ANALYSIS OF ANTIMICROBIAL, ANTIBIOFILM, AND HEALING ACTIVITY OF LIPID NANOCARRIERS BASED ON TUCUMAN BUTTER (*Astrocaryum vulgare*), FIXED OILS FROM MICROALGAE *Chlorella homosphaera* AND FROM UVA SEED *Vitis vinifera*

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Abstract

Therapeutic alternatives of natural origin have been arousing the interest of large research centers that are looking for new bioactive molecules to treat numerous diseases in the context of public health. Among them, infectious diseases, which present antimicrobial resistance, deserve attention. The present study aimed to evaluate the antimicrobial activity of lipid nanocarriers (CLN), as well as the healing activity, arising from the association of tucumã butter with grape seed oil and another one containing microalgae oil. Two formulations were prepared using the high-speed homogenization technique, which was evaluated for antimicrobial action for 10 strains of great clinical importance, including a multiresistant and healing activity. The formulation with Chlorella homosphaera oil showed growth inhibition for the 10 strains tested, in addition to antibiofilm activity for 8 strains, bactericidal action for 3 of 3 isolates, and satisfactory healing action in 48 hours.

Keywords: Nanotechnology, Nanosciences, Microbiology.

1. Introduction

Nanoparticles emerged as a novelty that, little by little, proved to be important therapeutic tools, due to their physicochemical properties on a nanometric scale. His contribution has impacted several areas, one of them being research on new therapies that solve the alarming problem that is antimicrobial resistance,

which has spread every day. There are many studies using nanoparticles as antimicrobial agents or carriers of substances for this purpose, as the size of a nanoparticle is compatible with the biomolecular and cellular system of bacteria, providing an interaction between the bacteria and the nanomaterial. In addition, the high surface area to the volume of nanomaterials allows a high use of therapeutic agents, with natural actives being one of the biggest current bets because they are compatible with the human body and sustainable (TAVARES 2002; MULHING et al., 2009).

Studies combining natural bioactives, such as oils and nanotechnology, are being used as adjuvants in therapeutic processes such as healing, antimicrobial action, antibiofilm, among others. Among the natural actives is *Astrocaryum vulgare* butter, Tucumã, a fruit from the Amazon region, used both in food and in the area of beauty, has high lipid content, large amounts of carotene, vitamin A precursor, high fiber content, and vitamin E (FERREIRA et al., 2008; SOUZA FILHO et al., 2013; MATHIAS et al., 2014; DA SILVA, 2015). Due to its rich composition of polyphenols, studies report that the fruit has, in addition to healing activity, antimicrobial and antibiofilm activity (DAGLIA, 2012; JOBIM et al., 2014; DA SILVA et al., 2013).

Another potential ally in research is grape seed oil, known for having high levels of vitamin E, vitamin C, and β-carotene, in addition to material rich in tocopherols, steroids, and various fatty acids. In addition, it acts as a fat-soluble antioxidant in plasma and tissue membrane, presenting antimicrobial activity due to its chemical composition. It stands out for being a source of linoleic and oleic acid, containing traces of linolenic and palmitoleic, which are essential for the proper functioning of various organs and are essential to maintain cell membranes with normal functioning (CHOI; LEE, 2009; ANDRADE et al., 2012; DA SILVA et al., 2013; MEDINA et al., 2015).

Fixed microalgae oils are a promising asset that has been increasingly included in the research, in particular the Microalgae *Chlorella homosphaera* oil, this asset has gained prominence mainly due to issues involving sustainability. It has a high content of proteins, fatty acids, minerals, and carbohydrates. Because it has relatively easy ways of cultivation and extraction, the use of microalgal oils is growing and diversified (MORAIS; COSTA, 2007; RADMANN, 2007; PIRES; VIEIRA, 2017).

Thus, like most actives of natural origin, despite the numerous properties and perspectives they present, they suffer interference, such as rapid degradation, oxidation, difficult penetration, and low solubility; To protect oxidative molecules that degrade easily, such as vitamin C, stabilize substances, enable greater penetration and controlled release of actives, there is the application of nanotechnology. (FATHIA et al., 2012; BOLZANI, 2016; FERREIRA et al., al., 2017).

Lipid nanocarriers are widely used in industry to convey active substances of medical and pharmaceutical interest, which have a high acceptability rate, since the systems are well tolerated by the skin, as they have lipids similar to the physiological ones in their composition, thus enhancing the action of the active principle, in addition to being biocompatible and biodegradable. (FATHIA et al., 2012; JAEGER, 2012; CAMPOS et al., 2015).

In this context, the present study aimed to evaluate the nanocarriers containing the association of Tucumã butter with grape seed oil and microalgal, regarding their antimicrobial, antibiofilm, and healing action.

2. Materials and Methods

2.1 Acquisition of butter and oils

The company Amazonoil-Industry Pará-Amazonas Brasil (Amazon oil, 2019) provided Tucumã butter. The grape seed oil was kindly provided by the pharmacology laboratory at Franciscan University. The microalgae oil was extracted in the Nanotechnology laboratory at Franciscan University.

2.2 Microalgae Cultivation

The microalgae cultivated was Chlorella homosphaera, production was carried out using modified BG11 medium (ACREMAN, 2017), and enriched with Vitamin B (GUILLARD; RYTHER 1962; GUILLARD 1975), with a volume of 300 mL of stock culture in a flask of 3 L containing the medium. The culture was subjected to constant aeration at an airflow rate of 2 L min-1 maintained under artificial light with a photoperiod consisting of a 14:10 light/dark cycle, provided by 40 W fluorescent lamps. The room temperature was maintained at around 24 °C. Cultivation was followed for 14 days.

2.3 Extraction and characterization of microalgae oil

For extraction, the method chosen was Soxhlet, a conventional method often used to extract natural products from organic matrices. For this, a mass of 3 g of dried microalgae was used, in an oven at 60 °C, for each extraction. The biomass was added to the extraction chamber, a solvent mixture (250 mL of chloroform: methanol, 2:1 v/v) was added to the distillation flask for lipid extraction (RAMOLA et al., 2019). The solvent was refluxed for 8 h, passing through the evaporation, condensation, and solvent percolation phases through biomass, the flask containing the mixture of solvent and extracted lipids was removed and the solvent was evaporated in a rotary evaporator to recover the lipids.

2.4 Preparation and characterization of lipid nanocarriers

Lipid nanocarriers based on tucumã butter were prepared using a method developed by pre-formulation tests, using the Ultra Turrax® equipment from the high-speed homogenization technique. Two formulations were prepared: Tucumã butter (*Astrocaryum vulgare*) with grapeseed oil (*Vitis vinifera*) - Nano 1 and Tucumã butter (*Astrocaryum vulgare*) with microalgae oil (*Chlorella homosphaera*) - Nano 2. The formulations were prepared in n = 3 and kept under different temperature and storage conditions (refrigerator at -4 ºC, oven at 40 ºC, room temperature with exposure to light and room temperature dark) for 60 days.

The characterization of nanoparticles was performed by analyzing parameters such as pH, polydispersion index (PDI), size, and zeta potential. The diameter and PDI determinations of the nanoparticles were performed using dynamic light scattering; The zeta potential was measured by electrophoresis (Zetasizer® nano-Zs model ZEN 3600, Malvern), the determination of the pH of the nanoparticles was carried out in a pH meter.

2.5 Evaluation of antimicrobial activity

2.5.1 Determination of the minimum inhibitory concentration (MIC)

Ten bacterial strains were tested, namely: *Aeromonas hydrophila, Enterococcus faecalis,*

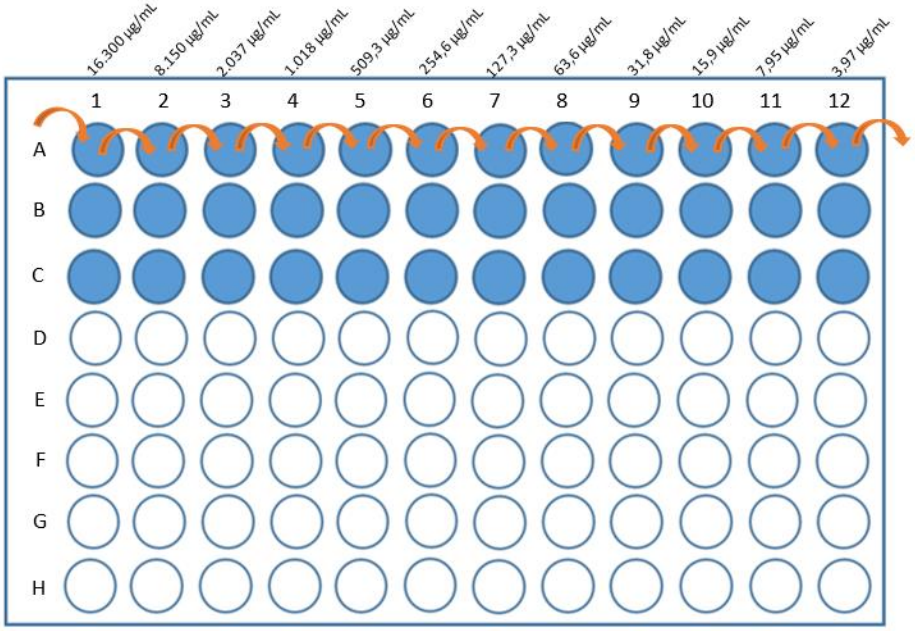
Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, methicillin-resistant Staphylococcus aureus (MRSA), Staphylococcus epidermidis, Streptococcus mutae. These microorganisms are part of the collection of strains at the Microbiology Laboratory at the Franciscana University, Santa Maria – RS. The strains of *Aeromonas hydrophila* and *Pseudomonas aeruginosa* were provided by the Laboratory of Microbiology and Pathology at the Federal University of Santa Maria - UFSM, Santa Maria - RS, Brazil.

The bacterial samples were cultivated in Muller Hinton agar (MH) and after the colonies were inoculated in 5 mL of sterile saline, the absorbance was controlled until obtaining a transmittance of 0.5 on the Mac Farland scale (1 x 106 a 5 x 106 cells/mL), then a 1:10 dilution in MH broth was carried out resulting in a concentration of 104 cells/mL.

After preparing the inocula, MIC was performed in 96-well polystyrene plates. The tests were carried out in triplicate wherein wells 1 to 12 were added 100 µL of M.H.

After adding the culture medium, 100 µL of the working solution was added to the first well and then serial dilutions were carried out up to the well 12 equivalent to the working concentrations (16,300 µg/mL, 8,150 µg/mL, 2,037 µg/mL, 1,018 µg/ mL, 509.3 µg/mL, 254.6 µg/mL, 127.3 µg/mL, 63.6 µg/mL, 31.8 µg/mL, 15.9 µg/mL, 7.95 µg/mL and 3.97 µg/mL) discarding after well 12 (Figure 1).

Figure 1. Schematic representation of the serial dilution with the concentrations used.



Source: Author's construction

Soon after, the inoculation of microorganisms was performed, where 10 µL of standardized inoculum was added to each well of the microdilution plate. After pipetting the plates were incubated at 37 ºC for 48 hours for bacteria. The reading of the plates was performed by adding 20 µL of a 1% solution of the dye 2,3,5-triphenyl tetrazolium chloride (Vetec®), to help reveal the MIC.

2.5.2 Determination of antibiofilm activity

The determination of antibiofilm activity was performed with clinical emergency strains, namely: *Aeromonas hydrophila, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, methicillin-resistant Staphylococcus aureus (MRSA), Staphylococcus epidermidis, Streptococcus*. To reduce the possibility of contamination, the steps were carried out in a biological safety cabinet. Furthermore, each test was performed in triplicate.

To prepare the bacterial inoculum, the species were subcultured in sterile Petri dishes with Mueller Hinton Agar, incubated at 35 ºC for 24 hours. Colonies were suspended in sterile 0.85% saline solution. To prepare the fungal inoculum, the species were subcultured in sterile Petri dishes with Sabouraud Dextrose Agar, incubated at 35 ºC for 24 h for *Candida* species, and cultured at 35 ºC for 48 h for *Cryptococcus* species, then the colonies were suspended in sterile 0.85% saline solution. Cell density was adjusted in a spectrophotometer to obtain a transmittance equivalent to a 0.5 tube on the McFarland scale (1 x 106 to 5 x 106 cells/mL), at a wavelength of 630 nm.

In sterile polystyrene 96-well plates, 180 µL of TSB Brain Heart Infusion (BHI) broth supplemented with 1% glucose were added and then 20 µL of the inoculum of each microorganism was dispensed into each well and 50 µL of the Solid Lipid Nanoparticles solution for test the antibiofilm activity of nanoparticles. For the negative control, only Brain Heart Infusion (BHI) broth supplemented with 1% glucose was added, and for the positive control, the culture medium plus the solution containing the microorganisms was added. After pipetting, the plates were incubated at 35 ºC for 24 h for bacteria and 48 h for fungi.

After incubation, the contents of the wells were discarded in a suitable container. Each well was washed three times with 200 µL of sterile phosphate buffer saline (PBS; pH 7.2). Washing the biofilm is another important step, as it removes all non-adherent cells while preserving the integrity of the biofilm. After washing, fixation is performed with 150 µL of methanol for 20 min, after which the plates are emptied and left to dry in an inverted position in the temperature room.

The adherent biofilm layer formed in each well of the plate was stained with 150 µl of crystal violet (2% Hucker's crystal violet) for 15 min at room temperature, followed by washing in running water and drying at room temperature. After the microplate was air-dried at room temperature, the cell-bound dye was resolubilized with 150 µl of 95% ethanol per well. Ethanol is gently added and subsequently, the plate is covered with the lid (to minimize evaporation) and then left at room temperature for at least 30 min. The addition of ethanol allows indirect measurement of bacteria bound to both the bottom and walls of the wells. Ethanol must be added gently to the wells and it is forbidden to shake the microtiter plate to speed up the resolubilization process.

To visualize the antibiofilm activity, the absorbance reading was performed in a spectrophotometer, with a wavelength of 570 nm (STEPANOVIC et al., 2007).

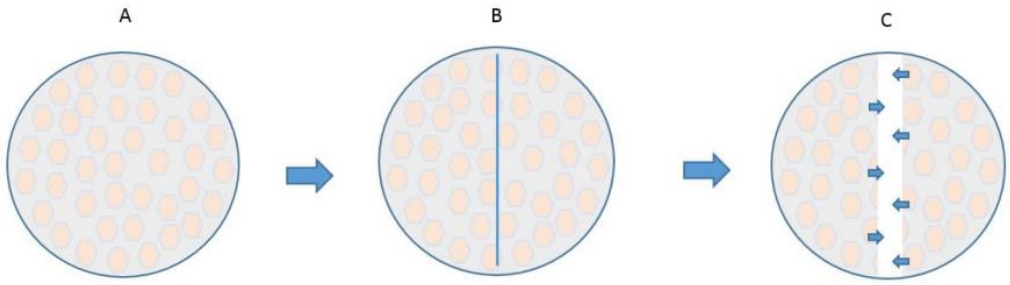
2.6 Assessment of healing activity

2.6.1 In vitro Scratch Assay

For this assay, HFF-1 human fibroblast strains, commercially obtained from the Rio de Janeiro Cell Bank, were used. After the confluence of the cells in the 96-well plate, the culture medium is removed from

the wells, and with the aid of a tip (tip 200), a continuous scratch is made on the medial surface of each well (Figure 2). This procedure causes a break in the contact between the cells and their removal from a certain region of the plate, resulting in the formation of a mechanical lesion in the monolayer. This marking establishes a visual field in the well, which will be analyzed later after establishing the "cut" in the monolayer, at times zero, 24, and 48 hours.

Figure 2. Illustration of the in vitro Scratch Assay, where after plating each well (A), it was subjected to mechanical stress disrupting the cell monolayer (B), after each well it was observed that adjacent cells tend to proliferate towards blank space (C)



Source: Adaptation of Santos (2019).

The wells were washed with 1X PBS (Gibco®), composed of sodium chloride and sodium phosphate, to remove debrided cells.

Subsequently, the follow-up was done through photographic records of the proliferation of adjacent cells towards the free space on the plate, because after removing the cells that are no longer adhered, the adjacent cells tend to proliferate towards the free space on the plate (VEDULA et al., 2013).

To analyze the images referring to the healing process of the various types of treatments, a script was developed in the Python programming language. Initially, the conductor of the experiment generated an image, 50x50 pixels in size, which represented a sample of cells, that is, a completely healed region. From this image, the average and standard deviation of the color of its pixels in RGB format was calculated (Red - Red, Green - Green, and Blue - Blue), whose values for each color range from 0 to 255.

From the calculated average, the analysis of the healing process of the images was started, analyzing them pixel by pixel. A pixel's color is considered a cell if its RGB value is greater than or equal to the mean minus the standard deviation, or less than or equal to the mean plus the standard deviation. Otherwise, the pixel is considered to be non-cell. Thus, it was calculated how many pixels, for each image, are within the cell and non-cell range, as well as calculated the cell and non-cell percentages to the total number of pixels.

Finally, the script generated a file, in a table format, which could be imported into Excel software with the data obtained from the analysis of each sample to be statistically analyzed using GraphPad Prism 5.

3. Results and discussion

3.1 Extraction and characterization of microalgae oil

After the cultivation of the alga *Chlorella homosphaera*, extraction, and characterization as described in the materials and methods item, it can be seen that the oil obtained a yield of approximately 40%.

3.3 Characterization of lipid nanocarriers

As described above, two formulations of lipid nanocarriers were prepared using the Ultra Turrax® device using the high-speed homogenization technique. One was constituted by Tucumã butter and grape seed oil and the other by Tucumã Butter and microalgae oil, both were formulated containing 0.47% solid lipid (Tucumã Butter), 2.86% liquid lipid (oil of grape seed or microalgae), and 2.65% of surfactants (SPAN 60® and TWEEN 60®).

Table 1 presents the results of the characterization of the nanocarriers, where, at time 0, that is, on the day of manufacture, the formulation 1 consisting of Tucumã butter and grape seed oil presented a monodisperse distribution of particles with an average PDI value of 0.260, where according to the literature the ideal value is <0.3 (MACHADO et al., 2019); The average size of the CLN is approximately 181.3 nm. The value for the zeta potential was -12.2 mV. Formulation 2, consisting of Tucumã butter and microalgae oil, also presented a monodisperse distribution of particles with an average PDI value of 0.200, an average size of approximately 105.8 nm, and the value for the zeta potential was -9.00 mV.

Table1. Representation of the parameters analyzed during the characterization of the formulations at time 0. Values are shown as mean ± standard deviation.

Nanocarrier 1 (UVA)			Nanocarrier 2 (ALGA)		
PDI	**Zeta Potential**	**Size**	**PDI**	**Zeta Potential**	**Size**
0.260 ± 0.1	-12.2 ± 2.8	181.3 ± 2.7	0.200 ± 0.009	-9.00 ± 1.3	105.8 ± 19.3

Source: author's construction

Through the results of the characterization of the nanoparticles, we know that they have adequate homogeneity, taking into account that the formulations must have a polydispersion index PDI <0.3 to be considered monodisperse and a diameter smaller than 300 nm. The zeta potential reflects the electrostatic repulsion between particles, and the suggested default value for zeta potential is equal to or greater than +/- 30mV, being associated with stable solutions; Analyzing Table 1, we can say that the prepared formulations are stable because they meet these parameters (MACHADO et al., 2019).

3.4 Evaluation of antimicrobial activity

3.4.1 Determination of minimum inhibitory concentration (mic)

To assess the antimicrobial activity, this study analyzed the effects on ten species of microorganisms of medical and hospital interest. The minimum inhibitory concentration values of lipid nanocarriers containing *Chlorella homosphaera* oil are shown in Table 2. This formulation demonstrated antimicrobial activity against the 10 bacterial strains of great clinical importance, most of them being gram-negative.

Table 2. Demonstration of the minimum inhibitory concentrations obtained after treatment with Nanocarrier based on Tucumã Butter and *Chlorella homosphaera* oil.

Nanocarrier based on Tucumã Butter and *Chlorella homosphaera* oil	
Microorganisms	**MIC (µg/mL)**
Aeromonas hydrophila	2.037
Enterococcus faecalis	1.018
Escherichia coli	2.037
Klebsiella pneumoniae	16.300
Pseudomonas aeruginosa	8.150
Staphylococcus aureus	2.037
Staphylococcus aureus (MRSA)	509.3
Staphylococcus epidermidis	1.018
Streptococcus agalactiae	1.018
Streptococcus mutans	8.150

Source: author's construction

Of the 10 strains tested, the formulation containing grape seed oil showed antimicrobial activity for 3 of them, the majority being gram-positive. The MIC values are shown in Table 3.

Table 3. Demonstration of the minimum inhibitory concentrations obtained after treatment with Nanocarrier based on Tucumã Butter and Grape Seed oil.

Nanocarrier based on Tucumã Butter and Grape Seed oil	
Microorganisms	**MIC (µg/mL)**
Enterococcus faecalis	4.075
Klebsiella pneumoniae	2.037
Staphylococcus aureus	2.037

Source: author's construction

The present study demonstrated antimicrobial activity of the formulations against strains of *Aeromonas hydrophila, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus,* and methicillin-resistant Staphylococcus aureus (MRSA), *Staphylococcus epidermidis, Streptococcus agalactiae, Streptococcus mutans*, where the formulation containing *Chlorella homosphaera* oil (table 2) demonstrated antimicrobial activity for all microorganisms and the formulation containing grape seed oil demonstrated activity against *Enterococcus faecalis, Klebsiella pneumoniae, Staphylococcus aureus* (Table 3). The bacteria tested in this study are of great clinical and hospital importance. Although reference strains have been used, these species may have highly virulent and/or antibiotic-resistant strains or serotypes.

The antimicrobial action reported in this study may be related to reactive oxygen species and also the antimicrobial properties were given to nanoparticles due to size, as mentioned above, however, studies report that in addition to the property such as size, it provides the capacity of the nanoparticles crossing the bacterial membrane, a mechanism that greatly favors the action, the composition of the bioactives that form

the nanoparticles are also extremely important to understand the mechanisms of action (MULHING et al., 2009).

3.4.2 Minimum Bactericidal Concentration (CBM)

In addition to the analysis of the Minimum Inhibitory Concentration (MIC) and antibiofilm action, for the strains, *Aeromonas hydrophila, Pseudomonas aeruginosa, and methicillin-resistant Staphylococcus aureus (MRSA),* the analysis of the Minimum Bactericidal Concentration (CBM) were performed with the nanocarrier containing oil of *Chlorella homosphaera*. From sowing, it was possible to observe that the Nanocarrier completely inhibited bacterial growth.

The Minimum Bactericidal Concentration (CBM) was performed only for *Aeromonas hydrophila, Pseudomonas aeruginosa, and Staphylococcus aureus (MRSA)* with the formulation of Nanocarriers based on Tucumã Butter and *Chlorella homosphaera* oil following the method described by Cabral et al. (2009) with minor modifications. After performing the CIM, the samples were striated in Petri dishes with MH agar (Muller Hinton Agar) and incubated for 24 h at 37 °C. Plates, where there was no bacterial growth, were considered the minimum bactericidal concentration.

The relationship between the antimicrobial action of nanoparticles and their size occurs because they are compatible with the size of the bacteria, as they can access its interior, through physical interactions with the microbial cell, acting by disturbing the DNA during replication division, which compromises the integrity of the bacterial membrane generating disorganization and even cell death (THEKKAE; CERNIK, 2013; FRANCI et al., 2015).

3.4.3 Determination of antibiofilm activity

Taking into account that the antimicrobial action may be related to the composition of the assets, we suggest that the indices found in this study corroborate some evidence, such as the second study carried out by Rossato et al. (2019) where the tucumã, for having molecules of polyphenols and fatty acids, presented antimicrobial activity against microorganisms.

In addition to the likely antimicrobial action related to the composition of tucumã, the grapeseed oil that is also part of this study, showed antimicrobial activity rates due to phenolic compounds and gallic acid, in a study where it was tested against fifteen bacteria, including *E. coli, S. aureus, and S. typhimurium* (BAYDAR et al., 2004).

In line with the present study, analyzes indicated antimicrobial activity of extracts obtained from microalgal cells against *S. aureus and Staphylococcus sp*. the activity being also attributed to the phenolic compounds of such oils (PARISI et al., 2009; BHAGAVATHY et al., 2011; CAVALCANTI et al., 2013).

According to Almeida et al. (2012), lauric acid has antimicrobial activity, inactivating Gram-positive and negative bacteria. This compound is present in all active nanocarriers studied in this research.

Given this scenario, in the use of plants with antimicrobial action, there is a need to further explore their effects on biofilm formation, as it plays an important role in pathogenicity and also in the resistance of microorganisms (MARTINEZ; FRIEZ, 2010).

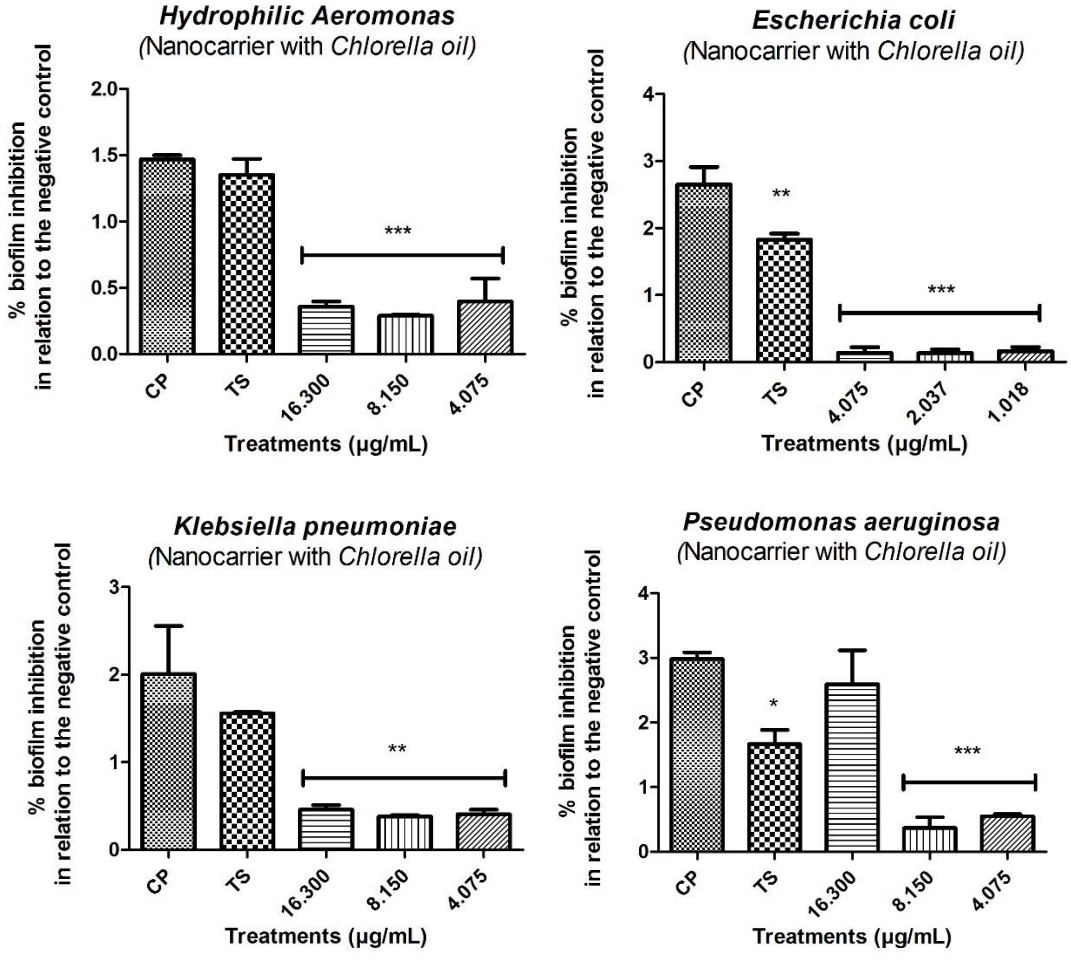
Faced with this need, we also evaluated the action of the nanocarriers synthesized here against the formation of biofilm with bacteria that showed susceptibility; As can be seen in Figure 3, the nanocarriers

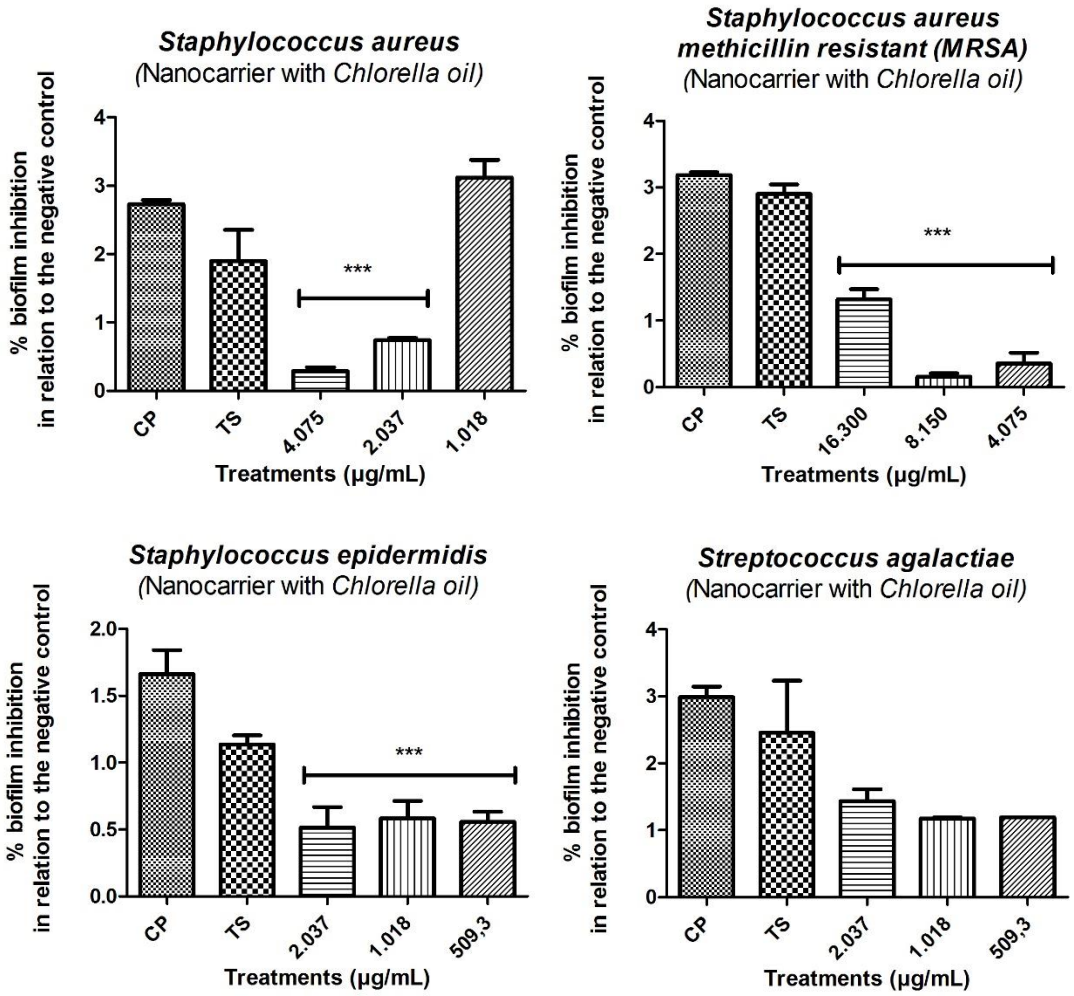
containing *Chlorella homosphaera* oil showed the ability to inhibit bacterial biofilm in *Aeromonas hydrophila, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus aureus resistente à meticilina (MRSA), Staphylococcus epidermidis e Streptococcus agalactiae.* The nanocarriers containing grape seed oil inhibited the bacterial biofilm in *Klebsiella pneumoniae* and *Staphylococcus aureus* (figure 4). Analyzing the MICs and the bacterial biofilm inhibition concentrations, it was observed that most of the tested microorganisms were able to inhibit the biofilm formation at concentrations lower than the respective MIC.

As discussed earlier on antimicrobial activity, studies report that nanoparticles also provide alternative pathways to combat biofilm infections with significantly lower bacterial resistance over time. The antimicrobial effects of nanocarriers against such strains are extremely important since all studied microorganisms represent importance at the clinical and/or hospital level (JOBIM et al., 2014).

In figure 3 we can see the results of the inhibition of the biofilm through the treatment with the formulation containing *Chlorella homosphaera* oil. The graphs show that there was inhibition of biofilm formation for 8 microorganisms tested, at sub-inhibitory, inhibitory and higher concentrations, that is, at the MIC concentration one below and one above.

Figure 3. Demonstration of the antibiofilm activity obtained by the Nanocarrier based on Tucumã Butter and *Chlorella homosphaera* oil. Positive control (CP) and surfactant control (T).

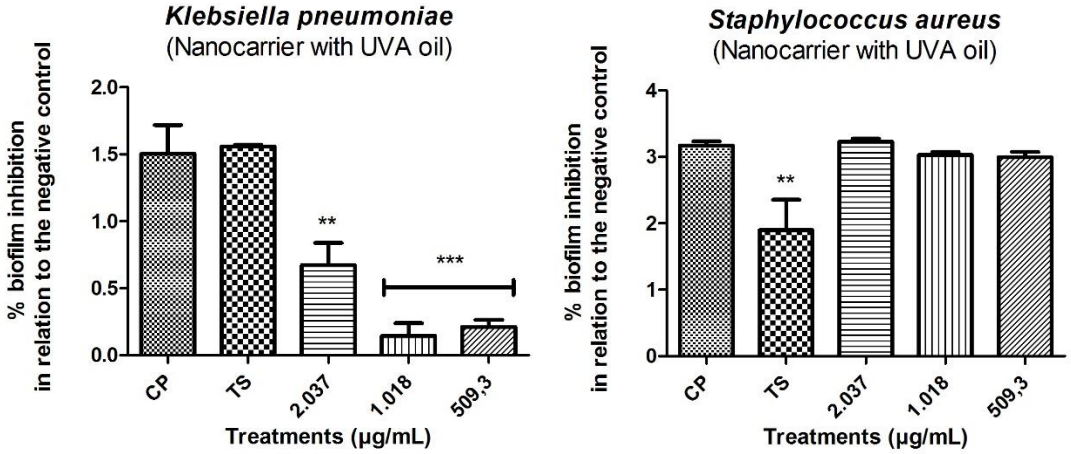




Results were expressed as a percentage of positive control (100%). Data were expressed as mean ± standard deviation (SD). Analyzes were performed by 1-way ANOVA, followed by Dunnett's post hoc test. Values with $p < 0.05$ were considered statistically significant.

In figure 4 we can see the results of the inhibition of the biofilm through the treatment with the formulation containing grape seed oil. The graphs show that there was inhibition of biofilm formation for 2 microorganisms tested, at sub-inhibitory and inhibitory concentrations and at higher concentrations, that is, at the MIC concentration one below and one above.

Figure 4. Demonstration of the antibiofilm activity obtained by the Nanocarrier based on Tucumã Butter and Grape Seed Oil. Positive control (CP) and surfactant control (T).



Results were expressed as a percentage of positive control (100%). Data were expressed as mean ± standard deviation (SD). Analyzes were performed by 1-way ANOVA, followed by Dunnett's post hoc test. Values with $p<0.05$ were considered statistically significant.

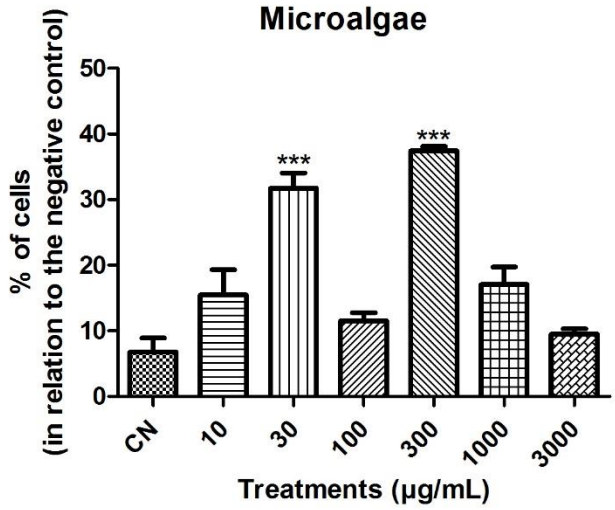
Currently, the search for natural bioactives with antimicrobial properties is increasing, as humanity needs new therapies to treat antimicrobial resistance, it also needs them to be sustainable, biodegradable, and biocompatible alternatives, as gram-positive bacteria have shown considerable changes in their sensitivity to antimicrobials over the years, especially staphylococci. Furthermore, the use of antibiotics can cause hematological, neurological, pulmonary, cardiac, gastrointestinal, hepatic, nephrotoxicity, and hypersensitivity side effects (TAVARES, 2002).

3.5 Evaluation of healing activity

3.5.1 In vitro scratch assay

Through the in vitro Scratch assay, we were able to analyze the healing trend of the compounds in the formulations. After mechanical damage to the cell monolayer, they tend to proliferate to cover the cell-free area. As shown in Figure 5, all concentrations treated with nanocarriers based on tucumã butter and *Chlorella homosphaera* oil showed an increase in the percentage of cells, and for concentrations of 30 µg/mL and 300 µg/mL, the increase was still more statistically significant.

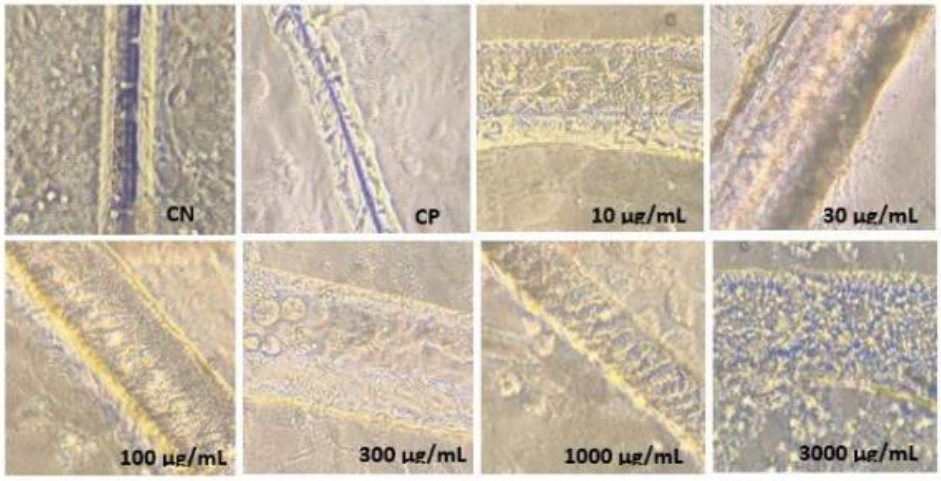
Figure 5. Results of the healing evaluation of the microalgae oil formulation.



Results were expressed as a percentage of positive control (100%). Data were expressed as mean ± standard deviation (SD). Analyzes were performed by 1-way ANOVA, followed by Dunnett's post hoc test. Values with $p<0.05$ were considered statistically significant.

Figure 6 shows the images obtained through optical microscopy, after 48 h of incubation, where cell proliferation is visible towards the mechanical injury caused.

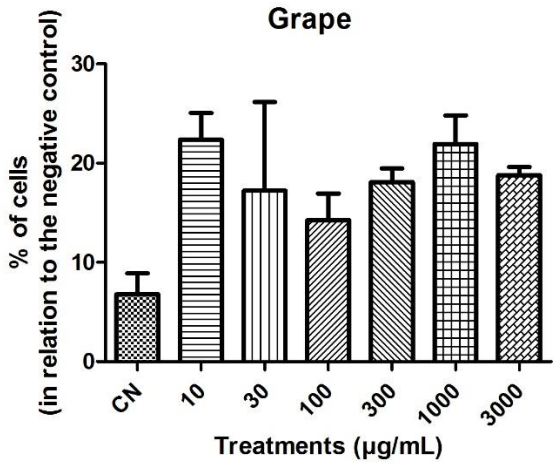
Figure 6. Demonstration of images obtained after 48 h of incubation with microalgae oil.



Source: Author's construction

When the cells were treated with the formulation containing grape seed oil, there was no significant increase in the percentage of cells at the concentrations analyzed. Figure 7 represents the healing results with the formulation containing grape seed oil.

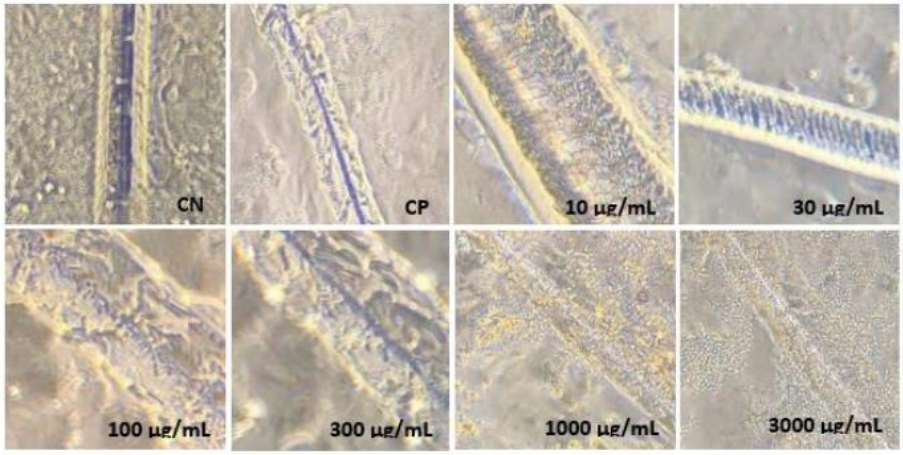
Figure 7. Results of the healing evaluation of the grape seed oil formulation



Results were expressed as a percentage of positive control (100%). Data were expressed as mean ± standard deviation (SD). Analyzes were performed by 1-way ANOVA, followed by Dunnett's post hoc test. Values with $p<0.05$ were considered statistically significant.

In figure 8 we can see the images obtained through microscopy after 48 h of incubation, where we can observe a certain cell proliferation towards the mechanical damage caused.

Figure 8. Demonstration of images obtained after 48 hours of incubation with grape seed oil.



Source: Author's construction

Nitric oxide is an endogenous molecule considered to be an important mediator of several biological actions such as vasodilation, neurotransmission, and inhibition of platelet adhesion and aggregation. Furthermore, it is recognized as a modulator of the healing phases, controlling collagen deposition, angiogenesis, cell proliferation, and even apoptosis (AMADEU et al., 2008; CHAN et al., 2015). In this way, the healing potential that the formulations presented may be related to the production of nitric oxide that was evidenced in previous studies and also the chemical constitution of bioactives, mainly microalgae oil and tucumã butter, where studies have reported the use of such oils for wound healing and anti-inflammatory treatments, as they have levels of vitamin E, which acts as a powerful antioxidant, helping

to hydrate, tone and revitalize the skin, having a healing and stimulating effect on collagen production (ZANETTI, 2009; MONTEIRO et al., 2005).

5. Conclusion

The formulation containing microalgae oil showed excellent antimicrobial action against all strains tested (10 out of 10 analyses), bactericidal action for strains of great clinical and hospital importance, including a multiresistant strain, as well as satisfactory antibiofilm action for most microorganisms tested (8 out of 10 reviews).

The formulation containing grape seed oil also showed antimicrobial activity, but for a smaller number of those tested. It is suggested that the antimicrobial action shown is related to the phenolic extracts presented by the constituents of the nanocarriers, as well as the action that the nanoparticles themselves have due to their size.

The formulation containing *Chlorella homosphaera* oil showed satisfactory healing action in a period of 48 hours.

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