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"*In Vitro*" Antibacterial Activity of the Hidroalcoholic Extract of the *Schinus terebinthifolius* Raddi Barks

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Abstract

The *Schinus terebinthifolius* Raddi is a native plant of South America popularly known in Brazil as *aroeira.* It is a medium-sized plant, which demonstrates a high adaptive potential in various environments, besides having various medicinal properties such as anti-inflammatory and antidiarrheal. Bacterial susceptibility tests were carried out and the minimal inhibitory concentration (MIC) was obtained. The results were interpreted based on the conventionally microbiological protocols and data from the CLSI. For the microbiological tests, microorganisms obtained from the American Type Culture Collection (ATCC), specifically, (*Pseudomonas aeruginosa, Staphylococcus aureus* and *Encherichia coli*) were used. Of the tested bacterial strains, only the *Staphylococcus aureus* 6538 presented susceptibility to the *aroeira*'s hydroalcoholic extract, forming zones of inhibition of 8.0 mm of diameter up to the MIC of 35.3 mg/mL, while the other tested strains showed to be resistant in all the concentrations of hydroalcoholic extract of the *Schinus terebinthifolius* Raddi bark. Our aim is to analyze the "*in vitro*" antibacterial potential of the 70% hydroalcoholic extract of the *Schinus terebinthifolius* Raddi.

Keywords

***Aroeira*, Antibacterial Activity, *Schinus terebinthifolius* Raddi, Hydroalcoholic Extract**

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1. Introduction

Schinus terebinthifolius Raddi is a plant popularly known as *aroeira*. This plant belongs to the Anacardiaceae family, which contains 70 genus and more than 600 species of trees and bushes [1] [2]. It is native of South America, where it stands out in countries such as Paraguay, Peru, Argentina and Brazil. But it may also be found in semitropical and tropical regions of the United States, Africa and even in Oceania [3] [4]. In Brazil due to the great variety and diversity of the vegetation, it is easily found; as it is present from the state of Pernambuco to the state of Rio Grande do Sul [5].

The *aroeira* is a medium-sized plant, demonstrating a high adaptive potential in various environments [6]-[8]. This plant has various medicinal properties such as: antidiarrheal, astringent, anti-inflammatory, febrifuge and anti-allergic [1] [9] [10]. It is popularly used as a healing agent, in inflammations and ulcers, against toothache, hematomas, contusions and hemoptysis [7] [10] [11]. Furthermore, the *Schinus terebinthifolius* is not only utilized for medicinal use, but also for dental treatments and as a base for some medicines [12]-[14].

Due to the evolution and dissemination of the antimicrobial resistance owing to the excessive and indiscriminate use of antibiotic agents in the health sector, the use of natural bioactive agents seems promising to us [15] [16]. The *S. aureus* is among the most frequent nosocomial pathogens, as the resistance to multiple drugs is common and contributes to the difficulty in the treatment of infections [17]-[19].

For economic reasons the large pharmaceutical industries have abandoned the search for new antimicrobial compounds. But in the past this was the main objective for the development of antimicrobial agents. However, the search for new alternatives for the antibacterial therapy has been focused in the search for new antibiotics that act on new molecular targets of the pathogens and are not affected by the resistance mechanisms, as well as in the search for inhibitors of these resistance mechanisms [20]. In light of this context our aim is the analysis of the *in vitro* antibacterial activity of the hydroalcoholic extracts of *Schinus terebinthifolius* Raddi.

2. Materials and Methods

2.1. Harvesting, Drying and Elaboration of the Crude Extract of the *Aroeira*

The vegetal natural product, (*Schinus terebinthifolius* Raddi barks) were appropriately harvested in the woods of a region of the northeast of Brazil. The barks were processed away from the light at a temperature of 60˚C for the removal of its humidity [21] [22]. After the drying the vegetal material was ground and stored in tubes, where were added 15 mL of 70% alcohol for the elaboration of the hydroalcoholic solution and posteriorly submitted to the TS-2000 VDRL SHAKER, for 120 min. Then the product was vaporized in the Rotavapor Fisatom 801, until the total vaporization and attainment of the crude extract. The 170 mg of the crude extract were diluted in 430 µL of 0.9% physiological solution to obtain an approximate concentration of 283 mg/mL.

2.2. Preparation and Use of the Bacterial Strains

The *in vitro* antibacterial properties of the 70% hydroalcoholic extract of *Shinus terebinthifolius* Raddi and all the inocula were prepared and standardized from the work culture by conventional microbiological protocols, and in conformity with the methodology proposed by the CLSI [23] [24]. We used bacterial strains obtained from the American Type Culture Collection (ATCC), where the initial cultures of *Pseudomonas aeroginosa* 8027, *Staphylococcus aureus* 6538 and 25,925 and *Encherichia coli* 10,536 were cultivated during 24 h and diluted with broth Brain Heart Infusion (BHI) culture medium for an approximate density of 7.0×10^6 CFU/mL (CFU: colony forming units).

2.3. Microbiological Tests

The bacterial suspensions were obtained by means of inoculation in enriched broth BHI, and then incubated at 37˚C for 24 h. After this period, the bacterial growth was observed by the turbidity of the samples and the inocula in agar. Of the bacterial strains about 100 µL were inoculated in Mueller-Hinton (MH) agar, and using a Drigalky strap the Spread Plate technique was carried out. The filter paper discs of 5.0 mm diameter were positioned on the surface of the medium, and about 25 µL of the crude extract was deposited after micro-dilutions in a Kline plate. The plates were incubated in a bacteriological incubator at 37˚C and were analyzed after 24 hours.

3. Results and Discussion

The internal stability of the bacterial cells depends on the interaction between a series of physiological factors, and the disturbance of this stability, may determine the bacteria's death or the inhibition of its growth [25]. To provide products, which reduce the toxicity risk and at the same time are obtained from a new natural and renewable source becomes a growing and economically viable option. The use of vegetal extracts for antibacterial activity is a consummated fact [20].

Of the bacterial strains submitted to the microbiological tests, only the *Staphylococcus aureus* 6538 presented inhibitions zones in agar The *Schinus terebinthifolius* Raddi extract in its maximum concentration produced the formation of sensitivity zones of 14.0 mm. In agar with the dilution of 1:2, the sensitivity zone was of about 12.0 mm, in the dilution 1:4 the sensitivity zone measured 10.0 mm and in the dilution of 1:4 the sensitivity zone measured 8.0 mm. On the plates containing the dilutions 1:8 the sensitivity zone measured 8.0 mm. On the plates containing the dilutions 1:16 and 1:32 was no formation of inhibition zones or the zone was of an insignificant size (**Table 1**). All the microbiological tests were performed in duplicate.

The results obtained from the 70% hydroalcoholic extract of the *aroeira* barks on some strains of *Staphylococcus aureus* 6538 showed intense inhibitory activity on the bacterial growth, from the maximum concentration (283 mg/mL) to the dilution of 1:8 corresponding to the minimal inhibitory concentration (MIC) of (35.3 mg/mL). This result corroborates with other studies in which the hydroalcoholic extracts of the *Schinus terebinthifolius* Raddi barks prepared with other percentages, had a similar action on the microorganisms that formed dental biofilm in dilutions of up to 1:4 [11]. Likewise other studies revealed that the 20% *aroeira* bark dye presented *in vitro* antibacterial activity on *Streptococcus mutans* in dilution of up to 1:8 [4].

Besides the antibacterial activity, the hydroalcoholic extract also has an efficient action on the healing process of wounds on rats' skin [17] [21]. *Schinus terebinthifolius* Raddi has shown in other studies a great development potential of new products , based on plants, however little has been done to identify and characterize its chemical components which certainly is a niche that needs to be better explored [5] [18] [19] [22].

4. Conclusions

The *Staphylococcus aureus* 6538 showed to be susceptible to the hydroalcoholic extract of *Schinus terebinthifolius* Raddi in dilutions of up to 1:8, concentrations of 35.3 mg/mL, being the MIC. While the strains of *Pseudomonas aeruginosa* 8027, *Staphylococcus aureus* 25,925 and *Escherichia coli* 10,536, showed to be resistant to the crude extract of the *aroeira* bark.

Hence, the obtained results show that it is of great importance to also carry out research *in vivo*, in order that the *aroeira*'s extracts may be clinically used in the treatment of various bacterial conditions. The results obtained in this study show the importance of the therapeutical indications of the medicinal plants as alternative and of low cost methods, at production level, once that the hydroalcoholic extracts of *aroeira* showed, *in vitro*, important antimicrobial activity.

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Table 1. Minimal inhibitory concentration (MIC) of the 70% hidroalcoholic extract of *Schinus terebinthifolius* Raddi for *Staphylococcus aureus* 6538.

Concentration (mg/mL)	Size of the zones (mm)
1 - 283.0 mg/mL	14.0 mm
2 - 141.5 mg/mL	12.0 mm
3 - 70.7 mg/mL	10.0 mm
4 - 35.3 mg/mL	8.0 mm
5 - 17.6 mg/mL	0.0 mm
6 - 8.8 mg/mL	0.0 mm

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