous solution of 2,3,5-Triphenyl Tetrazolium Chloride (TTC) at 0.5% has been added to each of the holes, and microplates were again reincubated for three hours at 35° C. After this last incubation, the presence of a red color in the holes was interpreted as negative evidence of the extract's inhibitory effect, while the absence of red staining is considered positive evidence for that

MIC was defined as the lowest concentration of extract µg/mL, which is able to prevent the microbial growth and was interpreted as follows: MIC samples $\leq$ 100 µg/mL represented a good activity; 100 < MIC $\leq$ 500 µg/mL was considered as a moderate inhibitory activity; 500 < MIC $\leq$ 1000 µg/mL as a weak inhibitory activity; and MIC $\geq$ 1000 µg/mL was considered inactive and, therefore, resistance [15].

Cell Viability Assay [16]

The feasibility of macrophages was performed using the method of MTT (3 - [4,5-dimethylthiazol-2-yl] -2,5-diphenyltetrazolium bromide). It is based on the cell mitochondria activity test, by the reduction of MTT by succinate dehydrogenase enzyme present in the active mitochondria responsible for the cleavage of the tetrazolium salt (yellowish). It results in the formation of formazan crystals (blue staining dark), which mean that the amount of crystal formed is directly proportional to the number of viable cells. Thus, if the color at the end of the reaction is darker, the cell viability is higher. The spectrophotometer determines the optical density of the resulting MTT assay. The cell manipulation was performed inside a biosafety cabinet with laminar flow, where a animal cell line was isolated, centrifuged and subjected to herbal extracts.

In this test, peritoneal lineage macrophages of Swiss mice were plated (3 x 10^5/well) and stayed "over night" for best adhesion of the cells to the plate. The next day, this macrophages were exposed to different extracts' concentrations (1000, 100, 10:01 µM) for a period of 48 hours. The control wells contained cells that were cultured only with culture medium and grown in the presence of DMSO diluent substances.

After the incubation period, the supernatant was discarded and then, 100 µL of MTT solution (10% in DMEM medium)was added to each well. The plates were reincubated for 4 hours at 37° C and 5% CO2 and held by spectrophotometer reading at 530 nm. The results were expressed as the percentage of cell death, considering the control as 100% count of viable cells in unstimulated cultures.

Assay Evaluation of free radical scavenging capacity

Qualitative tests

Extracts from the leaves, bark of the trunk and branches were dissolved in appropriate solvents, applied on cromatoplacas silica gel 60 F_{254}, and eluted in eluent systems previously defined. After the elution, the plates were dried at surrounding temperature. From the right side of the plate, at a half height, the (+)-catechin (1 mg/mol in MeOH and $CHCl_3$) was applied as a positive control. At the next step, the cromatoplacas were immersed for 10 seconds in a methanol solution of DPPH (0.4 mM). After drying at room temperature, the appearance of yellow stains in a purple background in positions of restraint factors (RFS) of substances suggests a possible activity when compared with the (+)-catechin.

Quantitative assays

Quantitative assays were performed against the synthetic DPPH with four study samples that showed positive results in qualitative assays [17, 18].

The free radical DPPH in methanolic solution is purple, absorbing at the wavelength of 515 nm. However, it forms the diphenyl-picryl hydrazine with yellow coloration, when it reacts with a radical species (R ') or it is reduced by an oxidizing agent..

Thus, the decrease in absorbance monitored at UV visible spectrophotometer (UV/VIS - Mini 1240, Shimadzu), at a wavelength of 515 nm, represents the use of the radical by the sample, indicating its free radical scavenging capacity.

Initially, 50 mL of a stock solution of DPPH were prepared in methanol (MeOH) spectroscopic grade (µg/mL ~ 100 µMol/L), which was protected with aluminum foil to avoid the direct light action, and also was kept under refrigeration until its use. From this solution, serial dilutions (35, 30, 25, 20, 15, 10, 5 e 1 µg/mL) were made for the calibration curve and subjected to analysis by the UV/VIS at 515 nm. Measurements were made in triplicate, using polystyrene cuvettes with optical path of 1cm, containing 1 mL of each solution. As a positive control (white), spectroscopic grade MeOH was used.

The calibrated curve was elaborated from the absorbance's medium values of each solution of DPPH versus the concentrations (1 a 40 µg/mL). For that, the linear regression analysis was applied using the *Microcal Origin Pro 8.0* software. This analysis provided the mathematical equation of the calibration curve: Y = A + Bx, in which: "A" is the linear coefficient; "B" means the slope or gradient of the line; and "x" is the concentration of DPPH through the 60 minutes; with its standard deviation (SD) and correlation coefficient (R).

After obtaining the calibration curve of DPPH, individual experiments were performed with each of the solutions (25 a 200 µg/mL) of samples from the controls (150 a 5 µg/mL). In polystyrene cuvettes, reaction mixtures consisting of 0.1 ml of the test solution and 0.9 ml of the stock solution of DPPH (40 µg/mL) were prepared. These solutions were homogenized and protected from light throughout the experiment. The absorbance measurements were done at 515 nm in triplicate every 15 minutes for one hour (at 0, 15, 30, 45 and 60 minutes). The absorbance of the solutions was recorded against a white (spectroscopic grade MeOH).

From the mathematical equation: y = A + Bx and the absorbance's values obtained by the average time of 60 minutes, along with linear and angular coefficients of the straight calibration curve, it was possible to determine the value of "x", which is the concentration of DPPH remaining in the medium after the reaction with the samples. The remaining percentage of DPPH (% $DPPH_{Rem}$) in the reaction medium was obtained according to the following equations [17].

$$\mathbf{Y = A + B.x}$$

Where:

Y = the average absorbance at t_{60};

A = linear coefficient of the straight line of the calibration curve;

B = slope of the calibration curve;

x = concentration of DPPH at 60 minutes (t_{60}).

$$\%(DPPH)_{Rem} = \frac{(DPPH)T = t}{(DPPH)T=0} * 100$$

Where:
% (DPPH) $_{Rem}$ = Percentage of remaining concentration of DPPH, that is, left;
(DPPH) T = t = concentration of DPPH after 60 minutes (t_{60});
(DPPH) T = 0 = initial concentration of DPPH in the middle (40 mg/L).

To determine IC_{50} (concentration of each sample required to decrease the concentration of DPPH by 50%), a curve from the $DPPH_{Rem}$% versus the concentrations of the samples (25 a 200 μg/ml) and controls (100 a 5 μg/ml) was plotted by linear regression *in Microcal Origin Pro 8.0* software. After obtaining the curve and from the mathematical equation: Y = A + Bx, substituting, in that, the values obtained from their linear and angular coefficients (A and B) and Y by 50 to get x the IC_{50}. As higher is the consumption of DPPH in a sample, lower is its IC_{50} and greater is its ability to scavenge free radicals.

For each data set, the average standard deviation (SD) and coefficient of variation (CV) measurements were obtained as to express the variability of the results, and correlation coefficient (CC) between the two variables. These variables are the concentrations tested independent variables, and the percentages of $DPPH_{Rem}$ for each concentration of dependent variables.

In this assay, the concentration of sample required to decrease the initial concentration of DPPH by 50% (IC_{50}) was determined. IC_{50} is the measure of the efficiency of the analyzed samples. IC_{50} values are inversely proportional to the activity, which mean that as lower is the value, greater is the antioxidant capacity. Ascorbic Acid (IC_{50} 37.37, with rapid kinetics) and BHT (IC_{50} 97.86, with slow kinetics) were used as positive standards. Thus, the samples that showed IC_{50}> 200 μg/mL were considered inactive.

RESULTS AND DISCUSSION

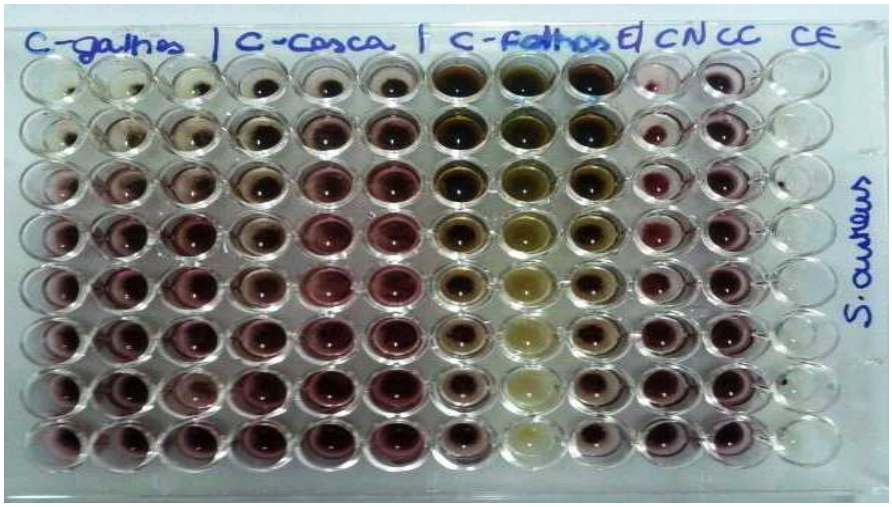
From the samples, only the crude ethanolic extract of the leaves demonstrated some inhibitory antimicrobial activity in two of the nine microorganisms used. This sample showed moderate inhibitory activity against the strain of *S. aureus* with MIC ≥ 312.5 μg/mL, and weak inhibitory activity against *S. epidermidis* with MIC ≥ 625 μg/mL. The MIC ≥ 1. μg/mL of the same extract against the strains of *Acinetobacter calcoaceticus* and *Enterococcus faecalis* is highlighted. The remaining samples did not reveal inhibitory action on the assessed bacterial strains (MIC ≥ 1000 μg/mL) (Table 1).

Table 1. Minimum Inhibitory Concentration of fractions and crude extract of Tabebuia species impetiginosa, facing the strains of the evaluated microorganisms. Maceió-AL, Brazil, 2014.

	Minimum Inhibitory Concentration – MIC (μg mL^{-1})			
Microorganism	Branches Crude Ext. EtOH	Stem bark Crude Ext. EtOH	Leaves Crude Ext. EtOH	Leaves Crude Ext. Hexane
S. Aureus	5.000	10.000	312,5	10.000
S. epidermidies	5.000	10.000	625	10.000
E. faecalis	10.000	10.000	1.250	10.000
E. aerogenes	10.000	10.000	2.500	10.000
S. flexneri	10.000	10.000	5.000	10.000
A. baumannii	5.000	5.000	1.250	5.000
S. entérica	10.000	10.000	2.500	10.000
E. Coli	10.000	10.000	5.000	10.000
P. aeruginosa	10.000	10.000	2.500	10.000

** Test performed in triplicate*

Figure 1. MIC, in triplicate, of the crude extract and fractions against the strain of S. aureus. The absence of staining showed inhibition of bacterial growth.



Cell viability assay revealed that the ethanol extract of the leaves and the stem bark did not exhibit cytotoxicity (Table 2), since the LC_{50} value was greater than the highest tested concentration (1000 µg/mL The hexane extract of the leaves and twigs of the ethanol were cytotoxic for presenting LC_{50} of 550 and 546.7 µM, respectively.

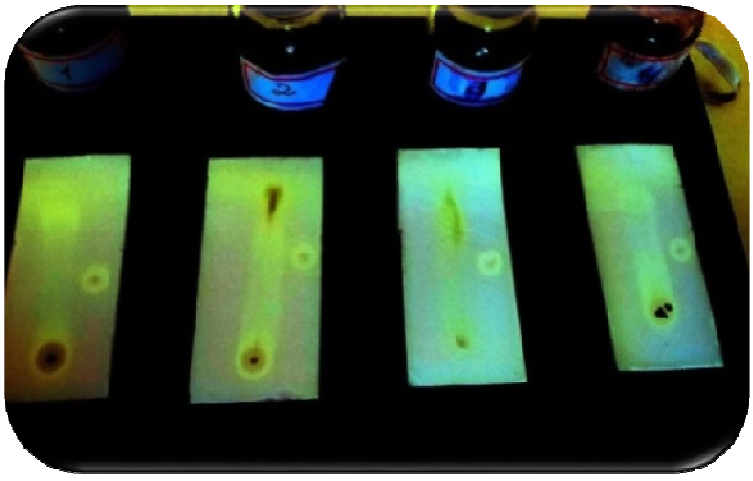
Table 2. Determination of cytotoxicity of the extracts against T. impetiginosa peritoneal macrophages. Maceió-AL, 2014.

Extracts	CL_{50} (µM)[a]	Maximum cytotoxicity (%)[b]
Leaves (Hexane)	550 ± 0,0	100 ± 0,0
Stem bark (ETOH)	> 1000	NT
Branches (ETOH)	546,7 ± 3,3	98,5 ± 2,0
Leaves (ETOH)	> 1000	NT

NT: nontoxic

All samples submitted to qualitative evaluation of the free radical scavenging ability against DPPH synthetic demonstrated positive results, since the presence of yellow stains on a white background, suggested a possible activity when compared with the (+)-catechin.

Figure 2. Thin-layer chromatography of qualitative assays



Of the four samples that showed positive results in qualitative assays, only three of them were subjected to quantitative evaluation because the hexane sample of leaves was not soluble in MeOH, and because of this, it has not been tested. In this assay, the IC_{50} values found for tested samples ranged 2509-14930 (Table 3), which mean that all the samples tested showed IC_{50} higher than 200 µg/mL. It demonstrates inactivity of the free radical scavenging ability.

Table 3. Evaluation of the extracts against DPPH. Maceió-AL, 2014.

Parts of the plant/ Sample	DPPH (Qualitative)	DPPH/ CI_{50}
Branches (EtOH)	+	14.930
Leaves (EtOH)	+	4.728
Leaves (Hexane)	+	NT
Stem bark (EtOH)	+	2.509
Positive Patterns		
Ascorbic	+	37,7
BHT	+	97,86

Species of the genus Tabebuia have been used empirically as anti-inflammatory, antitumor and antimicrobial agent in rural areas of Brazil, Colombia, Bolivia and other Latin American countries [19]. Results of ethnobotanical and ethnopharmacological studies, demonstrating the great potential of this genus of plants to treat a variety of diseases, have encouraged the search for new herbal medicines using plant biodiversity [20].

The main active constituent of plants of the genus Tabebuia is lapachol, a biologically active naphthoquinone, which has activity against various pathogens such as bacteria, fungi, viruses and parasites, and therefore has been used as a target for the synthesis of naphthoquinone derivatives with potent antimicrobial activity [11].

A study with ethyl acetate extract obtained from the stem bark of *Tabebuia ochracea* and *Tabebuia impetiginosa*, demonstrated the ability to inhibit the growth of *S. aureus* [21].

However, no inhibitory activity was found in acetate extracts of *Tabebuia ochracea* and *Tabebuia rosea* against strains of *Escherichia coli* and *Pseudomonas aeruginosa* [20]. It corroborates with the findings of this study, since these plants belong to the same genus of plant species studied.

This can be explained by the fact that Gram-negative rods possess anti-adhesion and phagocytosis mechanisms, endotoxins and some exotoxins, and also an extra protection promoted by an outer membrane that surrounds the cell wall. It makes them difficult to be removed and eliminated, making their toxins prolong the inflammatory response in a chronic process [22]. In this study, a strain of *S. aureus* was moderately sensitive to the ethanolic leaf extract of *Tabebuia impetiginosa,* confirming the findings of this research. This antibacterial activity may be associated with the presence of several active principles or phytochemicals, such as flavonoids and phenolic compounds present in the extracts of *Tabebuia impetiginosa* [23]. In Hospitals, primarily in nurseries and intensive care units (ICUs), the isolation of patients colonized with certain microorganisms is a routine. Among these, *S. aureus is mentioned by* bringing risk, especially for patients on dialysis, burnt, diabetics and HIV-positive, as they can cause various infectious processes, ranging from relatively benign chronic skin infections until infections systemic, potentially fatal [24].

Staphylococcus aureus is a spherical bacterium, from the group of Gram-positive cocci, often found on the skin and in the nasal passages of healthy people. However, it can cause illness ranging from a simple infection (pimples, boils, and cellulitis) to severe infections (pneumonia, meningitis, endocarditis, toxic shock syndrome, septicemia, and others) [25].

Articles that examine the antimicrobial activity of plant extracts against the strains of *Acinetobacter* sp. are extremely rare in the literature. In this study, the ethanolic sample of the purple "ipê" leaf showed MIC ≥ 1. µg/mL against the *A. calcoaceticus* strain. A sample that shows a MIC greater than 1,000 µg/mL is considered inactive [15]. However, because it is a Gram-negative microorganism quite sturdy and considering that it comes from crude extract, in which many substances are present, this result can be considered promising with the isolation of the substances present in the extract.

Other Gram-positive microorganism commonly associated with nosocomial infections is *S. epidermidis*. This organism does not show a large arsenal of enzymes and toxins, and, therefore, the infection progress is characterized by sub exacerbation or chronicity. The success of this organism as a pathogen is related to its ability to adhere to polymer surfaces, forming biofilms that are characterized as important virulence factors, reduce the immune response and interfere with the mechanisms of host defense [26].

Research that held a screening of the plant extract collection in Cerrado, on bacteria pathogenic to humans, showed that the methanol fraction of extracts of *Tabebuia craiba* has antibacterial activity against strains of *S. epidermidis* [27].

During the last two decades, *Staphylococcus epidermidis* and others *Staphylococcus* have emerged as significant causes of nosocomial infection [28]. In the present study, the ethanolic sample of the purple "ipê" leaf presented antibacterial activity against this type of microorganism, confirming the above findings, although it has been evaluated as a weak activity. As previously mentioned, because it is a crude extract, this result can be considered more promising if, in the future, antimicrobial tests are performed with isolated substances.

Besides microbicidal activity, lapachol and its synthetic derivatives have demonstrated in recent years, significant cytotoxic actions against several tumor cell lines [29]. A study demonstrated high cytotoxicity of naphthoquinones isolated compounds and other derivatives of lapachol, cell viability by MTT test [30].

Cytotoxic effects of these quinones are mainly due to the following two factors: inhibition of DNA topoisomerase and formation of semiquinone radical that can transfer an electron of oxygen to produce superoxide. Both (semiquinone and superoxide) can generate the hydroxyl radical, which is the cause of DNA breaks chain [30].

In the present study, it can be stated that some parts of the purple "ipê" (stem bark EtOH and leaves EtOH) presented viable cells, suggesting the continuation of *in vivo* studies under concentration of 100 µg/mL.

Also, the antioxidant activity of *T. impetiginosa* was evaluated in this study, showing the absence of free radical scavenging activity against the synthetic DPPH. It corroborated with a survey that evaluated the antioxidant potential of plants in the semiarid region of Bahia, and demonstrated that the extract of purple "ipê" showed no *in vitro* antioxidant activity [31].

Tabeuia impetiginosa is widely recognized for its biological activities and also by containing other active ingredients such as flavonoids, substances with phenolic groups and that may have antioxidant activity [9]. However, no antioxidant activity of the tested samples in this study may be justified due to a lower concentration of flavonoids, since they are crude extracts.

CONCLUSION

The ethanolic extract of the *T. impetiginosa* species leaf evidenced a promissory antimicrobial activity against the Gram-positive *S. epidermidis* and *S. aureus*trains strains. This ethanolic extracts from the leaves and branches did not show citotoxicity. These results represent proofs of safety in the therapeutic use of plant species, for realizing *in vivo* tests in the future. The study also reveals that *T. impetiginosa* does not have scavenging incapacity of free radical.

Thus, these findings scientifically prove the antibacterial activities of the species *T. impetiginosa*, but new research with chemical, pharmacological and clinical approach should be undertaken with this species, since this study assessed a crude extract and its isolated compounds certainly present greater antimicrobial activity. This study highlights the expanded perspective on health, from the nursing context, as a way to promote technological innovation in the treatment of skin infected wounds therapy, by promoting the incorporation of new knowledge to nursing science.

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