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# Microbiological analysis of chitosan polymer membranes associated with *Schinus terebinthifolius* for treating skin wounds

## Análise microbiológica de membranas poliméricas de quitosana associadas à *Schinus terebinthifolius* para o tratamento de feridas cutâneas

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## Abstract

**Objective:** To develop and evaluate chitosan membranes containing *Schinus terebinthifolius* bark extract for topical use in skin lesions. **Methods:** Chitosan membranes were prepared with different concentrations of *Schinus terebinthifolius* extract. The resistance to folding, the pH of the membranes, and the antimicrobial activity of the extract were evaluated using the disc diffusion technique on Mueller-Hinton agar. The bacterial inoculum used was *Staphylococcus aureus* ATCC® 25923. The diameters of the inhibition zones were measured with a caliper to determine the sensitivity of the bacteria. The interpretation of the results followed the cutoff points for minimum inhibitory concentration (MIC) and halo diameters from the Brazilian Committee on Antimicrobial Susceptibility Testing. **Results:** After incubation at 30°C for 24 hours, the *Schinus terebinthifolius* extract at concentrations of 43.0 µg and 21.5 µg showed the best inhibition zones for *Staphylococcus aureus* (16 mm and 14 mm, respectively). The membrane with 5% extract exhibited greater resistance to folding, supporting more than 300 folds before breaking. In contrast, the 10% membrane became very rigid and had low adhesion after only 12 folds. The pH of the mem-

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branes was 5.5 in both concentrations, without a significant difference**. Conclusion:** The best antimicrobial results from *Schinus terebinthifolius* extract were at concentrations of 43.0 µg and 21.5 µg. The membrane with 5% of the extract showed the best adaptation, while the one with 10% became excessively rigid. Further studies must explore lower concentrations of the extract.

**Keywords**: Chitosan; Plant extracts; Schinus; Wound healing

## Resumo

**Objetivo:** Desenvolver e avaliar membranas de quitosana contendo extrato de entrecasca da *Schinus terebinthifolius* para uso tópico em lesões cutâneas. **Métodos:** Foram elaboradas membranas de quitosana com diferentes concentrações de extrato de *Schinus terebinthifolius*. Avaliaram-se a resistência ao dobramento, o pH das membranas e a atividade antimicrobiana do extrato usando a técnica de difusão de discos em Ágar Müeller Hinton. O inóculo bacteriano utilizado foi *Staphylococcus aureus* ATCC® 25923. Os diâmetros dos halos de inibição foram medidos com paquímetro para determinar a sensibilidade da bactéria. A interpretação dos resultados seguiu as tabelas de pontos de corte para concentração mínima inibitória e diâmetros de halos do *Brazilian Committee on Antimicrobial Susceptibility Testing*. **Resultados**: Após incubação a 30°C por 24 horas, o extrato de *Schinus terebinthifolius* nas concentrações de 43µg e 21,5µg mostrou os melhores halos de inibição para *Staphylococcus aureus*, com 16 mm e 14 mm, respectivamente. A membrana com 5% de extrato teve maior resistência ao dobramento, suportando mais de 300 dobragens antes do rompimento, enquanto a de 10% suportou apenas 12 dobragens, mostrando-se muito rígida e de baixa aderência. O pH das membranas foi de 5,5 em ambas as concentrações, sem diferença significativa. **Conclusão:** Os melhores resultados antimicrobianos do extrato de *Schinus terebinthifolius* foram nas concentrações de 43µg e 21,5µg. A membrana com 5% do extrato mostrou a melhor adaptação, ao passo que a de 10% tornou-se excessivamente rígida. Recomenda-se explorar concentrações mais baixas do extrato em futuras pesquisas.

**Palavras-chave**: Quitosana; Extratos vegetais; Schinus; Cicatrização

## INTRODUCTION

Covering the wound with membranes can replace lost skin function, protect the wound from fluid and protein loss, prevent bacterial invasion, and reduce mechanical stress, improving and stimulating healing. These advances in wound care and treatment are often costly, as restoring tissue requires time and numerous supplies, which can impact the quality of life for patients and lead to additional health issues[1,2].

Various types of coverings are available on the international market for treating wounds and burns, including synthetic and biological options. Polymer-based products are progressive, and dressings are useful in these treatments due to their porosity, epithelialization, mechanical

performance, as well as biodegradability and biocompatibility, as long permanent consolidation techniques occur to prevent resorption and material failure[3].

Chitosan (QTS) is a polymer with applications in several areas, such as food, waste treatment, pharmaceutical industry, and the principles of filtration and agronomy. However, QTS showed promising results in the biomedical sector. Its relevance as a barrier in the healing process correlates with its biological properties, such as antimicrobial, biocompatibility, biodegradability, low toxicity, and low immunogenicity, which facilitate tissue renewal, while relating to other established treatments[4].

The bark of the aroeira tree (*Schinus terebinthifolius*) has great therapeutic potential, with high levels of antioxidant compounds (flavonoids, tannins, terpenes, and phenols) that draw attention, as these compounds exhibit various pharmacological activities in the plant specimen, including anti-inflammatory, antifungal, anticancer, and healing properties. The ease with which these plants can be acquired and their cultural compatibility are important factors in the development of these technologies, especially in Northeast Brazil, where the use of medicinal plants for preparing home remedies to treat various diseases is commonly underused[5,6,7,8,9].

An interest exists in obtaining and developing products from this plant species for wound treatment. Thus, this study aimed to develop and evaluate QTS membranes containing *Schinus terebinthifolius* bark extract for topical use on skin lesions.

## METHODS

### Preparation of *Schinus terebinthifolius* extract

The maceration method used 500 g of the bark of the *Schinus terebinthifolius* plant, which was cut into small pieces and crushed to increase the area of contact with the solvent, facilitating the extraction of the desired compounds. The solvent chosen for this extraction was 1 liter of 70% ethanol due to its efficiency in extracting polar and semi-polar compounds, such as flavonoids, tannins, and terpenes. This ratio was chosen to ensure that the solvent could dissolve the bioactive compounds present in the bark without wasting solvent or having a very low concentration of the extract. A ratio of 1:2 (bark:solvent) is common practice, as it guarantees a good concentration of bioactive substances in the final solution[7]. The maceration occurred in a hermetically sealed container to prevent solvent evaporation and ensure efficient extraction of the compounds. The process was conducted over 72 hours, with periodic stirring every 12 hours to ensure uniform contact of the solvent with the bark and maximize the extraction of the compounds. After the maceration period, the mixture was filtered to remove the solid bark. Last, a rotary evaporator was used to remove the excess ethanol, resulting in a more concentrated extract. The extract was stored in amber glass bottles to protect it from light, which can degrade some bioactive compounds.

**Film development**

The films were developed from QTS solutions prepared in a 1% (v/v) acetic acid solution to obtain a final concentration of 1% (m/v). The following samples were produced from this polymer solution: QTS film (F1), QTS with 5% mastic extract (F2), and QTS with 10% mastic extract (F3). The samples were prepared under magnetic stirring for 24 hours. The resulting solutions were poured into plastic Petri dishes with a diameter of 5.5 cm and a volume of 10 mL and then placed in an air circulation oven at 50 ºC for 24 hours to promote solvent evaporation and film formation. The formulations were developed at the Pharmaceutical Product Development and Characterization Laboratory at the State University of Paraíba.

**Antimicrobial activity**

The antimicrobial activity of *Schinus terebinthifolius* extract was evaluated using the disk diffusion technique on solid Mueller-Hinton agar culture in a 60 x 15 mm glass Petri dish. The bacterial inoculum of *Staphylococcus aureus* ATCC® 25923 was scattered using a sterile swab.

The cultures were prepared and incubated in the microbiology laboratory at the Federal University of Pernambuco. Gram-positive bacterial strains (i.e., *Staphylococcus aureus* ATCC® 25923) were used in the antimicrobial sensitivity tests. The bacterial strains were activated in test tubes containing nutrient agar and kept in an oven at 37°C for 18 hours. Then, a 0.9% saline solution was prepared, and a bacterial inoculum was created, corresponding to a MacFarland scale of 0.5, which is approximately equivalent to $10^8$ colony-forming units per milliliter (CFU/mL). From a culture of the selected strain, a single colony was implanted and incubated at 37 °C for about 18 hours. After clouding the medium with a sterile swab previously dipped in the bacterial suspension, the culture was sown by streaking the Mueller-Hinton agar plates in two directions, over the entire surface of the medium and then passing the swab around the entire plate, leaving the inoculum to dry for 5 to 10 minutes.

Using sterile tweezers, seven sterile diffusion discs with a diameter of 9 mm and 250 g/m² were soaked in *Schinus terebinthifolius* crude extract at a concentration of 43 mg/mL, previously diluted in distilled water, establishing the seven concentrations tested: 43 µg, 21.5 µg, 10.75 µg, 5.37 µg, 2.68 µg, 1.34 µg, 0.67 µg, respectively, being deposited on the inoculated plates and incubated (inverted) at 36 °C for 24 hours in a bacteriological oven.

The use of several concentrations of the extract enables a detailed assessment of its antimicrobial activity, providing data on the minimum inhibitory concentration (i.e., the lowest concentration capable of inhibiting microbial growth) and the minimum bactericidal concentration. The concentrations used were chosen based on previous studies on the antimicrobial activity of *Schinus terebinthifolius* and standard protocols for testing antimicrobial activity, such as the agar diffusion method. This range of concentrations allows the efficacy of the extract to be assessed over

a range of doses, ensuring that both antimicrobial activity and potential toxicity are analyzed[10].

The choice of these concentrations is also in line with incremental dosing studies, in which it is possible to identify the relationship between the concentration of the extract and the diameter of the inhibition zone. As the concentration of the extract increases, it is expected that the inhibition halo will also increase, indicating greater antimicrobial efficacy. This strategy enables a quantitative evaluation of the potency of the extract and allows a precise comparison between different concentrations[10].

The diameter of the inhibitory zone was measured in millimeters, considering the size of the disc (9 mm). The analysis was performed in triplicate with three repetitions, and the result was presented as the mean (± standard deviation).

The plates were read using calipers to determine the diameters of the inhibition zones, corresponding to the sensitivity of *Staphylococcus aureus* to *Schinus terebinthifolius* extracts. The interpretation of the results was based on the new methodology of cut-off point tables for the interpretation of minimum inhibitory concentrations and inhibition zone diameters recommended by the Brazilian Committee on Antimicrobial Susceptibility Testing, with the version valid as of March 15, 2023.

**Bending resistance test**

A folding resistance test was conducted by manually folding the film at the same point repeatedly until it broke or was folded up to a maximum of 300 times, based on a previous study[11]. The number of bends without any breakage gave the exact value of its resistance. The analysis was performed in triplicate at the Functional Practices Laboratory of the Faculdade de Medicina de Olinda.

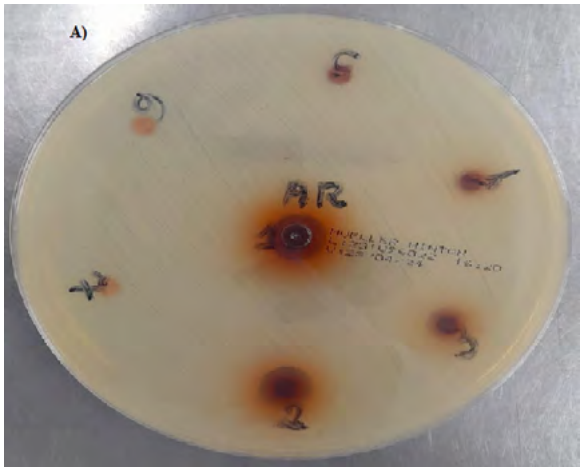
**Determining pH**

The pH was determined using the potentiometric method with a Tecnal model TEC-2 digital meter, calibrated with pH 4.0 and 7.012 buffer solutions. The results were expressed in pH units.

## RESULTS AND DISCUSSION

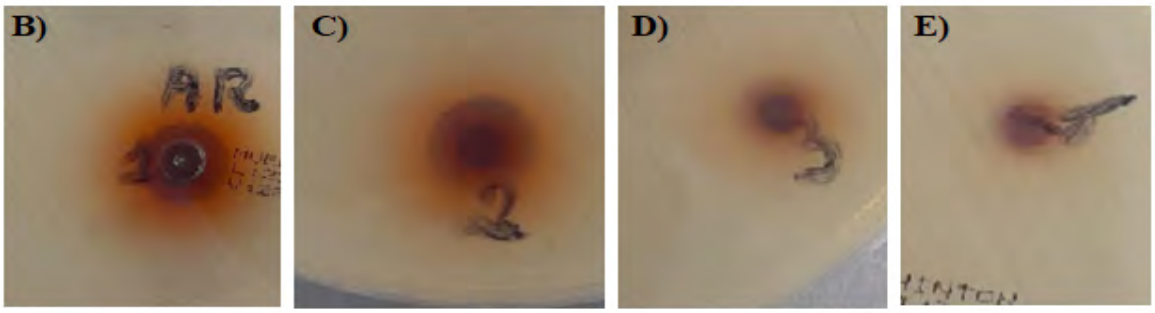
The results, after incubation at 37 °C for 24 hours, macroscopically demonstrated the formation of inhibition zones in 1, 2, 3 and 4 (Figure 1) at the concentrations 43 µg, 21.5 µg, 10.75 µg, and 5.37 µg, corresponding to the sensitivity of the *Staphylococcus aureus* bacteria to the extract with the formation of halos 16 mm, 14 mm, 11 mm, and 9 mm in diameter (Figure 1). For the 2.68 µg, 1.34 µg, and 0.67 µg concentrations, no inhibition zones were observed, indicating a lack of microbial inhibitory activity.

**Figure 1.** Inhibition zones (mm) of *Schinus terebinthifolius* extract against *Staphylococcus aureus.*




Source: Research data.

**Figure 2.** Formation of inhibition zones with different concentrations of the extract.




Source: Research data.

In the detailed image (Figure 2) of the plate streaked with *Staphylococcus aureus*, in terms of the formation of inhibition zones, the concentration in Figure B showed the greatest inhibition of growth with the formation of an inhibitory zone of 16 mm from the *Schinus terebinthifolius* extract at a concentration of 43 µg against *Staphylococcus aureus*. The concentration in Figure C showed an inhibition of 14 mm caused by the extract at the 21.5 µg concentration. Figure D shows an inhibition of 11 mm caused by the extract at a contraction of 10.75µg. Finally, the concentration in figure E showed a slight inhibition of 9 mm caused by the extract in the concentration of 5.37 µg.

In this study, the inhibitory capacity of the *Schinus terebinthifolius* extract was demonstrated at the following concentrations against *Staphylococcus aureus*: 43 µg, 21.5 µg, 10.75 µg, and 5.37 µg. The best results for the antimicrobial activity of the *Schinus terebinthifolius* extract were observed at concentrations of 43 µg and 21.5 µg, in which inhibition zones of 16 mm and 14 mm were noted for *Staphylococcus aureus,* respectively. The data obtained in this study, when bacterial inhibition was evaluated at a concentration of 5.37 µg with an inhibition zone of 9mm in diameter, was similar to the data already described in the literature, which used the ethanolic extract

of red mastic fruits at a concentration of 100 mg/mL to inhibit the microorganism *Staphylococcus aureus* (ATCC 6538), the result was an inhibition of 9 mm in diameter[13].

*Schinus terebinthifolius* is an extract composed of triterpenoids (3-hydroxymasticadienoic acid), phenols, and triterpenes (simiarenol). One possible explanation for why these compounds inhibit the microorganism is that their chemical structures interact with the peptide-glycan bonds in the cell wall, specifically targeting the D-alanine-D-alanine portion of the bacterial cell wall. These compounds bind to and form complexes with the units that would make up the peptide glycans of the new wall and interrupt the process of polymerization (formation) of the cell wall, leading to osmotic fragility of the bacterial peptide glycan wall, which leads to lysis of the bacteria near the diffusion discs and forms inhibition zone[14].

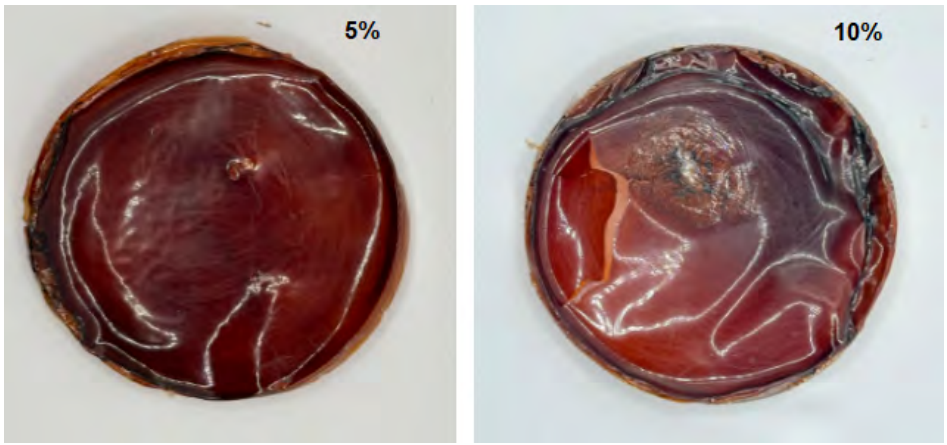
Folding resistance analyses are important for identifying the comfort of the film when applied to the wound site[15]. QTS films with *Schinus terebinthifolius* extract showed significant differences between the FQ5% and FQ10% samples. The FQ5% sample, containing 5% extract, showed substantially greater resistance by withstanding bending to breakage before 300 bends. On the other hand, the FQ10% sample, which contained QTS and 10% extract, showed a lower resistance to folding, averaging only 12 folds. These differences suggest that the FQ10% film is more brittle, probably due to the presence of a greater amount of polyphenols from the *Schinus terebinthifolius* extract. The intermolecular interactions between the polymers reduce the flexibility of the membranes, potentially hindering the flexibility and strength of the material[16].

**Table 1.** Samples used in the study.

| Sample | Concentration | Bends | pH |
|---|---|---|---|
| F1 | 0% | 38 | 5.7 |
| F2 | 5% | 27 | 5.3 |
| F3 | 10% | 12 | 5.5 |

Source: Research data.

**Figure 3.** *Schinus terebinthifolius* extract (5% and 10%) applied to the membranes.




Source: Research data.

Soft, flexible, and non-irritating healing membranes are crucial for promoting proper recovery and minimizing discomfort during the healing process. The comfort they provide directly influences patient acceptance and adherence to treatment, impacting their quality of life by allowing them to move around and conduct daily activities without restrictions[17].

When analyzing the pH of the membranes, no significant difference was found between the 5% and 10% concentrations, with an average pH of 5.5 being identified in the triplicate test. As far as pH is concerned, inflamed tissue has a recognizably acidic pH, in which most of the bacteria found in the root canal system develop. The relationship between healing membranes and the pH of the membrane and the wound is a critical aspect to consider for optimizing the healing environment. Studies have highlighted the importance of maintaining an adequate pH to promote effective healing and reduce complications. The pH of the healing membrane influences enzymatic activity and cell proliferation, affecting the speed and quality of healing. Membranes that maintain an ideal pH favor cell migration, collagen synthesis, and the activity of growth factors, contributing to rapid healing and reduced scarring. Additionally, regulating pH helps control inflammation and reduces the risk of infections. Therefore, the pH is crucial in the development of effective healing membranes[18].

## CONCLUSION

The results obtained in this study showed that *Schinus terebinthifolius* extract, at concentrations of 43 µg, 21.5 µg, 10.75 µg, and 5.37 µg, inhibited the growth of *Staphylococcus aureus* in Mueller-Hinton agar cultures. In this study, new information was obtained regarding the potential antimicrobial action of bioactive compounds extracted from *Schinus terebinthifolius*. Regarding the folding resistance test, the presence of a greater amount of the extract, which is rich in polyphenols, makes the membrane more brittle and less suitable for this purpose. Due to its potential antibacterial properties, further studies are needed to investigate its more detailed chemical composition using molecular modeling programs and to examine its microbial activities, obtaining more precise information about *Schinus terebinthifolius* extract and expanding knowledge about its antimicrobial activities for future pharmacological applications.

## CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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## AUTHOR CONTRIBUTIONS

**SMLTC**: Conceptualization, Data curation, Data analysis, Investigation, Methodology, Project administration, Supervision, Data validation and experiments, Data presentation, Writing - original draft and Writing - review and editing. **GSPB**: Conceptualization, Data curation, Data analysis, Investigation, Methodology, Development, Data validation and experiments, Writing - original draft and Writing - review and editing. **LFMN**: Investigation. **CABL**: Conceptualization, Investigation, Methodology and Writing - original draft. **GGG**: Methodology. **LRT**: Conceptualization, Writing - original draft and Writing - review and editing. **TKBO**: Conceptualization, Data curation, Data analysis, Investigation, Methodology, Project administration, Supervision, Data validation and experiments, Data presentation, Writing - original draft and Writing - review and editing. All the authors approved the final version.

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