

Assessment of the Antiadherent Potential of *Pogostemon cablin* Essential Oil against *Staphylococcus aureus*

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ABSTRACT

Nosocomial pneumonia, an infection of the lung parenchyma caused by bacterial species, corresponds to a morbidity responsible for reducing the quality of life and further compromising the hospitalized patient. Among the bacteria that cause this infection is *Staphylococcus aureus*, which has proved resistant to the drugs used in its treatment. Therefore, the need for new studies is urgent and phytotherapy has been gaining space. The objective of this research was to evaluate the anti-adherent activity of *Pogostemon cablin* essential oil against a strain of *Staphylococcus aureus*. To obtain the Minimum Inhibitory Adherence Concentration (MIC), Muller Hinton broth with 5% sucrose was used, the Sa 101 strain of the bacterial species under study, as well as, the positive control with 0.12% chlorhexidine. The material was incubated at 37 °C for 24 hours for reading using fuchsin. Through analysis, it was found that the oil studied was able to inhibit the adhesion of bacteria to the wall of the tube at a concentration of 1:2, showing positive activity against the formation of biofilm by *Staphylococcus aureus*, although the result found is lower than that of chlorhexidine, which inhibited at a ratio of 1:8. It is concluded that *Pogostemon cablin* oil showed anti-adherent activity against the strain under study, however, inferior when compared to the positive control.

Keywords: Dentistry, hospital infection, phytotherapy, pneumonia.

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1. INTRODUCTION

Most hospitalized patients have reduced resistance factors and are susceptible to conditions that can trigger complications to their clinical picture. Nosocomial pneumonia, an infection of the lung parenchyma acquired after a 48–72-hour hospital stay, corresponds to morbidity responsible for reduced quality of life and even greater impairment to the hospitalized patient. In Intensive Care Units (ICUs), the most common pneumonias that affect patients are those associated with mechanical ventilation and may occur from 8% to 38% of individuals undergoing this breathing support [1]–[3].

From the perspective of factors related to pulmonary infection, in 1970 the presence of dental biofilm bacteria in the oropharynx was discovered, thus emerging the

hypothesis of the relationship of this bacterial plaque with nosocomial pneumonia [3]–[7].

Dental biofilm is defined as a community of microorganisms colonizing the oral cavity that in the absence or deficiency of hygiene can cause deleterious oral diseases, such as dental caries [8]. Although it is not an etiological factor for this disease, the literature documents the presence of *Staphylococcus aureus*, a potential respiratory pathogen, in carious lesions. This can be explained by its ability to adhere and consequent biofilm formation [9].

When not disorganized, this pathogen can be aspirated or spread into the bloodstream through diseases in the periodontium and thus trigger an infectious process in the lungs [10], [11]. Thus, it is evident the importance of oral hygiene and early therapeutic intervention before

conditions that affect this cavity in hospitalized individuals. Nowadays, the use of antibiotic therapy against these bacteria is common, as well as the use of chlorhexidine digluconate to clean surfaces.

However, since the 1960s, *Staphylococcus aureus* has been an agent of great concern in the spectrum of resistance to drugs of antimicrobial nature, due to this, even today the infections associated with it are configured as a problem of high relevance [12]–[14].

With regard to chlorhexidine digluconate, although it acts in the reduction and formation of biofilm with a broad antimicrobial spectrum, with good activity against gram-positive bacteria such as *Staphylococcus aureus*, adverse effects of chronic use of this substance, such as teeth pigmentation, unpleasant taste, and oral scaling, are factors that limit its use [13].

From this perspective, it is evident the importance of the search for alternative drugs. In this context, phytotherapy presents itself as an important tool with broad possibilities for research. It is estimated that 25% to 30% of all drugs considered to be therapeutic agents derive from natural products. In dentistry, the study of herbal medicines for biofilm control is growing, either to obtain bactericidal or bacteriostatic compounds, or substances that hinder bacterial adhesion [15], [16].

Among the numerous researches with natural products to obtain medicines, a group that has been standing out are the essential oils, compounds derived from the secondary metabolism of plants and used in the prevention and treatment of various diseases in view of their documented pharmacological properties, such as antimicrobial, antifungal, antiviral, antitumor, spasmolytic, anticarcinogenic and anti-adherent action [17].

A species rich in essential oils is patchouli (*Pogostemon cablin*), an aromatic plant belonging to the Lamiaceae family whose oil extracted from its leaves is widely used as an important raw material in the manufacture of soaps, incense, cosmetics, and oral hygiene products. This oil is known for its antibacterial, insecticidal, antioxidant, repellent, and anti-adherent activities [18]–[20].

Given the importance of the species in the essential oil industry of *Pogostemon cablin*, the already use of this compound in the oral cavity and the capacity of adherence of *Staphylococcus aureus*, the aim of this research was to perform an *in vitro* investigation of the anti-adherent activity of this oil against this bacterium, considering that the mouth is the gateway for many systemic manifestations and that this pathogen presents itself with high virulence potential, as well as resistance to conventional antibiotic therapy.

2. METHODOLOGY

2.1. Test Substances, Bacterial Species and Culture Media

Pogostemon essential oil was purchased from Industria Herbia. 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 [21], [22].

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.* [23], 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°. The reading was done 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 used for the positive control, 0.12% chlorhexidine digluconate (Periogard®, Colgate-Palmolive Company, New York, USA). In the oil analysis, the lowest concentration of the agent in contact with sucrose that prevented adherence to the glass tube was considered the CIMA.

3. RESULTS AND DISCUSSION

Through the laboratory study, the results revealed that the lowest concentration of *Pogostemon cablin* essential oil able to inhibit the adhesion of bacteria to the tube wall was 1:2, showing positive activity against biofilm formation by *Staphylococcus aureus*, although the result found is lower than the control with 0.12% chlorhexidine digluconate, which inhibits biofilm formation at 1:8 concentration (Table I).

In line with what was found in the present research, after analyses of the study conducted by Bilcu *et al.* [24], Patchouli essential oil was found to inhibit biofilm adhesion by *Staphylococcus aureus* by combining the activity of the oil with the unique properties of magnetic catheter nanoparticles. The research aimed to obtain a novel nanobiosystem that would allow a film to form on the surface of catheter parts, exhibiting improved resistance to microbial adhesion and biofilm development by clinical strains of this bacterium, as the surface of these implanted biomaterials is covered by a protein-conditioning film that predisposes to microbial colonization, and consequently, biofilm-associated infections.

The anti-adherent activity, however, is not the only one of this oil known in the scientific environment. Due to the pharmacological properties of the secondary metabolites of this substance, Patchouli essential oil has been expressing varied and promising pharmacological results in numerous works in the literature. In this perspective, in a study by Pimenta *et al.* [25], by means of the broth microdilution technique for determining the Minimum Inhibitory Concentration (MIC), the antifungal capacity of this essential oil was verified against *Candida grabata* strains, responsible for an increasing amount of chest

TABLE I: MINIMUM INHIBITORY ADHERENCE CONCENTRATION (MIC) OF *Pogostemon cablin* ESSENTIAL OIL AND 0.12% CHLORHEXIDINE DIGLUCONATE AGAINST *Staphylococcus aureus* BACTERIAL STRAIN (SA 101)

<i>Pogostemon cablin</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).

and tracheobronchial infections with dissemination to the lungs in immunocompromised patients.

Among other examples of fungal pathogens affecting immunosuppressed individuals are *Candida tropicalis* and *Candida krusei*, whose systemic complications increase mortality rates in infected individuals. Research results conducted by Cavalcante *et al.* [26] and Alves *et al.* [27] using the same aforementioned methodology, reveal the antifungal ability of *Pogostemon cablin* essential oil against strains of these fungal species, respectively.

Wan *et al.* [28], in turn, evaluated the antibacterial activity of Patchouli alcohol, the major component of *Pogostemon cablin* essential oil, against 127 bacterial strains *in vitro* and *in vivo* tests, including common and drug-resistant bacteria. In *in vitro* evaluation using the double agar dilution method, the alcohol showed activity against some drug-resistant bacterial strains, such as methicillin-resistant *Staphylococcus aureus* (MRSA), whose resulting hospital-acquired infections have presented as a major clinical problem in hospitals.

Also, Fu *et al.* [29] isolated pogostone, another component of Patchouli, and verified the antibacterial activity of this substance against MRSA. In addition, anti-inflammatory, antiviral, antiemetic, antithrombotic, antioxidant and analgesic effects of this essential oil are widely reported [30]–[35].

Despite the wide scientific discussion about the varied pharmacological potential of *Pogostemon cablin* essential oil and the antibiofilm activity of this substance already known, there were no studies in the scientific literature, until then, that addressed the anti-adherent potential of this oil compared to the solution considered the gold standard in the lineage of oral antiseptics. Although the results found in this research are inferior in comparison to those evidenced with chlorhexidine digluconate, it does not reduce the importance and uniqueness for the scientific community, since it can be used as a reference base for future academic work.

4. FINAL CONSIDERATIONS

The *Pogostemon cablin* oil showed an anti-adherent potential, however inferior to the gold standard substance

in the line of oral antiseptics. Considering its vast pharmacological potential already documented in the literature, it is essential to carry out other studies with different types of microorganisms also involved in nosocomial lung infections.

CONFLICT OF INTEREST

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

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