

Assessment of the Antiadherent Potential of *Gaultheria procumbens* Essential Oil against *Staphylococcus aureus*

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ABSTRACT

Nosocomial pneumonia is an infection of the lung parenchyma caused by bacteria and acquired after a 48–72 hour period of patient hospitalization. Among the bacterial species causing this condition is *Staphylococcus aureus*, a pathogen that has proven resistant to commonly used antibiotics. In view of this, new therapeutic alternatives are necessary, and phytotherapy presents itself as a field of ample possibilities for study. The objective of this research was to evaluate the anti-adherent potential of *Gaultheria procumbens* essential oil against a strain of *Staphylococcus aureus*. To evaluate the Minimum Inhibitory Adherence Concentration (MIC) of the oil, the Muller Hinton broth with 5% sucrose, the Sa 101 strain of the bacterial species under study, as well as, the positive control with 0.12% chlorhexidine were used. The material was incubated at 37 °C for 24 hours for reading using fuchsin. As a result it was observed that the essential oil of *Gaultheria procumbens* inhibited the formation of biofilm in the proportion of 1:128, showing to be superior to the anti-adherent potential of chlorhexidine digluconate that inhibited in the proportion of 1:8. From the data, it was verified the anti-adherent potential of the essential oil of *Gaultheria procumbens* against the strain of *Staphylococcus aureus*, and thus, it can be an effective therapeutic option in intraoral antisepsis and surfaces of materials inserted in the oral cavity.

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1. INTRODUCTION

The human body is naturally colonized by different microorganisms that establish a symbiotic relationship with the host's defense mechanisms. Changes in the composition and size of microbial populations associated with deficiency of resistance factors of the individual can trigger a breakdown of this homeostasis and favor the emergence of pathologies [1], [2].

From this perspective, in the face of an imbalance, *Staphylococcus aureus*, a bacterium frequently found in the microbiota of healthy individuals, can trigger from superficial skin problems to lethal systemic infections [3]–[5]. This microorganism presents itself as an immobile gram-positive coccus, belonging to the Micrococaceae family,

and with a diameter ranging between 0.5 and 1.5 µm. It is a facultative anaerobic, mesophilic bacterium, with the ability to adhere to a wide variety of surfaces and thus with consequent ease of forming biofilm [3], [6], [7].

In the oral biofilm the microorganisms become less sensitive to the antibiotics used, moreover, poor choice, unnecessary prescription of an antibiotic or excesses in dosages can facilitate the acquisition of resistance by microorganisms and *Staphylococcus aureus* has been shown to be susceptible to this, which implies a relevant problem in the treatment of infections originating from this bacterium, whose primary solution is found in preventive measures through the correct hygienization of surfaces [2], [8].



Therefore, when the biofilm disorganization is not performed, this pathogen that can be present on dental surfaces, prostheses, oral cavity mucous membranes and endotracheal tubes in cases of mechanically breathing patients, can be aspirated or even enter the bloodstream through diseases in the periodontium, so as to start an infectious pneumatic condition. The immunocompromise of hospitalized individuals associated with the imbalance of *Staphylococcus aureus* populations may be responsible for a significant portion of these hospital-acquired infections, so as to contribute to the increase in the rates of health-related complications [2], [9].

Once acquired in the hospital, this infection is called nosocomial pneumonia, which occurs between 48 and 72 hours after patient admission [10], [11]. This condition is configured as one of the main causes of morbidity and mortality, which may be related to both the multiple resistance of the most common strains of this bacterium in hospital settings that limits the therapeutic options and prolongs the treatment time of these infections, and the poor sanitation of surfaces [9], [12].

Commonly, cleaning the oral cavity and structures inserted in it is performed with chlorhexidine digluconate 0.12%, a substance considered the gold standard in the lineage of oral antiseptics. However, when used chronically it can trigger some adverse effects, such as changes in the color of dental elements, restorations, prostheses and tongue, loss of taste, formation of supragingival calculus, xerostomia, mucosal ulcerations and unpleasant aftertaste in the mouth [13].

In view of this, new alternatives have been investigated to combat this pathogen and phytotherapy becomes a field of ample possibilities to remedy this problem. Phytotherapeutic substances, obtained from vegetable raw material, have been widely used in the treatment of various human diseases, and can be found in solid and liquid forms [14], [15].

Among the liquid extracts are the essential oils, compounds derived from secondary metabolism of plants, which as well as terpenes, major components of these oils, have several pharmacological properties, such as anti-adherent action. Given the high adherence capacity of *Staphylococcus aureus* and the resistance of clinical strains to commonly used antibiotics, substances with anti-adherent potential may play an important role in infections associated with this microorganism [16]–[18].

In this perspective, *Gaultheria procumbens* oil, composed mainly of methylsalicylate, a substance that can be metabolized in plant tissues into salicylic acid characterized as a phytohormone, induces pharmacological potential against microbial pathogens, presenting documented insecticidal, anti-inflammatory, antimicrobial and anti-adherent effects [19].

Given the importance of combating infections caused by multidrug-resistant bacteria and the already known antibiofilm activity of the aforementioned substance, this work aims to evaluate the anti-adherent activity of the essential oil of *Gaultheria procumbens* against *Staphylococcus aureus* compared to chlorhexidine digluconate 0.12%, in order to enable the development of a natural product with fewer adverse effects, and thus reduce or even prevent injuries to hospitalized patients.

2. METHODOLOGY

2.1. Test Substances, Bacterial Species and Culture Media

The essential oil of *Gaultheria procumbens* was purchased from Herbia Industry. For this study, *Staphylococcus aureus* strain Sa 101 maintained on Muller Hinton Agar (MHAG) medium at 4 °C was used for the tests. In addition, a bacterial inoculum of approximately 1.5×10^8 CFU/mL standardized according to the turbidity of the 0.5 tube of the McFarland scale was used in the research [20], [21].

2.2. Determination of the Minimum Inhibitory Adherence Concentration (MIC)

The Minimum Inhibitory Adherence Concentration (CIMA) of the oil was determined in the presence of 5% sucrose, according to Albuquerque *et al.* [22], using concentrations corresponding to the pure essential oil up to 1:1024 dilution. From the bacterial growth, the chosen *Staphylococcus aureus* strain was grown at 37 °C in Mueller Hinton broth (DIFCO, Michigan, United States), consequently, 0.9 mL of the subculture was dispensed into test tubes and then 0.1 mL of the solution corresponding to the essential oil dilutions was added. Incubation was performed at 37 °C for 24 hours with tubes tilted at 30°. Readings were then taken by visual observation of the adherence of the bacteria to the walls of the tube after shaking it. The assay was performed in duplicate. The same procedure was performed for the positive control, 0.12% chlorhexidine digluconate (Periogard®, Colgate-Palmolive Company, New York, USA). The CIMA was considered the lowest concentration of the agent in contact with sucrose that prevented adherence to the glass tube.

3. RESULTS AND DISCUSSION

Through the laboratory study following the methodology mentioned above, the anti-adherent activity of *Gaultheria procumbens* essential oil was evaluated. The results show that the lowest concentration of this substance able to inhibit bacterial adhesion to the tube wall was 1:128, showing positive and superior activity against biofilm formation by *Staphylococcus aureus* when compared to the positive control with 0.12% chlorhexidine digluconate, which inhibits biofilm formation at a concentration of 1:8 (Table I).

In a research conducted by Nikoli *et al.* [23] the chemical composition and biological activity of *Gaultheria procumbens* essential oil against food spoilage and oral microorganisms was examined. In the study, the essential oil inhibited the growth of all microorganisms tested and as verified in the present research, the anti-adherent activity was evidenced, however, against *Candida albicans* and *Streptococcus mutans* species. The latter is a cariogenic species known for its colonization on surfaces, and thus responsible for the initial phase of formation of oral biofilm.

Also, it was verified the antimicrobial potential against *Staphylococcus aureus* species, as well as, the antioxidant and moderate antiradical effect, reducing the amount of

TABLE I: MINIMUM INHIBITORY ADHERENCE CONCENTRATION (MIC) OF GAULTHERIA PROCUMBENS ESSENTIAL OIL AND 0.12% CHLORHEXIDINE DIGLUCONATE AGAINST STAPHYLOCOCCUS AUREUS BACTERIAL STRAIN (SA 101)

<i>Gaultheria procumbens</i>							
Concentration							
1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128
+	+	+	+	+	+	+	+
0,12% chlorhexidine digluconate							
Concentration							
1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128
+	+	+	+	—	—	—	—

Legend: (+) Without adhesion to the tube wall (—) With adhesion to the tube wall.

Source: Author himself (2022).

hydroxyl radicals produced [23]. Besides the aforementioned pharmacological activities, the insecticidal effect of this oil is also reported in the scientific literature [24].

Studies about the pharmacological potential of the essential oil of *Gaultheria procumbens* are scarce in the literature today, thus, methodologically reliable scientific studies about its antibiofilm potential against *Staphylococcus aureus* were nonexistent until the present moment, making evident the relevance of this research and the need for toxicological tests, as well as microbiological and pharmacological studies *in vivo*, for the interpretation of the mechanism of action of this natural product of anti-adherent potential.

4. FINAL CONSIDERATIONS

The oil of *Gaultheria procumbens* stands out for presenting high anti-adherent potential against the bacterial species under study and may be an effective therapeutic option in intraoral antisepsis and surfaces of materials inserted in the oral cavity. However, further studies are essential to emphasize its efficacy against different types of microorganisms and, subsequently, *in vivo* research to verify its behavior in the human body.

CONFLICT OF INTEREST

Authors declare that they do not have any conflict of interest.

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